

INNOVATIVE DEVELOPMENTS IN MULTI-MODALITY ELASTOGRAPHY

EDITED BY: Simon Chatelin, Javier Brum, Philippe Garteiser,
Jean-Luc Gennisson, Jing Guo and Najat Salameh
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INNOVATIVE DEVELOPMENTS IN MULTI-MODALITY ELASTOGRAPHY

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Editorial: Innovative developments in multi-modality elastography

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Editorial on the Research Topic

Innovative developments in multi-modality elastography

At the crossroads between biomechanics, medical imaging and wave physics, elastography has been widely developed over the last 30 years. These innovations go hand in hand with the tremendous expansion of knowledge and technological leaps medical imaging has undergone in the past decades. Whatever the modality, elastography relies on three key steps: 1) biological soft tissue is stressed; 2) the resulting displacement, strain or strain rate fields are encoded on images; 3) mechanical property maps are reconstructed from the previously encoded fields. Since the initial proposal of static elastography [1], numerous static [2,3], strain [4–7] and dynamic shear wave [8] imaging methods have emerged. Today, the most widely deployed, dynamic methods include vibro-acoustography [9], Acoustic Radiation Force Impulse (ARFI) [10–12], Transient Elastography (TE) [13–15], Shear Wave Elasticity Imaging (SWEI) [8,16], MRI Elastography (MRE) [17,18] and Optical Coherence Elastography (OCE) [19,20]. Reference is made here to some of the main founding studies of these different approaches, many of which have been widely extended subsequently. Each of these methods has its own advantages and disadvantages with respect to the application to which they are dedicated, and it is important to emphasize their complementarity. For example, MRE methods, even if more complex to implement, allow to obtain measurements with a contrast and an attenuation independent of the penetrated tissues when using ultrasonic or optical methods which, for their part, provide a much more “real-time” information. These aspects have been widely compared in the past in comparative studies and literature reviews [21,22]. However, and as it appears later *via* the different articles presented in this topic, the overlapping of these different methods

allows today to move towards complementary approaches tending to blur these differences by getting the best of each method according to the targeted clinical application. We can mention as an example the use of focused ultrasound for the generation of shear waves in MRE [23–26].

In addition to developments in imaging methods themselves, the most notable advances in recent years have focused on both *in vivo* soft tissue excitation methods and on methods for reconstructing mechanical maps. Even if they are not yet the most important developments, we can for example underline the emergence passive elastography [27–29] and Deep Learning [30,31] as excitation and reconstruction methods, respectively. Through *in vivo* mechanical characterizations across different scales and ranges of behavior of biological soft tissues, elastography enables today the exploration of a very wide spectrum of medical applications, ranging from diagnosis in clinical practice to the understanding and modeling of many healthy and pathological organs.

Current challenges and perspectives in elastography

From the early days, elastography methods have brought together physicists, engineers and physicians with diverse and complementary backgrounds, ranging from medical imaging to wave physics and mechanics. Recent developments have further broadened this spectrum of skills by including new fields of research and applications, such as data science, artificial intelligence, interventional radiology and organ modeling. The diversity in researcher profiles involved in elastography calls for the need of communication channels that facilitate exchanges and synergies between different scientific communities that traditionally do not speak to one another.

In this context, the present topic aims to capture the current elastography landscape, whatever the imaging modality, and highlight the transdisciplinary potential of the tools and methods developed by each of the communities. In order to fully understand these new developments as well as the issues and applications associated with them, this topic first proposes five literature reviews on:

- elastic waves generation: on the one hand for MRE specifically [Gnanago et al.](#), and on the other hand for passive elastography [Brum et al.](#);
- the use of machine learning for model-free mechanical property mapping [Hoerig et al.](#);
- multi-scale opportunities of ultrasonic and optical elastography methods [Ormachea and Zvietcovich](#);
- specific clinical applications of MRE for the characterization of malignant tumors [Pagé et al.](#)

In addition, original research papers of this topic focus on methodological developments for multimodal elastography, with particular emphasis on reconstruction methods for poroelasticity [Aichele and Catheline](#), [Sowinski et al.](#), [Theodorou et al.](#), scattering, strain [Liu et al.](#), [Rippy et al.](#) and multiscale mechanics [Garczyńska et al.](#), [Garczyńska et al.](#), as well as on the development of multimodal numerical models [Torres et al.](#) and experimental phantoms [Chatelin et al.](#), [Yushchenko et al.](#) Finally, it was shown in some clinical applications that multimodal elastography can be leveraged in order to take full advantage of the complementarities across modalities as illustrated in some clinical studies [Goudot et al.](#), [Kreft et al.](#), [Pan et al.](#), [Li et al.](#)

To conclude, this Research Topic successfully highlighted that despite using different approaches, strong interrelationships and transfers between different modalities appear most fundamental and beneficial for the future development of elastography.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Innovative Multiparametric Characterization of Carotid Plaque Vulnerability by Ultrasound

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Objective: The degree of stenosis of a carotid plaque is a well-established risk factor for ischemic stroke. Nevertheless, the risk of ipsilateral stroke in asymptomatic carotid stenosis remains low and new imaging markers are needed to better target which patients would benefit most from endarterectomy or intensive medical therapy. Ultrafast ultrasound imaging offers parameters helping at characterizing the carotid plaque by shear wave elastography and Ultrafast Doppler (UFD). We aimed at using these techniques to characterize 3 different ultrasound biomarkers: plaque stiffness heterogeneity, wall shear stress (WSS) and intraplaque micro-flows and to correlate these biomarkers with findings on computed tomography angiography (CTA) and the pathological examination.

Methods: We present the case of a multimodal evaluation of a carotid plaque using ultrasound. Elastography has been coupled to the WSS assessment and the detection of intraplaque micro-flows by UFD. The data have been compared to CTA and to the pathology examination of the tissue after carotid endarterectomy.

Results: Elastography allowed at identifying stiff areas corresponding to calcifications, as well as a soft area corresponding to an intraplaque hemorrhage. The flow evaluation with UFD showed an increase of the WSS along the plaque and identified the presence of a plaque rupture, confirmed by the pathologist.

Conclusion: Ultrafast ultrasound imaging is an innovative, easily accessible technique that provides imaging modalities on top of the conventional B-mode. Ultrafast ultrasound biomarkers such as plaque stiffness heterogeneity, WSS and intraplaque micro-flows could help to define the vulnerability of the carotid plaque in order to stratify patients that could benefit most from endarterectomy or intensive medical therapy.

Keywords: carotid plaque, plaque vulnerability, ultrafast ultrasound imaging, wall shear stress, elastography

INTRODUCTION

Carotid endarterectomy in case of asymptomatic plaque has been tested by 2 large randomized trials, ACAS and ACST trials (Walker et al., 1995; Halliday et al., 2010). A small but significant absolute risk reduction at 5 years of 5.9% (5.1 vs. 11.0% of ipsilateral stroke) and 5.4% (6.4 vs. 11.8% for any territory stroke) were respectively found. But these past studies overestimated the

stroke risk using old standards medical intervention (Naylor, 2011; Abbott et al., 2017). With lower thresholds for defining hypertension and hypercholesterolemia, better blood pressure lowering drugs, statins and more people giving up smoking, the risk of ipsilateral stroke from a carotid plaque has been reduced to approximately 1% per year since ACAS and ACST trials (Naylor et al., 2009), now making the relevance of endarterectomy debatable (Abbott, 2009). There are, however, still patients having asymptomatic carotid plaques at “higher-than-average” risk of ipsilateral stroke. For example, as reported by Nicolaides et al. (2010), asymptomatic patients with a 90–99% ECST stenosis, a history of contralateral transient ischaemic attack or stroke and no discrete white areas and a grayscale median over 30, had a risk of ipsilateral stroke over 5 years of 13.3% when plaque area exceeded 80 mm² (Nicolaides et al., 2010). Better risk stratification of people with carotid stenosis may mean better use of treatments to reduce the risk of ipsilateral stroke (as in the case of carotid procedural intervention) or all arterial disease complications (as in the case of medical intervention- lifestyle coaching and medications).

All persons with arterial disease should receive current optimal medical intervention to reduce the risk of stroke and other arterial disease complications using clinically identified risk factors (such as hypertension, hyperlipidaemia or smoking) for risk stratification. This is because current best evidence indicates a likely overall patient benefit using this approach. In addition, arterial imaging technology could be used to spare people from unnecessary and potentially harmful procedures or other treatments. It could be also used to better select persons for carotid procedures or particularly effective but risky or expensive medical treatments.

Biomarkers to identify plaques at higher risk of ipsilateral stroke have been proposed with different imaging modalities, magnetic resonance imaging (MRI), ultrasound, and computed tomography angiography (CTA) (Aboyans et al., 2017; Naylor et al., 2018; Saba et al., 2019). These biomarkers are based on comparisons with the histological profiles of vulnerable plaques, determined by the presence of a ruptured fibrous cap or an intra-plaque hemorrhage for instance (Lovett et al., 2004). Using ultrasound imaging, plaque vulnerability can be assessed by measuring plaque B-mode echogenicity (Geroulakos et al., 1993; Topakian et al., 2011), estimation of plaque volume (Nicolaides et al., 2010), stenosis progression (Kakkos et al., 2014), impaired vascular reserve (King et al., 2011) and spontaneous embolization on transcranial Doppler (Markus et al., 2010). The combination of combined markers would thus make it possible to identify the most at-risk plaques.

The aim of our research is to provide biomarkers with ultrafast ultrasound imaging that could easily be combined to identify patients who would benefit most from endarterectomy or intensive medical therapy. In order to maintain the benefit of such interventions, the objective would be to identify plaques with an annual risk of ipsilateral stroke over 3%. In our laboratory we developed specifically dedicated sequences for the evaluation of the carotid plaque by

using ultrafast imaging modalities. We present here the multimodal evaluation of a patient at “higher-than-average” risk of ipsilateral stroke based on the stenosis progression and a large plaque area. The aim of this case is to show a proof of concept on the feasibility of ultrafast imaging evaluation by combining several methods of tissue and blood flow analysis.

CASE

A 68-year-old male with a medical history of hypertension and hyperlipidaemia was monitored every year by Doppler ultrasound for an atherosclerotic stenosis of the left carotid bifurcation. He was non-smoker, he received valsartan 160 mg, amlodipine 10 mg, pravastatin 40 mg and aspirin 75 mg daily. Still asymptomatic, without ischemic complication, the degree of stenosis increased up to 70% NASCET compared to 55% one year before. The plaque was large, at 84 mm², with a grayscale median at 64 (no unit). A CTA confirmed the presence of a 70% NASCET carotid stenosis associated with calcifications (**Figure 1**). The patient was therefore referred for a carotid endarterectomy. After being informed of the risks of this procedure, the patient accepted the surgery and signed a written statement of consent. The UF-plaque study evaluating the usefulness of ultrafast ultrasound imaging (UF) multiple parameters to better define the plaque vulnerability was further presented to the patient. He signed the consent for inclusion and publication of this case report. We present below the results of this UF evaluation.

Elastography Evaluation

Shear wave elastography is an ultrasound-based technique for real-time and quantitative imaging of soft tissue viscoelastic properties. UF, using a frame rate of 5,000 frames per second, allows tracking transient mechanical shear waves generated by the ultrasonic probe through the acoustic propagation. The velocity of shear waves is directly linked to the tissue stiffness (Bercoff et al., 2004). The elastography mode, by the use of an Aixplorer® ultrasound scanner (SuperSonic Imagine®, Aix-en-Provence, France) and a linear probe (SL10-2, central frequency of 7.5 MHz), provides stiffness mapping of the arterial wall superimposed to the B-mode image (Tanter and Fink, 2014). This technique can be applied to the analysis of carotid plaques (**Figure 1**) (Ramnarine et al., 2014; Garrard et al., 2015; Lou et al., 2017). In our case, we observed heterogeneity of the local elastic Young modulus, corresponding to the arterial wall stiffness, with the presence of a stiff area (area's mean elasticity of 79.3 ± 33.3 kPa) in the posterior wall, and a low stiffness area (area's mean elasticity of 30.6 ± 7.0 kPa) in the anterior wall. The posterior wall had scattered echoes with shadow artifacts in B-mode ultrasound that could correspond to the calcifications depicted on the CTA. The anterior wall had a hypoechogenic segment in B-mode ultrasound that could correspond to the low-density area on the CTA (**Figure 1** and **Supplementary Figure S1**).

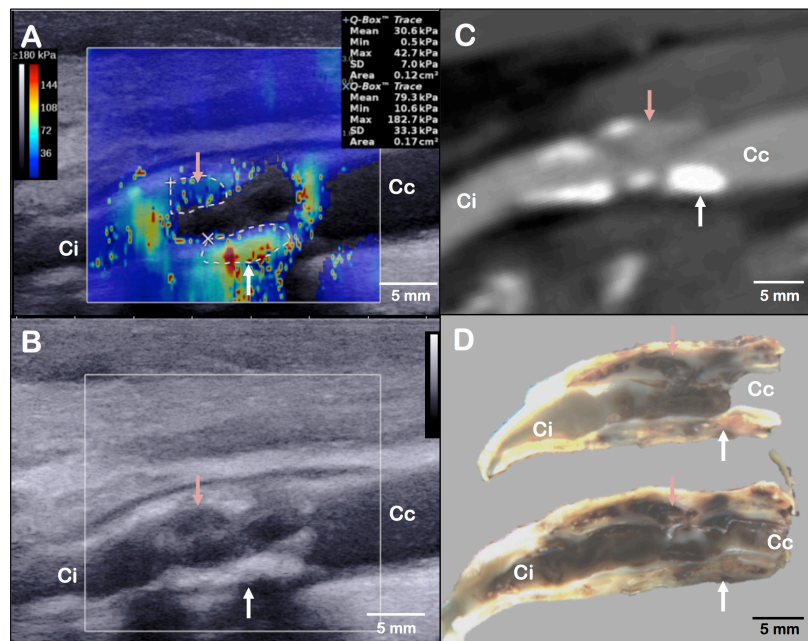


FIGURE 1 | Layout of elastography assessment (A), B-mode ultrasound acquisition (B), computed tomography angiography (C) and gross examination (D) of the same plaque in a longitudinal axis view. The red arrow indicates the main haemorrhagic area on panel (D). It corresponds to a low stiffness (A), hypoechoic (B) and low-density (C) area. The white arrow indicates a fibrous and calcified area on panel (D). It corresponds to a high stiffness (A), hyperechoic (B) and high-density (C) area. Ci, internal carotid; Cc, common carotid.

While the stiff part of the plaque corresponded to surface and deep calcifications, the low-stiffness part of the plaque could correspond to the intraplaque hemorrhage or the necrotic lipid core located at the most stenotic segment found on gross examination (Figure 2).

Ultrafast Doppler (UFD) Evaluation

Two UFD imaging sequences have been used here: the first sequence was designed for ultrafast vector flow imaging (angle-independent quantitative Doppler mode) and the second sequence was designed for ultra-sensitive Doppler imaging of low velocity blood flows. To map the flow dynamics correctly in the carotid artery, it is imperative to resolve the beam-flow angle dependence problem. Vector Doppler imaging can estimate flow velocities at each point of the lumen without any angle correction (Dort et al., 2012; Jensen et al., 2018), giving access to the wall shear stress (WSS) (Poelma et al., 2012; Leow and Tang, 2018; Goudot et al., 2019a,b): the tangential force exerted by the blood on the vessel wall. Vector Doppler imaging relies on Doppler estimation performed at different beam-flow angles, with a minimum of two angles, to obtain two independent frequency shift equations and derive the absolute velocity of each point. The ultrafast vector flow imaging used the same Aixplorer® scanner, the same linear probe and a sequence of 3 plane waves [-10° , 0° , 10°] leading to a frame rate of 5,000 Hz. The WSS data were generated by computing the gradients of velocity in the vicinity of the arterial wall multiplied by the blood viscosity. In this case, the evaluation

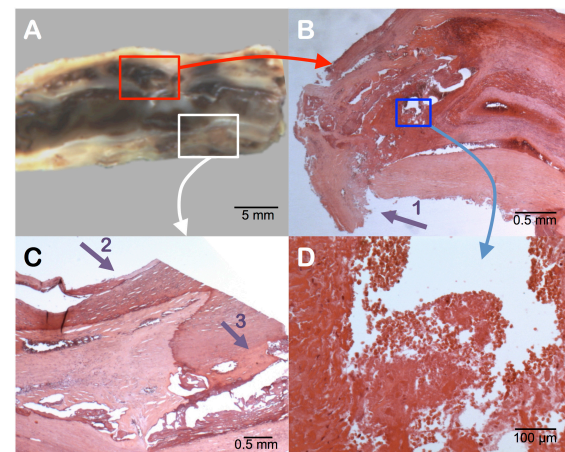


FIGURE 2 | Pathology examination of the carotid plaque. Gross longitudinal section (A). Presence of an intraplaque hemorrhage (surrounded by the red box) in the upper section, as seen in cross-sectional microscopic analysis (B). Identification of the rupture zone of the fibrous cap (arrow 1). At higher magnification of the blue box (D) the intraplaque hemorrhage contains numerous red blood cells with intact membranes, confirming a recent hemorrhage. The posterior part of the plaque (white box) shows surface calcifications (arrow 2) and deep calcifications (arrow 3) (C).

showed an increase of the WSS along the carotid plaque from 3.82 Pa at the plaque ascent, to a maximum of 4.71 Pa at the most stenotic segment (plaque's peak) and

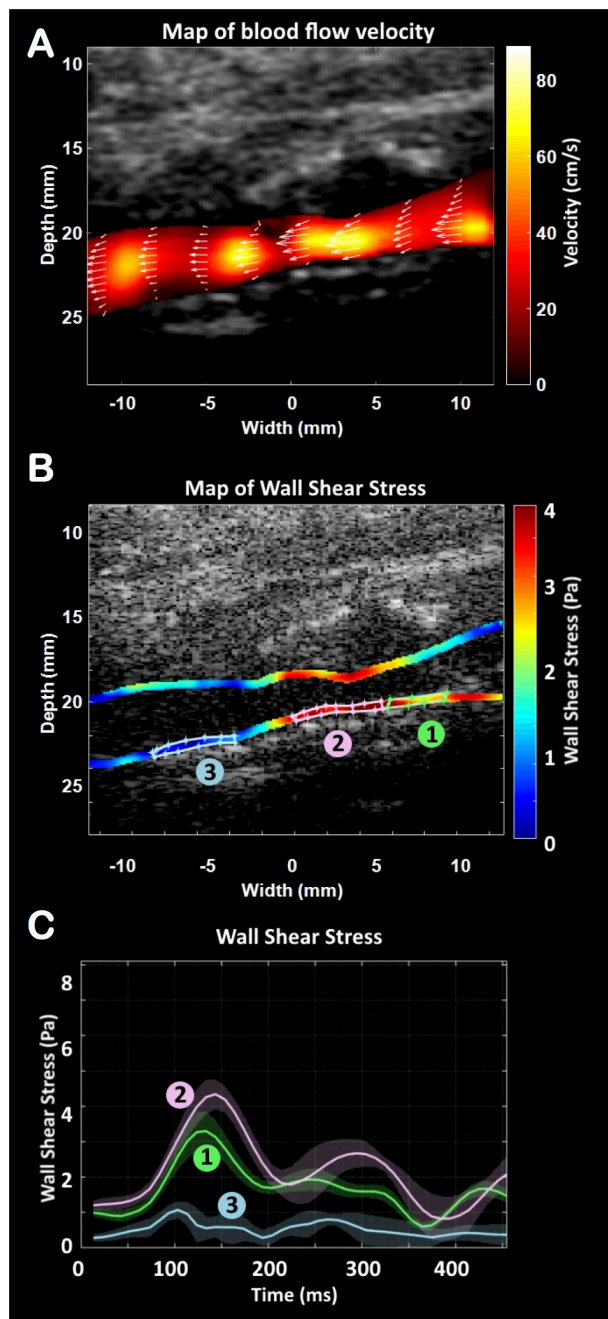


FIGURE 3 | Ultrafast vector flow imaging. Representation of the velocities map (A). Representation of the wall shear stress (WSS) map along the carotid walls (B) and the changes over the cardiac cycle (C) at the plaque's ascent (1), plaque's peak (2) and plaque's descent (3).

dropped below 1.45 Pa at the plaque descent (Figure 3 and Supplementary Video S1).

Unlike the vector flow imaging mode, ultra-sensitive Doppler mode has been developed to enhance the detection of low velocity flows with a high spatial resolution (Macé et al., 2011). Applied to the carotid artery, the objective was to detect

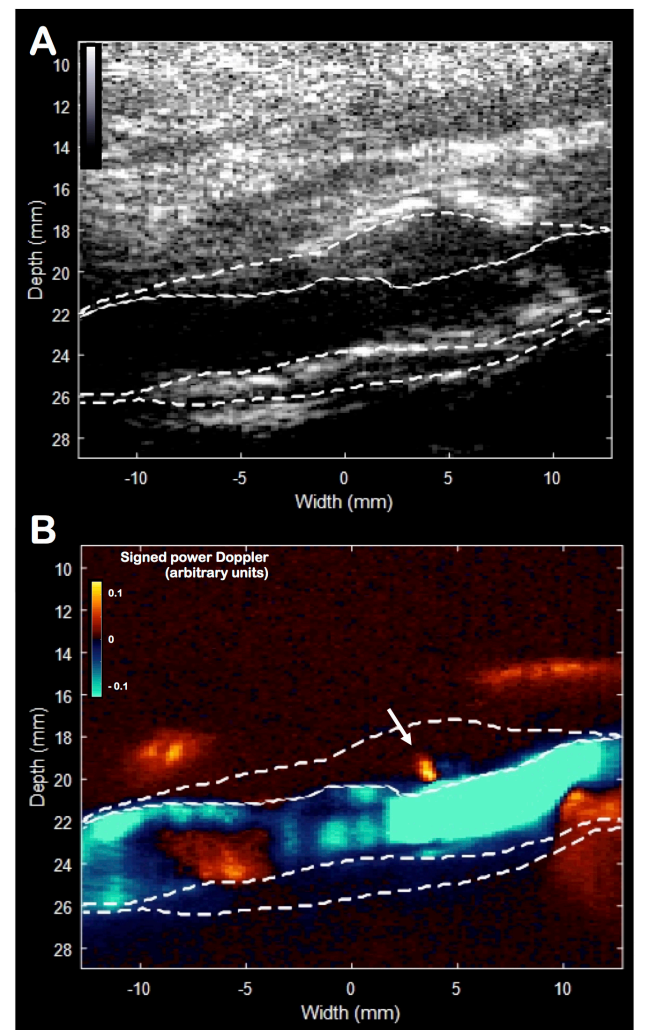


FIGURE 4 | Ultra-sensitive Doppler imaging. B-mode picture (A) and the signed power Doppler picture (B). The plaque was manually delineated. The white arrow indicates the presence of an isolated centrifugal micro-flux originating from the lumen inside the plaque's wall, which may correspond to a rupture zone of the fibrous cap.

micro-flows within the plaque. The sensitivity of detection is improved by using ultrafast Doppler imaging and plane wave compounding (Bercoff et al., 2011) combined with a spatio-temporal singular value decomposition clutter filter (Demené et al., 2015). The ultra-sensitive Doppler imaging used the same ultrasound scanner, the same probe and a sequence of 16 plane waves $[-15^\circ, -13^\circ, -11^\circ, -9^\circ, -7^\circ, -5^\circ, -3^\circ, -1^\circ, 1^\circ, 3^\circ, 5^\circ, 7^\circ, 9^\circ, 11^\circ, 13^\circ, 15^\circ]$ leading to a frame rate of 950 Hz. Compared to conventional Doppler, the compounding of these 16 plane waves increases the sensitivity to 30 times (Macé et al., 2011) showing very small flow changes. In this case, the presence of a centrifugal micro-flow from the lumen was identified within the anterior wall (Figure 4 and Supplementary Video S1) and located in the area of soft stiffness described above. Ultra-sensitive Doppler

allows to identify ascending and descending flows. During the cardiac cycle, the ascending flow occurs in systole, and the descending flow occurs in diastole, as shown in the movie. We believe that this flow, not observable in conventional Doppler technique, is the witness of an ulceration in the plaque's anterior wall.

Surgical Observation

During the intervention, the surgeon noticed the presence of a severe and calcified stenosis with a plaque ulceration. No complication, either immediately after surgery or after 1 year of follow-up, were noticed. Post-operative Duplex ultrasound revealed no residual stenosis.

Pathology Examination

Gross examination of the removed plaque was assessed on a longitudinal section (**Figure 2**). It noticed the presence of a large intraplaque hemorrhage and a plaque rupture located in the anterior wall. Histology revealed diffuse microcalcifications and thick calcifications in the posterior wall. According to Lovett et al., the presence of a rupture of the fibrous cap and an intraplaque hemorrhage were significantly associated with irregularity of the carotid plaque on angiography, which appeared as a strong independent predictor of ipsilateral ischemic stroke on medical treatment at all degrees of stenosis (hazard ratio 1.80) (Rothwell et al., 2000), categorizing the plaque as vulnerable (Eliasziw et al., 1994; Streifler et al., 1994; Rothwell et al., 2000; Lovett et al., 2004).

DISCUSSION

This case illustrates a multiparametric assessment of a carotid plaque by UF. The vulnerability of the plaque was confirmed by the presence of a plaque ulceration and a large intraplaque hemorrhage described on gross examination. These characteristics had not been seen neither by Duplex ultrasound nor by CT scan. In addition to conventional B-mode, UF enables us to better identify structures by stiffness analysis and WSS measurement. In this case, UF identified a soft area with a low velocity flow disappearing in the anterior wall of the carotid artery. These data may attest the presence of a hemorrhage and ulceration in the plaque's wall. The ultrafast vector flow imaging, recently developed, offers the unique opportunity to directly measure WSS by ultrasound. MRI previously tried, first by Computational Flow Dynamics and recently by 4D flow sequences with to address the direct assessment of the WSS. High WSS values were found associated with the presence of intraplaque hemorrhage (Tuenter et al., 2016), as we report in our case. Moreover, as reported by Slager et al. (2005) the WSS may play a role in the generation of rupture-prone vulnerable plaque. As the intraplaque hemorrhage is known to be associated with an increased risk of stroke in carotid plaques, presence of high WSS values could argue for a vulnerable plaque.

Lastly, the ultra-sensitive Doppler imaging aims at detecting micro-flows within tissue without the need for any contrast

agents. This method can identify a flow irrigating into the plaque, which can indicate the presence of a plaque ulceration, a characteristic associated with an increased risk of embolic events (Eliasziw et al., 1994; Handa et al., 1995). The high frame rate (950 Hz) allows here to detect low velocity flows, not accessible with all conventional imaging techniques (ultrasound, CT and MRI) (Macé et al., 2011).

The main limitation regarding the parameters presented is related to the presentation of a single case. The sole objective was to illustrate the feasibility of these ultrafast imaging modalities and no conclusion on the validation of the parameters presented can be provided here. A prospective validation is indeed required to demonstrate the value of these markers.

CONCLUSION

In conclusion, UF is an innovative technology that conveys, on top of a B-Mode imaging, a shear wave elastography mode and an ultrafast Doppler mode leading to new parameters to better characterize the carotid plaque by measuring stiffness, WSS and micro-flows. The UF-plaque study (NCT03234257) is currently on-going aiming at demonstrating the correlation between stiffness measurement, WSS, micro-flows detection with plaque vulnerability criteria determined by histology on a prospective cohort of patients with carotid endarterectomy. For the future, the local evaluation of the carotid plaque by UF could provide an opportunity to perform a multimodal analysis including clinical criteria (history of previous cerebral infarction or transient ischemic attack), and association with brain imaging criteria (silent infarction), in order to select patients at higher than average risk of stroke that would most benefit from carotid endarterectomy or intensive medical treatment.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the *Comités de Protection des Personnes*, CPP N° 2017T2-13. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GG, LK, and TM wrote the manuscript. GG and EM performed the ultrasound analysis. PJ and J-MA performed the surgery.

PB and KA performed the histological analysis. MP, OP, and JP performed the post-processing of the ultra-fast Doppler acquisitions. TM performed final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2020.00157/full#supplementary-material>

FIGURE S1 | Carotid plaque contouring visualized by different imaging modalities: B-mode ultrasound (A), shear wave elastography (B), ultrasensitive Doppler (C), computed tomography (D). The plaque is contoured on gross analysis after a longitudinal section of the carotid bifurcation (E). The areas of calcifications (gray) and intra-plaque hemorrhage are shown on the carotid stenosis figure (F). Ci, internal carotid; Cc, common carotid.

VIDEO S1 | Evaluation of blood flow by Ultrafast Doppler at the carotid plaque level over the cardiac cycle. Representation of flow velocity vectors (1), wall shear stress (2), and micro-flow obtained with the ultra-sensitive Doppler sequence (3).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Changes in Liver Mechanical Properties and Water Diffusivity During Normal Pregnancy Are Driven by Cellular Hypertrophy

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During pregnancy, the body's hyperestrogenic state alters hepatic metabolism and synthesis. While biochemical changes related to liver function during normal pregnancy are well understood, pregnancy-associated alterations in biophysical properties of the liver remain elusive. In this study, we investigated 26 ex vivo fresh liver specimens harvested from pregnant and non-pregnant rats by diffusion-weighted imaging (DWI) and magnetic resonance elastography (MRE) in a 0.5-Tesla compact magnetic resonance imaging (MRI) scanner. Water diffusivity and viscoelastic parameters were compared with histological data and blood markers. We found livers from pregnant rats to have (i) significantly enlarged hepatocytes ($26 \pm 15\%$, $p < 0.001$), (ii) increased liver stiffness ($12 \pm 15\%$, $p = 0.012$), (iii) decreased viscosity ($-23 \pm 14\%$, $p < 0.001$), and (iv) increased water diffusivity ($12 \pm 11\%$, $p < 0.001$). In conclusion, increased stiffness and reduced viscosity of the liver during pregnancy are mainly attributable to hepatocyte enlargement. Hypertrophy of liver cells imposes fewer restrictions on intracellular water mobility, resulting in a higher hepatic water diffusion coefficient. Collectively, MRE and DWI have the potential to inform on structural liver changes associated with pregnancy in a clinical context.

Keywords: liver stiffness, viscosity, pregnancy, hypertrophy, magnetic resonance elastography (MRE), diffusion weighted imaging (DWI), water diffusion, hepatomegaly

INTRODUCTION

Pregnancy is a dynamic process involving a series of maternal physiological changes and adaptations that occur to support fetal growth and development. The changes are driven by maternal and placental hormones (estrogen, progesterone, prolactin, and others; Napso et al., 2018) and require considerable morphological and physiological flexibility of the maternal body, both

locally and systemically (Moll, 2001; Baeyens et al., 2016; Soma-Pillay et al., 2016; Napso et al., 2018).

The liver is the largest gland in the human body and plays a central role in metabolism. Hepatocytes participate, inter alia, in glucose, lipid, protein, and peptide metabolism (Pedrycz et al., 2014). As a central metabolic organ, the maternal liver undergoes significant changes induced by higher estrogen levels during normal pregnancy. In early pregnancy, when fetal demands are still relatively low, the maternal body stores energy through increased glucose intake and lipogenesis, as well as glycogen accumulation in hepatocytes to prepare for the higher energy consumption by the developing fetus in late gestation. Therefore, maternal cholesterol, triglyceride, and phospholipid levels are elevated from the second trimester until the end of pregnancy (Bacq, 2000–2013; Lain and Catalano, 2007; Pedrycz et al., 2014; Soma-Pillay et al., 2016; Zhang et al., 2017; Napso et al., 2018). In light of these known physiological changes, adjusted standard reference levels of serum markers have been defined for pregnant women (Abbassi-Ghanavati et al., 2009; Jamjute et al., 2009).

In addition, pregnancy-related adaptation of the maternal liver also involves changes in the organ's biomechanical properties (Ammon et al., 2018; Stenberg Ribeiro et al., 2019). Elastography can quantify biomechanical properties such as stiffness and viscosity of the liver *in vivo*. Studies of pregnant women using ultrasound-based elastography reported that liver stiffness increased during normal pregnancy and returned to baseline values after delivery (Ammon et al., 2018; Stenberg Ribeiro et al., 2019) where intraabdominal pressure was considered an imported contributor. Additionally, it was shown that changes in liver stiffness related to normal pregnancy differed from those caused by pregnancy-related liver disorders such as pre-eclampsia (Frank Wolf et al., 2016; Ammon et al., 2018) and intrahepatic cholestasis of pregnancy (IHC; Cetin et al., 2017). These results indicate that elastography sensitively detects mechanical changes of the liver during pregnancy. However, due to a lack of histological evidence, the underlying biological causes of altered hepatic stiffness during pregnancy are not fully understood, the authors only hypothesized on a possible association of increased stiffness with altered liver perfusion and intra-abdominal pressure during pregnancy.

Magnetic resonance elastography (MRE), an emerging elastography modality based on magnetic resonance imaging (MRI), can quantify the mechanical properties of soft tissues both *in vivo* and *ex vivo*. A compact MRE modality tailored to *ex vivo* tissue investigation of small cylindrical samples has been introduced recently (Ipek-Ugay et al., 2015; Braun et al., 2018; de Schellenberger et al., 2019; Sauer et al., 2019; Everwien et al., 2020). This compact MRE technique enforces well-controlled (cylindrical) boundary conditions permitting analytical solutions of the inverse problem in MRE by utilizing Bessel function. As a result, compact MRE has been shown to provide consistent values of stiffness and attenuation (shear elasticity and shear viscosity) with little degradation by noise. Moreover, MRE examinations can be combined with determination of other quantitative MRI parameters including water diffusion. In order to correlate pregnancy-related biomechanical changes with underlying biology, we used compact MRE (Ipek-Ugay et al.,

2015; Braun et al., 2018; de Schellenberger et al., 2019; Sauer et al., 2019; Everwien et al., 2020) to investigate changes in stiffness and viscosity of *ex vivo* liver samples harvested from pregnant rats. With *ex vivo* samples, we examine mainly the dependency of mechanical properties on the underlying microarchitecture of the liver without influence from blood perfusion, which could be a confounding factor. MRE results were paired with diffusion-weighted imaging (DWI) to investigate the effect of pregnancy on hepatic water diffusivity. The water transport and viscoelasticity quantified by MRE and DWI, respectively, provided complementary information that are sensitive to the microstructure of biological tissues.

In addition, we performed extensive histological and biochemical analyses to elucidate possible structural causes of MRE and DWI parameter changes due to pregnancy. Our MRE results might shed light on clinically relevant biophysical changes of the liver detected by ultrasound elastography in pregnant women, however, one must take into consideration the difference between the two imaging modalities in terms of frequency range and data acquisition technique when comparing data obtained from MRE and ultrasound elastography.

MATERIALS AND METHODS

All procedures involving animals were approved by the local authority (Landesamt für Gesundheit und Soziales Berlin, Reg. No. T0280/10, T0212/19) and were performed according to institutional guidelines.

Sample Preparation

Livers were harvested from young adult female Wistar rats (Forschungseinrichtungen für Experimentelle Medizin, FEM, Berlin, Germany; Janvier Labs, Le Genest-Saint-Isle, France) of two groups: (i) pregnant group (P18; sacrificed on 18th day of gestation), $n = 13$; and (ii) non-pregnant group (NP), $n = 13$. The animals were kept in the same animal facility under standard housing conditions for at least 3 days before imaging.

To harvest the liver, rats were anesthetized with an overdose of isoflurane vapor and then decapitated with a rodent guillotine. A slice of liver (approximately 20 mm in height, 5–8 mm in width) was cut from the left lateral lobe of the fresh liver and transferred directly to the sample tube for MR imaging, while the remaining liver tissue was prepared for histological analysis. The width of the liver slice was trimmed to fit the diameter of the sample tube so that the liver slice can be slid into the tube easily without any compression. In 8 rats of each group, the freshly harvested livers were weighed and the blood collected—approx. 0.8 ml per rat in blood collection tubes containing EDTA and lithium heparin (Sarstedt, Germany)—for laboratory analysis.

Histology and Immunostaining

After harvesting, fresh liver tissue samples (from 8 rats per group) were fixed in 4 % formaldehyde solution (ROTI[®] Histofix 4 %, Roth, Karlsruhe, Germany) at room temperature for 24 h. After fixation, the samples were dehydrated for 24 h and embedded in paraffin (ROTI[®] Plast, Roth, Karlsruhe, Germany).

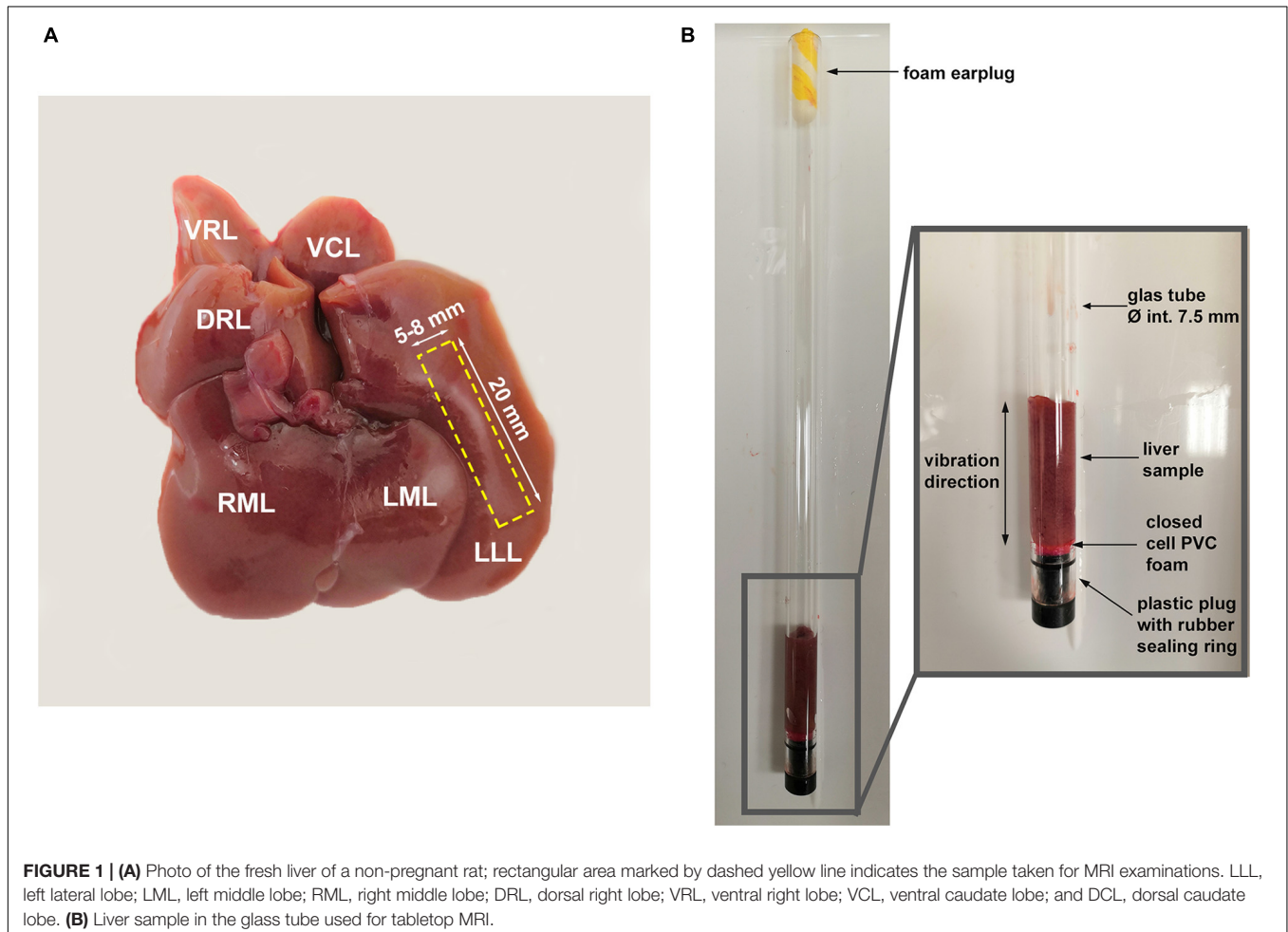
The tissue paraffin blocks were sliced into 2 μm thick sections and finally transferred onto Superfrost/Superfrost Plus slides (R. Langenbrinck GmbH, Emmendingen, Germany). As the mechanical properties quantified by MRE directly associate with microarchitecture of the tissue and the arrangement of the structure elements, especially those in the extra cellular matrix (ECM), we have selected the following staining methods for morphological characterization: hematoxylin and eosin (H&E; Mayer's Hemalum Solution, Merck, Darmstadt, Germany; Eosin Y solution, Sigma-Aldrich, Darmstadt, Germany) and Elastica van Gieson (Merck, Darmstadt, Germany). As hyperproliferation of hepatocytes has been reported in Bustamante et al. (2010), Dai et al. (2011) for pregnant rats, the liver tissue sections were also immunostained for Ki-67 protein. The primary antibody (clone SolA15, eBioscience™ from Thermo Fisher Scientific; Thermo Fisher Scientific Cat# 14-5698-80, RRID:AB_10853185) was pre-incubated with FabuLight secondary antibody (biotinylated goat anti-rat; Jackson ImmunoResearch). Biotin was detected by streptavidin coupled with alkaline phosphatase (ALP) and RED as chromogen [both Dako REAL™ Detection System, ALP/RED, Rabbit/Mouse (Agilent Technologies)], nuclei were counterstained with hematoxylin (Merck Millipore).

Images of stained sections were taken with a BZ-X800 fluorescence microscope (KEYENCE DEUTSCHLAND GmbH, Neu-Isenburg, Germany). Five high-power fields per animal were analyzed. Hepatocytes and Ki67-positive hepatocytes were counted per field of view (FoV) at 40x magnification in H&E-stained and immunostained sections, respectively, using ImageJ software version 1.52v (Schneider et al., 2012). Histological analysis was performed in a blinded manner.

Magnetic Resonance Elastography and Diffusion-Weighted Imaging

All tissue samples for MRI (P18, $n = 13$; NP, $n = 13$) were taken from the left lateral lobe of the liver which is the largest lobe, facilitating sample preparation (**Figure 1A**). The MRI measurements started 2 h post-mortem. Liver slices (approximately 20 mm in height, 5–8 mm in width) were cut from the liver. The samples were placed in a glass tube (**Figure 1B**), which was then inserted in a 0.5-T compact MRI device (Pure Devices GmbH, Würzburg, Germany) for both MRE and DWI.

The compact MRE setup (tabletop MRE) was described in detail in previous publications (Braun et al., 2018;



de Schellenberger et al., 2019; Sauer et al., 2019; Everwien et al., 2020). In short, a gradient amplifier (DC 600, Pure Devices GmbH, Würzburg, Germany) and a piezo-actuator (Piezosystem Jena, Jena, Germany) which was directly coupled to the glass sample tube, were integrated into the tabletop MRI device for generating mechanical vibrations and introducing them into the tissue sample. A spin-echo-based MRE sequence as described in Braun et al. (2018) was used for acquiring wave images.

Imaging parameters for tabletop MRE and DWI were similar to those described in Braun et al. (2018), de Schellenberger et al. (2019), Sauer et al. (2019), Everwien et al. (2020). In brief, mechanical vibrations of 800 Hz were excited for MRE acquisition. Dynamic wave propagation was recorded in eight time steps over a wave cycle in one axial 3-mm thick slice with a field of view of $9.6 \times 9.6 \text{ mm}^2$ (64×64 matrix size). With a repetition time (TR) of 0.5 s and an echo time (TE) of 20 ms, the total MRE acquisition time was 3 min. DWI was performed with a customized spin-echo sequence (Sauer et al., 2019) using seven b -values (50, 175, 300, 425, 550, 675, and 800 s/mm^2). One 3-mm single slice with an in-plane resolution of $600 \mu\text{m}$ was acquired with a TR of 1 s and TE of 8 ms, and total acquisition time was 5 min. During one imaging session, MRE and DWI were performed in an interleaved manner, and the MRE/DWI block was repeated five times, resulting in a total acquisition time of 40 min. Sample temperature was kept constant at 30°C for all MRI examinations. During the acquisition time, the liver sample which was sealed in the sample tube and kept at a constant temperature was considered well-conditioned.

For MRE data post-processing, shear wave speed (c in m/s) and penetration rate (a in m/s) were derived by taking the analytic solution of the fitting of the single profile of complex-valued wave along the z -direction based on a Bessel function, as described in Braun et al. (2018), de Schellenberger et al. (2019). c represents tissue stiffness while penetration rate a is inversely correlated with tissue viscosity. For comparing to results obtained by ultrasound elastography, c can be converted to Young's modulus (E) with: $E = 3\rho c^2$ where ρ is the density, which we assume to be 1 kg/l for all biological soft tissues. For DWI,

maps of apparent diffusion coefficient (ADC), which quantifies water diffusivity, were generated with mono-exponential fitting and linear regression analysis taking images at all seven b -values. Images with b -value of 50 were also used for fitting considering the absence of perfusion in our *ex vivo* samples. Data were post-processed using algorithms written in MATLAB (R2019b, The Mathwork Inc., Natick, MA, United States).

Statistical Analysis

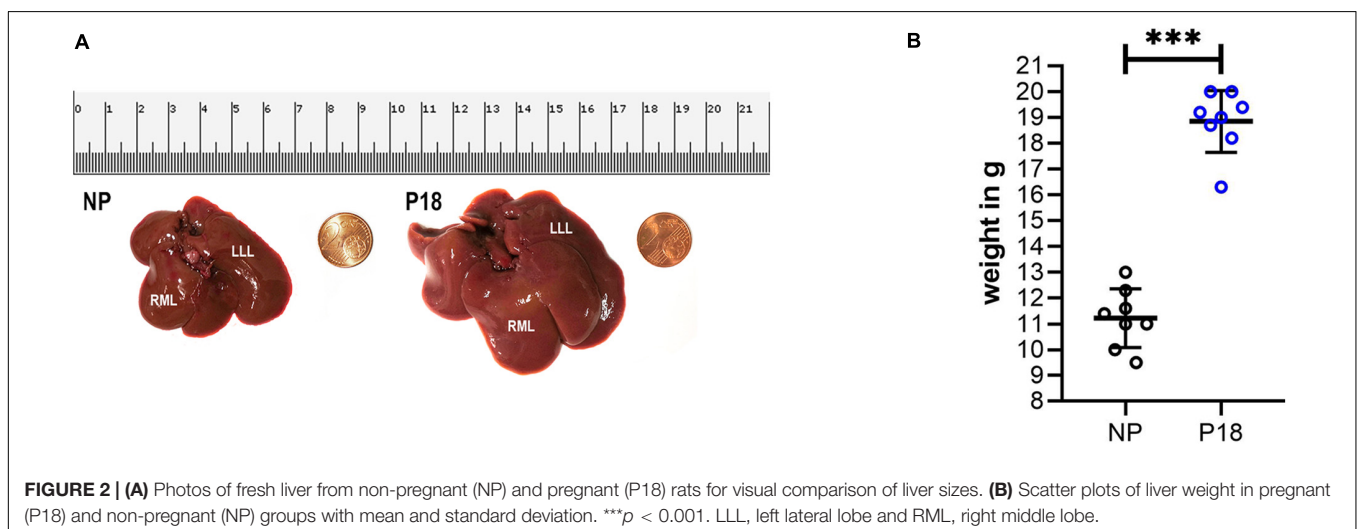
Mixed analysis of variance (ANOVA) was performed to account for the effects and interactions of two factors present in our data – acquisition time (within-subject factor) and pregnancy (between-subject factor). Normality was tested with both the Shapiro–Wilk test and the Kolmogorov–Smirnov test. Differences between the pregnant and non-pregnant groups were tested using the unpaired t -test for normally distributed data and the Mann–Whitney test for datasets that violated the normality assumption. Relationships between data were assessed by Pearson correlation ($n > 10$) and Spearman correlation ($n < 10$). P -values below 0.05 were considered statistically significant.

Graphical and statistical analysis was performed with GraphPad Prism (GraphPad Prism 8.01. for Windows, GraphPad Software, San Diego, CA, United States, www.graphpad.com; GraphPad Prism, RRID:SCR_002798) and SPSS 23 (SPSS Inc, Chicago, IL, United States; SPSS, RRID:SCR_002865).

RESULTS

General Characterization of Livers From Pregnant Rats

A representative photo of two rat livers is shown in **Figure 2A**. The liver from a P18 rat was visibly larger compared to that from an NP rat. A significant difference in liver weight was observed between the pregnant and non-pregnant groups (P18: $18.8 \pm 1.2 \text{ g}$ vs NP: $11.2 \pm 1.1 \text{ g}$, $p < 0.001$, $n = 8$ per group, **Figure 2B**). The livers from P18 rats were on average 40% heavier than those from non-pregnant rats.



Histological Evaluation

The number of hepatocytes per FoV at 40x magnification was significantly lower in the P18 rats than in the NP rats (P18: 104 vs NP: 140, $p < 0.001$, $n = 8$ per group), see **Figure 3**. This 26 % reduction in hepatocyte counts per FoV was characteristic of hepatocyte hypertrophy.

As Ki67 protein is present during all active phases of the cell cycle, it is indicative of cell proliferation. In our samples, we observed a 4-fold increase in Ki67-positive hepatocytes in livers from pregnant rats (P18: 12 vs NP: 3, $p < 0.001$, $n = 8$ per group, **Figure 4**).

Livers from P18 rats did not differ from those of NP rats in terms of hepatic collagen or elastin content, as shown in the H&E- (**Figure 3A**) and Elastica van Gieson (EvG)-stained slices (**Figure 5**). Moreover, no visible signs of liver pathologies such as ballooning, steatosis, and inflammation were present in H&E-stained slices in either group.

Additionally, the presence of erythrocytes was visually assessed in both H&E and EvG-stained slices and there was no visible difference between the NP and the P18 groups, as shown in **Supplementary Figure 1**.

Biochemical Analysis

A total of 14 serum markers were analyzed. While 4 markers [alanine transaminase (ALT), total protein, creatinine, red blood cells] showed no significant changes during pregnancy, total bilirubin (P18, $2.6 \pm 0.3 \mu\text{mol/l}$; NP, $2.1 \pm 0.7 \mu\text{mol/l}$, and $p = 0.049$) and triglyceride (P18, $3.9 \pm 1.4 \text{ mmol/l}$; NP, $1.9 \pm 0.6 \text{ mmol/l}$, and $p < 0.001$) were significantly increased while the remaining markers showed a significant reduction during pregnancy. The results of biochemical analysis are compiled in **Table 1**.

Magnetic Resonance Elastography

Based on the mixed ANOVA analysis of MRE parameters acquired at multiple time points of the P18 and NP groups, acquisition time, the within-subject factor, had no significant influence on MRE parameters (c , $p = 0.50$; a , $p = 0.18$) while pregnancy status, the between-subject factor, had a significant effect on both c ($p = 0.01$) and a ($p < 0.001$). Additionally, there were no significant interactions between these two factors for c ($p = 0.98$) or a ($p = 0.44$).

Since acquisition time had no effect on MRE parameters, we averaged both c and a values of five acquisitions over 40 min

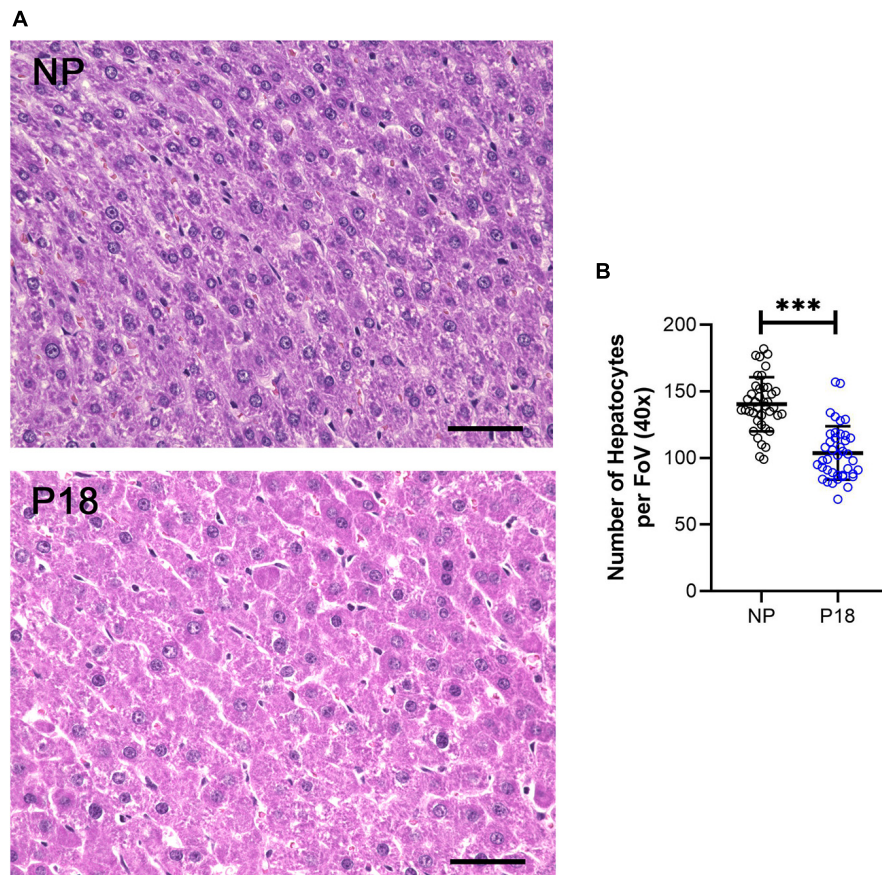


FIGURE 3 | (A) Representative H&E-stained liver sections from non-pregnant (NP) and pregnant (P18) rats. Scale bars correspond to 50 μm . Hepatocytes were counted at 40x optical field, five high-power images per animal were analyzed. **(B)** Scatter plots of numbers of hepatocytes per field of view (FoV) in pregnant (P18) and non-pregnant (NP) groups with mean and standard deviation. *** $p < 0.001$.

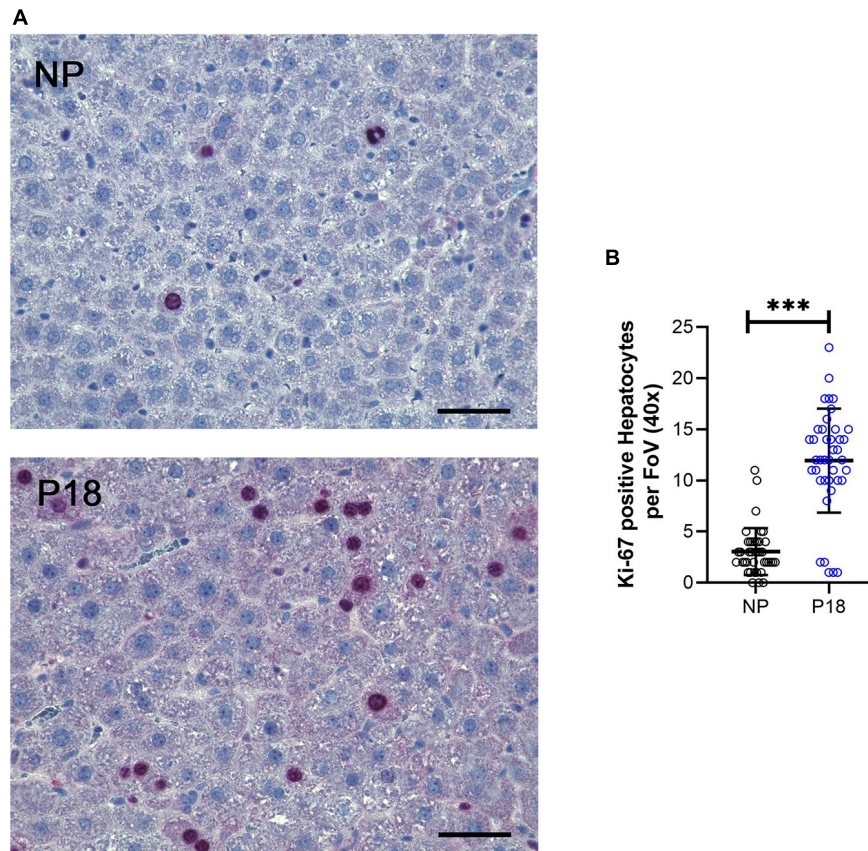


FIGURE 4 | (A) Representative Ki-67-immunostained liver sections from non-pregnant (NP) and pregnant (P18) rats. Scale bars correspond to 50 μ m. Ki-67-positive hepatocytes were counted at 40x optical field, five high-power images per animal were analyzed. **(B)** Scatter plots of numbers of Ki-67-positive hepatocytes per field of view (FoV) in pregnant (P18) and non-pregnant (NP) groups with mean and standard deviation. *** $p < 0.001$.

for each animal, and compared the difference between the P18 and NP groups using the t -test. As shown in **Figure 6A**, c of the P18 group was significantly higher than that of the NP group (P18: 3.8 ± 0.4 m/s vs NP: 3.3 ± 0.5 m/s, $p = 0.012$). Similarly, a significant increase in penetration rate a was observed in the P18 group compared to the NP group (P18: 2.1 ± 0.3 m/s vs NP: 1.6 ± 0.2 m/s, $p < 0.001$, **Figure 6B**).

Correlation analysis was performed by pooling the data from the P18 and NP groups. We observed a positive correlation between c and a (Pearson's $r = 0.49$; $p = 0.011$) and a negative correlation between a and the number of hepatocytes per FoV (Pearson's $r = -0.76$; $p = 0.002$), as shown in **Figures 7A,C**.

We also correlated MRE parameters with biochemical results. There were a total of five significant correlations: between c and ALP (Pearson's $r = -0.7$; $p = 0.003$), a and ALP (Pearson's $r = -0.65$; $p = 0.006$), a and albumin (Pearson's $r = -0.68$; $p = 0.004$), a and urea (Pearson's $r = -0.54$; $p = 0.033$), and a and glucose (Pearson's $r = -0.76$; $p < 0.001$).

Diffusion-Weighted Imaging

Mixed ANOVA analysis of the DWI data acquired at multiple time points in the P18 and NP groups revealed that the effect of acquisition time (within-subjects factor) was not significant

($p = 0.19$) while the pregnancy status (between-subject factor) significantly influenced the ADC ($p < 0.001$). Also, there was no significant interaction between these two factors ($p = 0.51$). Based on the results of mixed ANOVA analysis, we averaged the ADC values of the five acquisitions over 40 min for each animal and compared the P18 and NP groups with the t -test. As shown in **Figure 6C**, ADC values in the P18 group were significantly higher than in the NP group (P18: $0.47e^{-3} \pm 0.03e^{-3}$ mm²/s; NP: $0.42e^{-3} \pm 0.04e^{-3}$ mm²/s, $p < 0.001$).

For correlation analysis, ADC values from the P18 and NP group were pooled. We observed a positive correlation between a and ADC (Pearson's $r = 0.48$; $p = 0.013$) and a negative correlation between ADC and the number of hepatocytes per FoV (Pearson's $r = -0.67$; $p = 0.009$). Results of correlation analysis are shown in **Figures 7B,D**.

We also tested correlation between ADC values and biochemical parameters. Four biochemical parameters – bile acid (Pearson's $r = -0.66$, $p = 0.005$), triglyceride (Pearson's $r = 0.5$; $p = 0.047$), albumin (Pearson's $r = -0.65$; $p = 0.007$), and glucose (Pearson's $r = -0.65$; $p = 0.006$) – were found to be significantly correlated with ADC values.

Group mean values and standard deviations of the aforementioned imaging parameters are presented in **Table 2**.

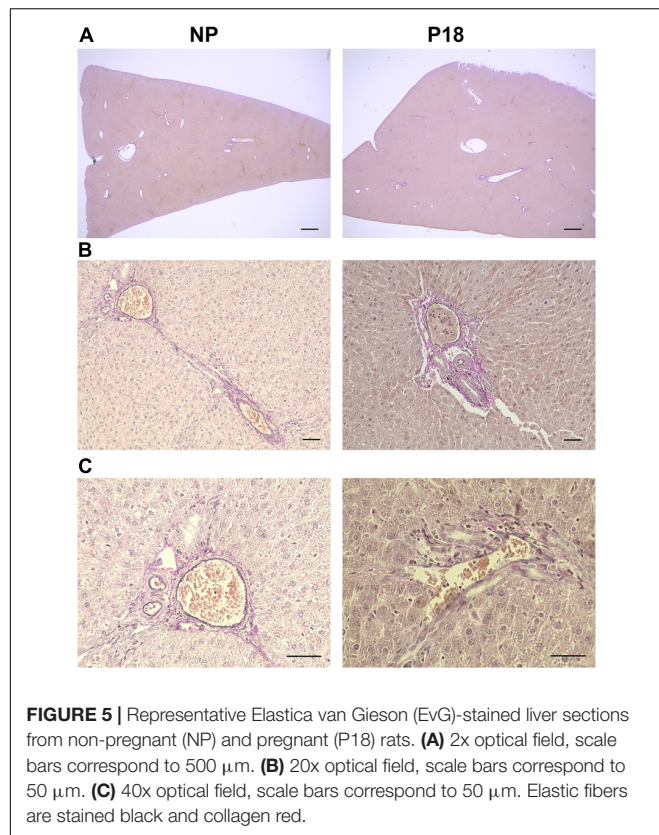


TABLE 1 | Mean serum markers with standard deviation of the pregnant and non-pregnant groups.

Parameter	Pregnant (n = 8)	Non-pregnant (n = 8)	p-value
ALP (U/l)	78.0 $\pm$ 25.0	116.0 $\pm$ 24.2	0.022
AST (U/l)	101.0 $\pm$ 14.2	145.6 $\pm$ 48.6	0.026
ALT (U/l)	69.9 $\pm$ 9.4	79.4 $\pm$ 13.6	0.133
GLDH (U/l)	4.7 $\pm$ 2.4	9.8 $\pm$ 5.5	0.033
Total bilirubin ($\mu\text{mol/l}$)	2.6 $\pm$ 0.3	2.1 $\pm$ 0.7	0.049
Bile acids ($\mu\text{mol/l}$)	14.3 $\pm$ 11.8	40.5 $\pm$ 28.8	0.026
Triglyceride (mmol/l)	3.9 $\pm$ 1.4	1.9 $\pm$ 0.6	0.001
Albumin (g/l)	31.0 $\pm$ 1.7	34.3 $\pm$ 2.0	0.003
Total protein (g/l)	58.8 $\pm$ 5.0	61.0 $\pm$ 3.6	0.319
Creatinine ($\mu\text{mol/l}$)	19.4 $\pm$ 1.4	21.7 $\pm$ 4.1	0.153
Urea (mmol/l)	6.2 $\pm$ 0.6	7.4 $\pm$ 0.9	0.011
Glucose (mmol/l)	5.1 $\pm$ 0.6	7.5 $\pm$ 0.9	< 0.001
Hemoglobin (g/l)*	110.0 $\pm$ 3.8	142.5 $\pm$ 4.4	< 0.001
Red blood cells (T/l)**	6.0 $\pm$ 0.2	6.7 $\pm$ 2.5	0.106

ALP, Alkaline phosphatase; AST, Aspartate Transaminase; ALT, Alanine transaminase; and GLDH, Glutamate dehydrogenase.*Pregnant n = 7 and non-pregnant n = 4; **pregnant n = 7 and non-pregnant n = 5- the amount of blood was insufficient to execute the test.

DISCUSSION

In this study, compact MRE and DWI were used to study biophysical changes occurring in the liver during pregnancy. Our results obtained in rat livers reveal that pregnancy increases

stiffness and water diffusivity while reducing viscosity. The biophysical changes identified with these two imaging techniques were correlated with and validated by extensive histological and biochemical analysis.

The most obvious change was a pregnancy-related increase in liver size caused by hepatocyte hypertrophy and hyperproliferation. A pregnancy-related increase in liver weight is well documented for animals (Rosenfeld, 1977; Nuwayhid, 1979; Buelke-Sam et al., 1982; Ahokas et al., 1984). Hollister et al. (1987) were the first to report hepatic growth in pregnancy as a result of hepatocyte hypertrophy. This was later confirmed by other studies (Bustamante et al., 2010; Gielchinsky et al., 2010; Milona et al., 2010; Dai et al., 2011) showing hyperproliferation of hepatocytes (Bustamante et al., 2010; Milona et al., 2010; Dai et al., 2011), increased hepatic DNA content, and an altered hepatic gene expression profile (Bustamante et al., 2010; Dai et al., 2011) during pregnancy. An enhanced liver metabolism during pregnancy could lead to the observed liver growth (Pedrycz et al., 2014). Additionally, elevated estrogen levels in pregnancy (Abbassi-Ghanavati et al., 2009) were reported to induce transient hepatocyte proliferation and liver growth (Fisher et al., 1984; Yager et al., 1994). In the current study, considering the absence of ballooning, steatosis and inflammation based on histopathology, the observed hepatocyte hypertrophy was a result of increased DNA content which was physiological during pregnancy as reported in Bustamante and Dai et al. (Bustamante et al., 2010; Dai et al., 2011). Furthermore, as our histological analysis revealed no evidence of altered structural elements such as collagen and elastic fibers, we conclude that pregnancy-related hypertrophy and hyperproliferation of hepatocytes contributed to the overall increase in liver size and weight in our experiments.

As mentioned in the Introduction, biochemical changes occurring in maternal livers during pregnancy are well studied in humans (Abbassi-Ghanavati et al., 2009; Jamjute et al., 2009; Cunningham, 2010). With the notion that the liver anatomy differs between rats and humans with rats' liver consisting of four main lobes (Kogure et al., 1999), we compared the biochemical changes in rat maternal liver to that of humans. Most of the changes in serum markers we observed in rat livers were in accordance with results obtained in pregnant women (Abbassi-Ghanavati et al., 2009; Jamjute et al., 2009). Similar to humans, pregnant rats showed an increase in hepatic triglycerides with a concomitant reduction in glucose, which is attributable to the high energy demands during pregnancy. Additionally, hemodilution due to a larger volume of circulating plasma leads to lower albumin levels during pregnancy, a phenomenon observed in both rats (De Rijk et al., 2002; Liberati et al., 2004) and humans (Moll, 2001; Carlin and Alfirevic, 2008; Pedrycz et al., 2014). Nevertheless, there were three main differences between our results and findings known from human studies: firstly, the ALP level decreased significantly in pregnant rats, whereas pregnant women may have up to 3 times higher amounts of ALP compared to non-pregnant women. This difference is due to the fact that ALP produced by rat placenta does not enter maternal serum whereas placental ALP in humans contributes to overall maternal ALP (Boles et al.,

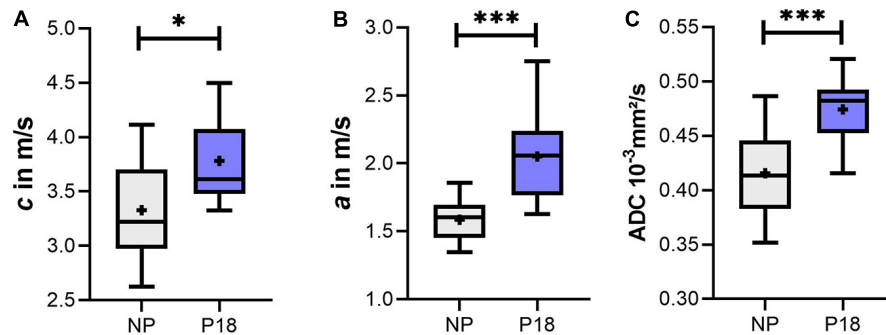


FIGURE 6 | Box plots of (A) shear wave speed (c), (B) penetration rate (a), and (C) apparent diffusion coefficient (ADC) of the liver in the pregnant (P18) and non-pregnant (NP) rat groups. Data are presented as minimum to maximum with interquartile range and median; + indicates means. * $p \leq 0.05$ and *** $p \leq 0.001$.

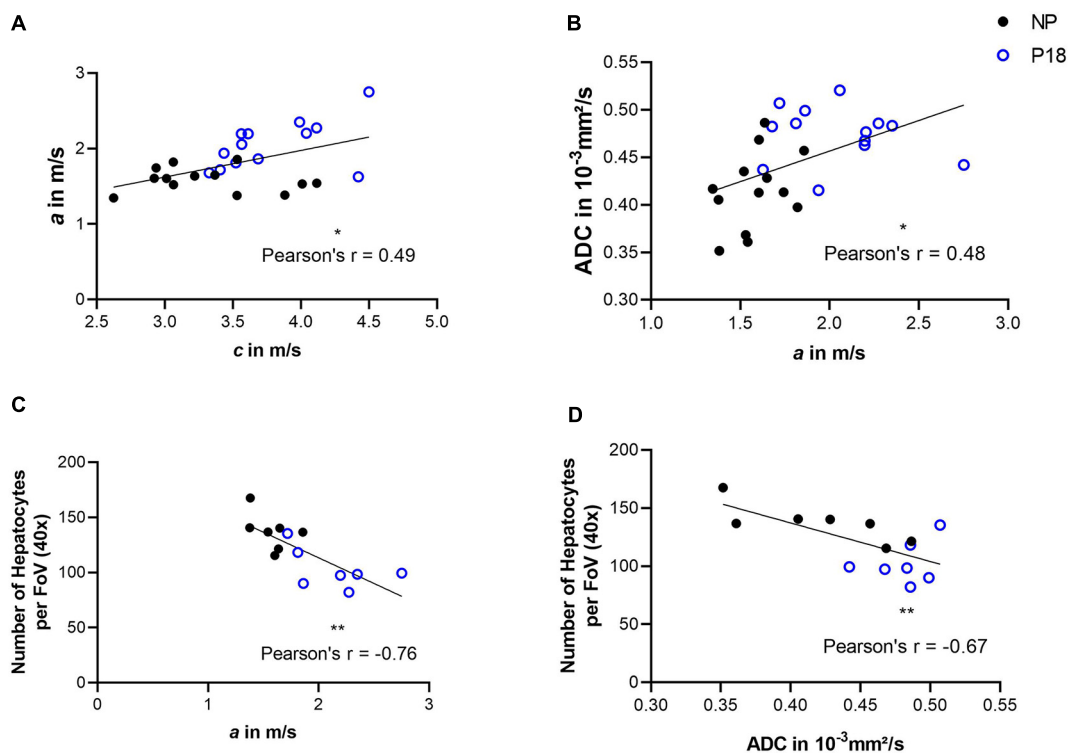


FIGURE 7 | Correlations between: (A) shear wave speed (c) and penetration rate (a); (B) a and apparent diffusion coefficient (ADC); (C) a and number of hepatocytes per field of view (FoV); and (D) ADC and number of hepatocytes per FoV. Solid black circles represent data from the non-pregnant group (NP) while open blue circles represent data from the pregnant group (P18). * $p \leq 0.05$ and ** $p \leq 0.01$.

1972; Pedrycz et al., 2014). Secondly, in contrast to pregnant women, whose bile acid level is normally elevated (Abbassi-Ghanavati et al., 2009; Jamjute et al., 2009; Pedrycz et al., 2014; McIlvride et al., 2017), rats showed reduced bile acids during pregnancy, which was due to hemodilution as reported in Zhu et al. (2013). Thirdly, unlike humans whose bilirubin level is slightly reduced during pregnancy (Abbassi-Ghanavati et al., 2009; Jamjute et al., 2009), we observed an increase in total bilirubin concentration in the pregnant rats which is accordance with previously published data obtained from Wistar rats (Liberati et al., 2004).

Firstly, liver stiffness of rats measured *ex vivo* in our study is higher than that obtained *in vivo*, as reported in Piecha et al. (2016), Elshaarawy et al. (2020). Aside from the difference between *ex vivo* and *in vivo* conditions and the technical dissimilarity between the two imaging modalities, the frequency used in the current study (800 Hz) was higher than that of Fibroscan used in Piecha et al. (2016), Elshaarawy et al. (2020), 573 Hz, leading to the higher stiffness values. Secondly, pregnancy-related increase in liver stiffness was observed *in vivo* by Ammon et al. (2018) and Stenberg Ribeiro et al. (2019). These authors discussed that elevated liver stiffness might be

TABLE 2 | Mean imaging parameters obtained by MRE and DWI and mean liver weight of the pregnant and non-pregnant groups.

Parameter	Pregnant (n = 13)	Non-pregnant (n = 13)	p-value
c in m/s	3.8 ± 0.4	3.3 ± 0.5	0.012
a in m/s	2.1 ± 0.3	1.6 ± 0.2	< 0.001
ADC in mm ² /s	0.47e ⁻³ ± 0.03e ⁻³	0.42e ⁻³ ± 0.04e ⁻³	< 0.001
Liver weight in g*	18.9 ± 1.2	11.2 ± 1.1	< 0.001

*n = 8 in both groups.

associated with increased blood flow to the liver and elevated portal pressure. In Ammon et al. (Ammon et al., 2018), the author also proposed a possible association between liver congestion and liver stiffness, however, judging by both the macroscopic liver appearance and the microscopic histologic features, we didn't observe signs of liver congestion in our samples. More importantly, as the *in vivo* factors such as blood flow and pressure were not present in our *ex vivo* study, we can exclude blood-flow related influences on our data and attribute the stiffness changes that we observed mainly to structural alterations. The expansion of hepatocytes exert force on the cell membrane which lead to elevated intra-cellular pressure and increased mechanical resistance. The observed increase in stiffness reflects the collective behavior of these enlarged cells and is the macroscopic manifestation of elevated intracellular pressure. The expansion and the proliferation of the hepatocytes in the pregnant liver could also reduce intercellular space which lead to decreased friction as reflected by the wave penetration rate (*a*). Thus, in our study, the liver of the pregnant rats appeared more solid-like with lower viscosity. Based on our histology results, there were no other pregnancy-related extracellular matrix alterations such as changes in collagen or elastin fiber content which potentially also influence the mechanical properties of the liver (Feng et al., 2016; Hudert et al., 2018; Heucke et al., 2019). In addition to the aforementioned structural elements that contribute to the mechanical properties of the liver, production of macromolecule such as glycogen which was reported to increase especially at the begin of the pregnancy might also influence the observed hepatic viscoelasticity. We suspect that the accumulation of the multibranched polysaccharide with linear chains could alter the microarchitecture of the hepatocytes, thereby changing the macroscopic viscoelasticity of the liver. However, our study couldn't provide further detailed insights regarding macromolecule production. Overall, based on our results, we concluded that hypertrophy and hyperproliferation of hepatocytes are the main contributors to the observed changes in hepatic mechanical properties during pregnancy.

Pregnancy-related changes seen in MRE were accompanied by marked increases in ADC values probably due to hypertrophy of the hepatocytes. As cell density per unit area decreases, there are fewer cell membranes, which act as barriers that restrict water mobility within the hepatocytes, and water diffusion increases. This is consistent with data (Kele and van der Jagt, 2010) showing that reduced cellularity (number of cells per area) due to cell hypertrophy increases water diffusivity. Although

the hyperproliferation of hepatocytes potentially reduces water diffusivity (Le Bihan, 2013), we assume that – in light of the considerably expanded liver volumes in our group of pregnant rats, effects of hypertrophy dominated over hyperproliferation in our ADC values.

The inverse correlations between the number of hepatocytes per FoV and imaging parameters *a* and ADC confirms liver hypertrophy to be the main contributor to both the pregnancy-related reduction of viscosity and increase of water diffusion.

Despite encouraging results, our study has limitations. Firstly, biochemical and histological examinations were performed only in a subgroup of rats. Secondly, we only imaged a small portion of the left lateral hepatic lobe, while clinical *in vivo* MRI normally covers the whole liver. However, as normal pregnancy usually affects the whole maternal liver, we do not expect significant regional difference. Finally, as we investigated only *ex vivo* liver samples, possible confounders for changes of the liver stiffness during pregnancy observed *in vivo* such as intra-abdominal pressure and blood perfusion (Millonig et al., 2010; Mueller, 2016; Piecha et al., 2016; Ammon et al., 2018) were not considered. To assess the influence of these factors, *in vivo* studies using animal models are warranted.

In conclusion, using a compact tabletop MRI scanner, we observed increased stiffness and water diffusion accompanied by decreased viscosity in *ex vivo* rat liver specimens obtained from rats with normal pregnancy. Our results suggest that these changes in biophysical properties were mainly caused by pregnancy-related hypertrophy and hyperproliferation of hepatocytes as supported by biochemical and histological examinations. Finally, the maternal liver during pregnancy mechanically transforms from a soft-viscous to a more solid-rigid state. MRE and DWI have the potential to inform on structural changes of the maternal liver in a clinical context.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Landesamt für Gesundheit und Soziales Berlin.

AUTHOR CONTRIBUTIONS

KG: data acquisition, investigation, formal analysis, data curation, and writing – original draft. HT and LL: software and validation. AK: histological investigation and writing – review and editing. A-SM: investigation and writing – review and editing. AH, ES, NB, and H-GH: writing – review and editing. JB: funding acquisition, resources, methodology, project administration, supervision, and writing – review and editing. IS: conceptualization, funding acquisition, resources, methodology,

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2020.605205/full#supplementary-material>

Supplementary Figure 1 | Selected (A) H&E-stained and (B) EvG-stained liver sections from non-pregnant (NP) and pregnant (P18) rats. Scale bars correspond to 500 μm . Visually, similar amount (both high and low) of erythrocytes were found in NP and P18, indicating that the number of erythrocytes is not uniquely different in the pregnant rats compared with the pregnant ones.

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Investigation of PolyVinyl Chloride Plastisol Tissue-Mimicking Phantoms for MR- and Ultrasound-Elastography

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Objective: Realistic tissue-mimicking phantoms are essential for the development, the investigation and the calibration of medical imaging techniques and protocols. Because it requires taking both mechanical and imaging properties into account, the development of robust, calibrated phantoms is a major challenge in elastography. Soft polyvinyl chloride gels in a liquid plasticizer (plastisol or PVCP) have been proposed as soft tissue-mimicking phantoms (TMP) for elasticity imaging. PVCP phantoms are relatively low-cost and can be easily stored over long time periods without any specific requirements. In this work, the preparation of a PVCP gel phantom for both MR and ultrasound-elastography is proposed and its acoustic, NMR and mechanical properties are studied.

Materials and methods: The acoustic and magnetic resonance imaging properties of PVCP are measured for different mass ratios between ultrasound speckle particles and PVCP solution, and between resin and plasticizer. The linear mechanical properties of plastisol samples are then investigated over time using not only indentation tests, but also MR and ultrasound-elastography clinical protocols. These properties are compared to typical values reported for biological soft tissues and to the values found in the literature for PVCP gels.

Results and conclusions: After a period of two weeks, the mechanical properties of the plastisol samples measured with indentation testing are stable for at least the following 4 weeks (end of follow-up period 43 days after gelation-fusion). Neither the mechanical nor the NMR properties of plastisol gels were found to be affected by the addition of cellulose as acoustic speckle. Mechanical properties of the proposed gels were successfully characterized by clinical, commercially-available MR Elastography and sonoelastography protocols. PVCP with a mass ratio of ultrasound speckle particles of 0.6%–0.8% and a mass ratio between resin and plasticizer between 50 and 70% appears as a good TMP candidate that can be used with both MR and ultrasound-based elastography methods.

Keywords: tissue modeling, phantom, elasticity imaging, indentation, magnetic resonance imaging, ultrasound imaging

INTRODUCTION

Over the past 3 decades, different methods have been developed for tissue elasticity measurement using medical imaging. All elastography approaches rely on the encoding of tissue displacement as a result of a force field that can be either external or internal, static or dynamic [1]. Displacements can be encoded by using medical imaging, such as ultrasound imaging, Magnetic Resonance Imaging (MRI) or optical imaging. Tissue-mimicking phantoms (TMP) are of primary importance during the development, validation and calibration processes in elasticity imaging [2, 3] and during operator training phases. Research-based and commercially-available TMP are today proposed for elastography, and are mostly dedicated to one specific elastography modality [4].

Ideally, elastography-dedicated TMP are expected to offer the following features: 1) Their mechanical properties (such as elasticity, viscosity, anisotropy, porosity or hyperelasticity) must be well controlled and must lie within typical values of soft tissues they are supposed to mimic; 2) They should offer particular ease of use in terms of storage conditions and durability; 3) They must be compatible with the medical imaging modality for which they have been developed. Several studies have proposed elastography TMP that display particular mechanical properties beyond linear elasticity, such as viscosity [43, 65, 74], poroelasticity [5], anisotropy [6–10], hyperelasticity [11–13], heterogeneity [14–19] and TMP including dynamic flow pulsations [20]. Inclusion of anisotropy [9] or porosity could be obtained through the addition of fibrin fibers or through the use of 3D-printing, [21, 22]. Important features that need to be accounted for are the preservation process, the stability over time and the inhalation toxicity during manufacturing. Depending on the imaging method, elastography TMP are mainly composed of a matrix, solvents and other additives [3]. Studies comparing elastography measurements obtained with different imaging modalities have illustrated the need for multi-modality elastography TMP [23, 24].

Synthetic phantoms have very interesting properties and are widely used as TMP. The most commonly used synthetic TMP are styrene-ethylene/butylene-styrene (SEBS) [2, 25] and silicone-based materials (more specifically Ecoflex gels) [74, 78–80]. Soft PolyVinyl Chloride (PVC) suspensions in a liquid plasticizer (plastisol or PVCP) have also been proposed as tissue-mimicking candidates for elasticity imaging [15, 26, 29, 37, 45, 69, 74, 81, 82]. These TMP are good candidates for mimicking different stages of liver fibrosis, by varying their mass ratio between resin and plasticizer $M_{Res/Plast}$ from 40 to 60% [26]. Soft PVCP has been proposed in robotics as TMP for needle insertion [27, 28], and also for Magnetic Resonance Elastography (MRE) [15, 24, 26, 45, 69, 81, 83, 84], sonoelastography [26, 29] and biomedical photoacoustic [30–35]. PVCP has been previously compared to silicone-based Ecoflex [24] and has been shown to be a very interesting candidate for MRE in terms of MRI and dynamic viscoelastic properties [36–38]. In

addition to their relatively low cost, PVCP TMP are simple to prepare, stable over time at room temperature and resistant to damage caused by typical handling procedures.

However, whether the same phantoms can be used simultaneously for sonoelastography and MRE remains poorly known. This study aims at investigating several key properties of the same PVCP phantoms for application to MRE and sonoelastography. In particular, the influence of added particles for ultrasound speckle on acoustic properties, MRI relaxation times and mechanical properties is investigated. Long term stability in mechanical properties is investigated over 43 days, and phantoms with different resin and speckle concentrations are tested using commercial MR and ultrasound elastography systems.

MATERIALS AND METHODS

Description of Polyvinyl Chloride Plastisol Samples

PVCP is widely used in industry, mainly textile, automobile and aeronautics. In this study, we consider its use specifically for MR and ultrasound elasticity imaging. PVCP is a combination of a PVC colloidal suspension in a liquid ester plasticizer, the role of which is to soften the final material. These gels are formed through the gelation-fusion process [39]. In the current study, after mixing the PVC resin suspension (40–80%) and the plasticizer (a Bis(2-ethylhexyl) adipate) (*Plastileurre Soft and Softener, Bricoleurre, Mont-Saint-Aignan, France*), the solution is heated. Complete gelation-fusion requires to reach a temperature of 160°C while the thermal degradation limit of PVCP is 190°C [40], hence the curing temperature should remain between 160°C and 190°C. Curing temperature can be achieved for instance through hot plate heating in an open beaker [28] or through bain-marie oil bath heating [31, 33, 35]. In this study, the solution is heated in an open glass beaker by means of a microwave oven (800W) with regular stirring (total heating duration of about 8 min including 5 s stirring every 2 min for the first 6 min and then every 20 s for the last 2 min) until reaching a target temperature of 180°C. Cellulose particles (Sigmacell Cellulose, Cotton linters type 50, 50 μm , SigmaAldrich, Saint Louis, MI, United states) are added as ultrasound speckle particles, once the gel has cooled down to a temperature of 80°C in order to avoid thermal degradation of cellulose. The gel is stirred with mass concentrations of 0, 0.6, 0.8, and 1% of cellulose particles. The solution is then degassed in a vacuum bell for 5 min and, poured into cylindrical molds. This process was repeated in order to make cylindrical samples of two different sizes: diameter 60 mm and height 30 mm for investigation of the imaging properties (ultrasound attenuation and MR relaxation times) and diameter 98 mm and height 75–90 mm for investigation of the mechanical properties (indentation, MRE and sonoelastography). The mass ratio between resin and plasticizer (mass fraction noted $M_{Res/Plast}$) as well as mass ratio between cellulose and PVCP solution (mass fraction noted $M_{Cell/Plast}$) can influence the mechanical

and imaging properties of the samples, as hereafter investigated. Each sample is tested at the earliest 1 day after preparation (day #1).

Influence of Acoustic Speckle on the Imaging Properties of Polyvinyl Chloride Plastisol

Influence on the Ultrasound Attenuation

The acoustic attenuation of PVCP samples is investigated using the method proposed in [41]. The reader is referred to Appendix A for more details.

In the present study, we consider the acoustic attenuation properties of PVCP at a single nominal frequency of 1 MHz. The density of water, the speed of sound in water, the density of the specimen (regardless of mass ratio between resin and plasticizer $M_{Res/Plast}$ in accordance with [42], who used PVCP from the same manufacturer) and the speed of sound in the specimen are assumed to have values of $1,000 \text{ kg.m}^{-3}$, $1,500 \text{ m.s}^{-1}$, $1,000 \text{ kg.m}^{-3}$ and $1,400 \text{ m.s}^{-1}$, respectively (values from Ref. 28, who used PVCP from the same manufacturer, and in accordance with [30, 32, 40, 43]). The values for the density and speed of sound are close to those of most biological soft tissues ($1,050 \text{ kg.m}^{-3}$ and $1,578 \text{ m.s}^{-1}$ for liver, $1,041 \text{ kg.m}^{-3}$ and $1,550\text{--}1,630 \text{ m.s}^{-1}$ for muscle, $1,035 \text{ kg.m}^{-3}$ and $1,562 \text{ m.s}^{-1}$ for brain, 928 kg.m^{-3} and $1,430\text{--}1,450 \text{ m.s}^{-1}$ for fat [44, 46]).

The acoustic test bench is composed of a single element ceramic focused transducer (23 mm diameter, nominal frequency of 1 MHz, PA-765, Precision Acoustics Ltd, Dorchester, United Kingdom) driven by a digital wave generator (33210A, Agilent Technologies Inc., Santa Clara, CA, United States), a 0.2 mm needle hydrophone (SN2319, Precision Acoustics Ltd, Dorchester, United Kingdom) connected in series with a preamplifier and an oscilloscope (TDS 2002B, Tektronix Inc., Beaverton, OR, United States). More details on both the measurement method and the experimental set-up are proposed and illustrated in the Appendix A.

Influence on the Nuclear Magnetism Relaxation Times

The potential of PVCP as a TMP for MRE is assessed through the evaluation of its NMR relaxation times (T_1 and T_2). The influence of the mass ratio between resin and plasticizer $M_{Res/Plast}$ and of the cellulose (ultrasonic speckle) mass ratio $M_{Cell/Plast}$ on the NMR relaxation times is evaluated.

The acquisitions are performed in a 1.5 T MRI scanner (MAGNETOM Aera, Siemens AG, Erlangen, Germany) on samples with mass ratio between resin and plasticizer $M_{Res/Plast}$ of 40, 50, 60, and 70%. The additional influence of ultrasonic speckle on the NMR relaxation times is also investigated by adding 0.6, 0.8, and 1%-concentrated cellulose. Overall, 16 samples are imaged.

The spin-lattice relaxation time T_1 at 1.5 T is evaluated using a turbo spin echo sequence (TE/TR 6/3,000 ms, turbo factor 7, image acquisition time 97 s) with selective inversion recuperation preparation pulse and varying

inversion times (TI) of 23, 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 800, 1,000, 1,500, 2,000, and 2,970 ms. The spin-spin relaxation time T_2 is evaluated using a spin echo sequence (TR 2500 ms, partial Fourier 6/8, acquisition time 363 s) with varying echo times (TE) 3.5, 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, and 200 ms. Common relevant imaging parameters for both sequences are: matrix 192×192 , field of view $340 \text{ mm} \times 340 \text{ mm}$, slice thickness 6 mm, bandwidth 789 Hz.px^{-1} .

Mechanical Properties

Based on the observations reported in the previous sections, standard MRE and sonoelastography (Acoustic Radiation Force Imaging–ARFI) protocols used *in vivo* in clinical practice are investigated in the same TMP and compared to a reference mechanical testing approach, namely, indentation. The mechanical stability over time of the gels is reported using these three modalities at day #1 and day #43 after the beginning of the gelation-fusion process. The mass fraction of cellulose particles to PVCP solution $M_{Cell/Plast}$ is fixed to 0.6% based on previous measurements of the acoustic attenuation.

Indentation Measurements

The linear elastic behavior is characterized against mass ratio between resin and plasticizer $M_{Res/Plast}$ from 50 to 80% with 10% steps.

The acquisitions are performed using a home-made dedicated indentation set-up (20 mm-diameter hemispherical shaped indenter mounted on a 1 degree-of-freedom translation motor and a force sensor) at room temperature (22°C) on cylindrical-shaped samples (98 mm and 75–90 mm in diameter and height, respectively). They are indented up to 11 mm depth at a speed of 7.6 mm.s^{-1} (corresponding to strain rates between 0.08 and 0.1 s^{-1}). For this range of deformations, the mechanical behavior of PVCP can be considered linear [28]. A scheme of the experimental set-up is available in the Appendix B. The elastic modulus E is computed using the continuous stiffness measurement [47] method and the Sneddon relationship [48], as described in Ref. 49. In order to investigate the capability of PVCP to mimic biological soft tissues for shear wave elastography and to compare with the results from MRE and ARFI measurements, the shear modulus G is deduced from: $G = E/2(1 + \nu) \approx E/3$. Each measurement is repeated five times and the mean values and standard deviations over these five measurements are reported.

In order to evaluate the stability over time of mechanical properties, indentation method is performed in the same samples over a period of 43 days (every 3–14 days) after the beginning of the gelatin-fusion process. The temperature is constant and equal to $T_0 \approx 22^\circ\text{C}$ for both storage and testing conditions.

Acoustic Radiation Force Imaging Measurements

Shear wave velocity c_s measurements are performed using Siemens Acuson S3000 ultrasound imager together with a

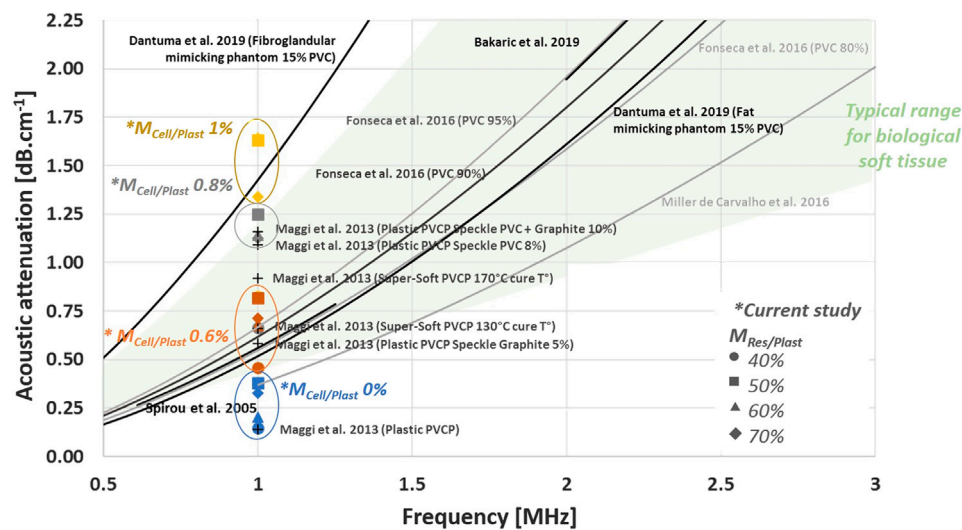


FIGURE 1 | The acoustic attenuation coefficients measured at 1 MHz in the tested Plastisol phantoms (colored signs) are compared to typical values from the literature for biological soft tissues (green colored area) [44] and to similar PVCP TMP (gray and black curves) [30, 32, 33, 35, 40, 51, 52] over the acoustic frequency range 0.5–3 MHz.

linear 9-L4 ultrasound transducer probe in the virtual touch quantification (VTq) ARFI mode (Siemens Medical Inc., Mountain View, CA, United States). For each sample, 10 measurements are performed and averaged, distributed along two lines at depths of 15 and 30 mm. To allow multi-modality comparison, the shear modulus is considered as $G = \rho c_s^2$.

Magnetic Resonance Elastography Measurements

MRE acquisitions are performed on a 1.5 T MRI scanner (MAGNETOM Aera, Siemens AG, Erlangen, Germany) using a standard *in vivo* MRE protocol with motion sensitizing gradient. The mechanical waves are generated using a commercial pneumatic driver system (Resoundant®, Mayo Clinic Foundation, Rochester, MN, United States). Scanning parameters, excitation frequency f of 60.1 Hz, echo time/repetition time (TE/TR) = (14.47 ms/50 ms), flip angle 25°, slice thickness 7 mm, acquisition matrix 128 by 102, reconstruction matrix 256 by 204, resolution 1.5625 mm by 1.5625 mm, and 1 slice parallel to the vibrating plate. Assuming pure elasticity and that the stiffness is defined using the scalar shear modulus $G = \rho(\lambda f)^2$ (λ being the wavelength), the mechanical parameters are estimated using the local frequency algorithm [50]. The results are given in terms of mean shear modulus and their standard deviation in cylindrical regions of interest (diameter 80 mm) at the center of each phantom.

ARFI and MRE measurements were performed at day #1 (beginning of the gelatin-fusion process) and day #43. A total of 5 gels with mass ratio between resin and plasticizer varying from 40% to 80% with 10% steps was tested with both MRE and ARFI sonoelastography.

RESULTS

Influence of Acoustic Speckle on the Ultrasound Attenuation

Depending on $M_{Res/Plast}$, the attenuation values obtained at 1 MHz vary from 0.146 to 0.381, 0.458 to 0.817, 0.665 to 1.251, and 0.827 to 1.641 dB.cm^{-1} for $M_{Cell/Plast}$ of 0, 0.6, 0.8, and 1%, respectively. The values are reported in **Figure 1** (and further detailed in Appendix C). The results are systematically compared to typical values found in the literature not only for biological soft tissues (for instance 0.45 dB.cm^{-1} for liver, 0.5–1.5 dB.cm^{-1} for muscle, 0.58 dB.cm^{-1} for brain, 0.6–0.8 dB.cm^{-1} for fat at 1 MHz [44]) but also for similar PVCP TMP, most often over a wider frequency range [30, 32, 33, 35, 40, 51, 52]. With the exception of the values obtained for a mass ratio of resin to plasticizer $M_{Res/Plast}$ of 50%, the acoustic attenuation values increase with $M_{Res/Plast}$. This is consistent with the observations found in the literature [32].

Influence of Acoustic Speckle on the Nuclear Magnetism Relaxation Times

Relaxation times T_1 and T_2 values evaluated at 1.5 T as a function of PVC and cellulose concentrations are reported in **Figure 2** (and further detailed in Appendix C). The presence of ultrasonic speckle does not appear to consistently affect T_1 and T_2 values of PVCP. T_2 values (averaged over all cellulose mass ratios) decrease by 10% (50 ms–44 ms) between mass ratio between resin and plasticizer $M_{Res/Plast}$ from 40 to 50%; for $M_{Res/Plast}$ from 50% to 70%, no further change in T_2 is observed. The T_1 values of PVCP (averaged over all cellulose mass ratios)

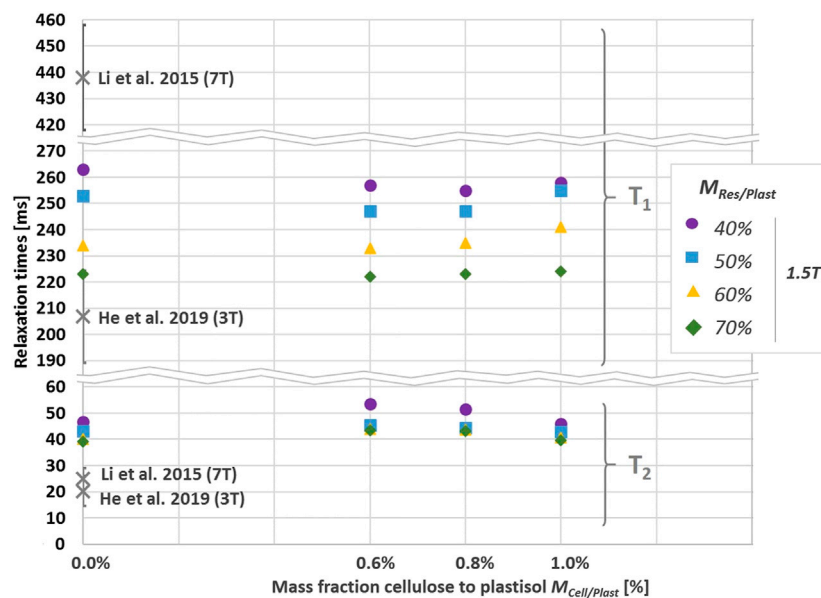


FIGURE 2 | Longitudinal T_1 and transverse T_2 NMR relaxation times acquired at 1.5 T for varying mass ratio between cellulose and PVCP $M_{\text{Res/Plast}}$ and mass ratio between resin and plasticizer $M_{\text{Res/Plast}}$. These values are compared to T_1 and T_2 values from the literature measured in similar PVCP TMP (not containing cellulose) at 3 [38] and 7 T [36] (gray crosses).

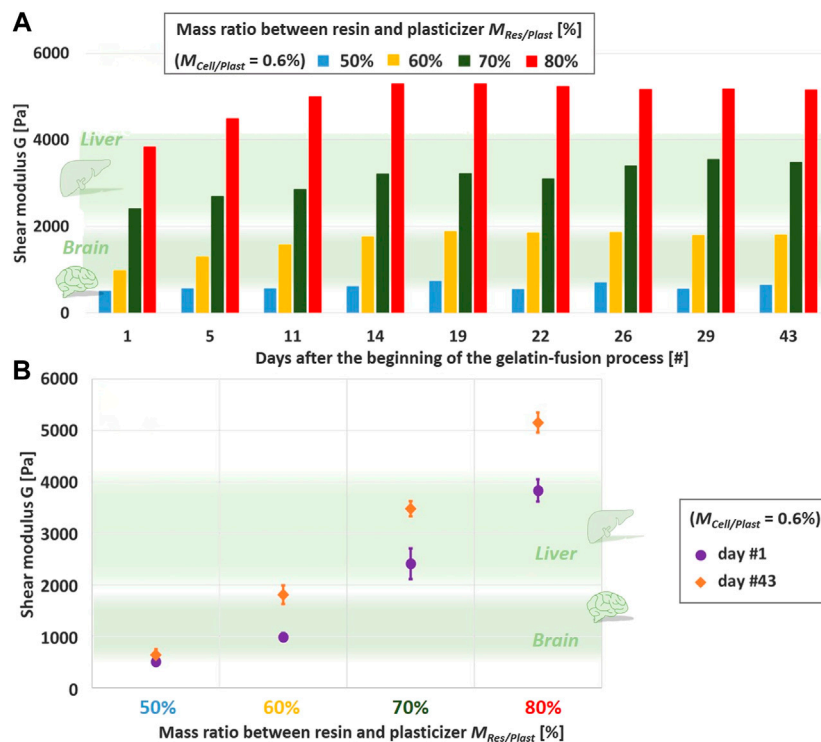


FIGURE 3 | The shear modulus against mass ratio resin to plasticizer is measured with indentation tests over a period of 43 days after gelation-fusion in order to study the stability of the PVCP gels in terms of mechanical properties **(A)**. Shear moduli measured directly after gelation-fusion process (purple) and after stabilization (orange) are represented against mass ratio between resin and plasticizer, and are compared to liver [64] and brain [59–63] values from the literature **(B)**.

decrease from 258 to 223 ms with increasing mass ratio between resin and plasticizer $M_{Res/Plast}$ 40–70%. The results obtained at 1.5 T are systematically compared to typical values found in the literature for similar PVCP TMP, most often at higher magnetic field, such as at 3 T [38] and at 7 T [36]. While T_2 values of PVCP are slightly shorter than those of healthy soft tissue at 1.5 T (54 ± 8 ms for liver, 35 ± 4 ms for muscle, 75–90 ms for brain, 90 ms for fat), T_1 values of PVCP are significantly shorter (600 ms for liver, $1,060 \pm 155$ ms for muscle, 500–750 ms for brain, 200 ms for fat) [53–58].

Mechanical Properties of the PVCP Phantom

The mechanical values obtained from indentation measurements up to 43 days after the beginning of the gelatin-fusion process are shown in **Figure 3A**. After a period of gelation-fusion of approximately 14 days, the linear mechanical properties are found to be stable for mass ratio between resin and plasticizer $M_{Res/Plast}$ 50–80%. These observations are consistent with the results obtained by MRE and dynamic mechanical analysis in [37]. After a 2-weeks stabilization period, PVCP appears to be mechanically stable over time (for at least up to 4 weeks) at room temperature, without any specific storage conditions, such as immersion or moistening.

The mechanical values obtained from indentation measurements on day #1 of the gelatin-fusion process (purple) or averaged over all measurements obtained after day #14 once mechanical stabilization of the phantom is observed (orange) are reported in **Figure 3B** and in Appendix C (for the immediate and long-term elasticity). The values are compared to typical values available from the literature for both brain [59–63] and liver tissues [64]. PVCP with a mass ratio $M_{Res/Plast}$ of 70% is an adequate candidate to mimic the elastic shear behavior of the liver tissue at small strain.

In accordance with the mechanical time-evolution observed from indentation measurements, the ARFI and MRE acquisitions are performed directly after (1 day) and 43 days after beginning of the gelatin-fusion process. The same increase over time is observed as with indentation measurements. MRE results are presented in **Figure 4** and in Appendix C. The results are given in terms of mean shear modulus and their standard deviation in cylindrical regions of interest. The values are very close to those found by indentation.

The results from ARFI mechanical measurements are presented in **Figure 4** and in Appendix C and compared to indentation measurements. The values at day 1 are higher than those obtained by indentation (differences of 52, 93, 21, and 18% for $M_{Res/Plast}$ of 50, 60, 70, and 80%, respectively) and MRE (differences of 3, 49, 14, and 9% for $M_{Res/Plast}$ of 50, 60, 70, and 80%, respectively). The same trend is observed at day 43 with values from ARFI measurements higher than those obtained by indentation (differences of 83, 23, 9, and 4% for $M_{Res/Plast}$ of 50,

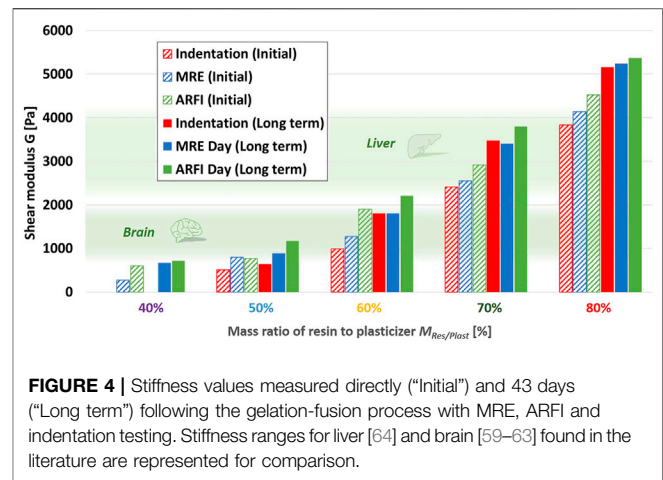


FIGURE 4 | Stiffness values measured directly ("Initial") and 43 days ("Long term") following the gelation-fusion process with MRE, ARFI and indentation testing. Stiffness ranges for liver [64] and brain [59–63] found in the literature are represented for comparison.

GENERAL DISCUSSION

The use of a single PVCP TMP for MRE and sonoelastography is investigated in this study. From our results, plastisol appears as a good candidate for mimicking soft tissues in terms of mechanical properties with T_1 and T_2 MR imaging values compatible with typical MRE pulse sequences. In addition, the ultrasound speckle can be adjusted by adding cellulose to PVCP without altering significantly its NMR properties. The current study provides indications for easily preparing PVCP phantoms for both MR and ultrasonography elastography, with readily available instruments, i.e., a microwave and a thermometer. Mechanical properties measured with indentation were found to stabilize 14 days after gelation-fusion, and remain mechanically stable until the end of our follow-up period of 43 days for PVCP gels with resin to plasticizer ratios of 50–80% and containing 0–1% mass ratio cellulose.

Many advantages for the use of PVCP in elastography phantoms can be listed, as attested by the current study: 1) short preparation time and ease-of-use; 2) fast solidification process, avoiding sedimentation of the acoustic speckle particles; 3) natural transparency to ultrasound, making it easy to control wave diffusion by adjunction of speckle particles; 4) long conservation time without any specific storage requirements, such as moistening or water bath required for other hydrogels such as PVA. Elasticity at small strains is simply controlled by varying the mass ratio between resin and plasticizer and consequently the PVC concentration. These specificities make it possible to combine both rheological and imaging properties close to those of biological soft tissues in a calibrated, robust TMP.

As previously reported, the acoustic attenuation of PVCP can mimic those of biological soft tissues through the addition of cellulose speckle particles. The results at 1 MHz are in the typical range of biological organs ($0.14\text{--}1.16\text{ dB}\cdot\text{cm}^{-1}$ at 1 MHz [51]) and a cellulose concentration of $0.6\%\text{--}0.8\%$ was found to mimic the acoustic attenuation of soft tissues. The mass ratio between resin and plasticizer $M_{\text{Res/Plast}}$ does not significantly influence the acoustic attenuation properties for concentrations higher than 50% in the studied PVCP. Limitations of this study in terms of measurement of the ultrasonic properties must be mentioned. First, the attenuation coefficient of PVCP TMP is characterized in this work at a single frequency (1 MHz), that lies below the center frequency of most clinical ultrasound transducers. How the studied PVCP TMP remains realistic in terms of acoustic attenuation over a wider frequency range remains unaddressed in this study. However, the values measured at 1 MHz agree with those found in the literature over a larger range of frequencies, as illustrated in **Figure 1**. Second, the density and speed of sound used in this study were taken from the literature [28, 30, 32, 33, 35, 40, 42, 43, 51, 52]. They are here assumed to be the same for all the mass ratios of resin to plasticizer.

NMR relaxation times of the studied PVCP phantoms were found to slightly depend on the mass ratio between resin and plasticizer. None of the tested PVCP composition reproduces both the T_1 and T_2 at 1.5 T of any biological soft tissues. The typical T_2 value of plastisol (45 ms) lies within the lower range of T_2 for biological soft tissues, including the liver and the heart. The T_1 values (220–260 ms) found for plastisol at 1.5 T are lower than those of most soft tissues. These relaxation times are different than those reported in the literature in PVCP without cellulose addition [36, 38], but direct comparison is not straightforward as measurement were performed at higher magnetic fields (3 and 7 T, respectively) on different PVCP. However, the objective of this study was to evaluate whether the studied PVCP is a good TMP candidate for typical MRE acquisition sequences, rather than to reproduce NMR relaxation times of biological soft tissues. Since MRE relies on the use of phase images alone for the estimation of elasticity, and not on the T_1/T_2 weighted magnitude images, the studied PVCP can be used with typical MRE acquisition sequences, including the clinical hepatic MRE protocol, as demonstrated here.

PVCP gels are good surrogate for biological soft tissues from a mechanical point of view. Despite the same samples being tested across the different modalities, small differences in the stiffness values can be observed between MRE, ARFI and indentation measurements. These can be explained by the different physical protocols and assumptions between the three methods (harmonic shear wavelength, quasi-static compression and impulse shear wave velocity for MRE, indentation and ARFI, respectively). Consequently, the so-called “stiffness” does not exactly correspond to the same parameter under the same conditions. For instance, the frequency and strain rate ranges are intrinsic to the aforementioned methods and do not necessarily coincide. Previous investigations from the literature suggest that PVCP gels are likely to exhibit relatively low viscosity at

the usual elastography frequency range and therefore moderate dependence of the elasticity with frequency [69, 74]. Due to different experimental strain rate (or frequency), the viscosity could be a possible reason for the differences observed between indentation, ARFI and MRE measurements. It appears that the higher the resin concentration is, the closest the results are between the different methods. This could be explained by the fact that viscous effects are less predominant compared to pure elastic effects at higher concentrations, thus resulting in decreased shear wave dispersion and therefore less sensitivity to differences in mechanical frequency content. If needed, additional investigation should be carried out in order to evaluate whether including additional compounds could increase PVCP viscosity, as oil does in gelatin phantoms [65].

The issue of cross-validation between elastography and rheological measurements or between different elastography methods is well known [18, 23, 24, 37, 62, 66–69]. As previously introduced, differences in frequency range and excitation modes—harmonic vs transient—make any direct comparison particularly difficult. PVCP with added cellulose offers the possibility of cross validating indentation with MR and US elastography methods. PVCP with added cellulose is a good, stable TMP for methods that require simultaneously both MR imaging and US attenuation for internally-generated displacements, such as MR-ARFI [70–72].

The stability of the mechanical properties of a phantom over time is of great importance for the development, the validation and the comparison of elastography protocols. For instance, extending the shelf-life and the mechanical stability of gelatin and agar-agar hydrogels is challenging and requires adequate storage conditions and addition of preservative, fungicides or bactericide agents [73]. The plastisol TMP of this study were found to have stable mechanical properties, as measured with indentation testing, from day 14 until day 43 (end of follow-up period) after being manufactured. Over a longer follow-up period, the mechanical properties of a PVCP gel were found stable during storage, up to six months [31].

SEBS, PDMS and silicon exhibit similarly high shelf life with no specific storage constraints. Some silicone-based materials (such as the silicone-based Ecoflex gels [74]) share other similar properties and advantages with PVCP, and a similar characterization study of their mechanical and MR/US imaging properties would be of interest. Silicone and PDMS gels have relatively similar imaging, mechanical and storage characteristics as PVCP. Indeed, they all provide a solid support to which acoustic scatterers can be easily added, they are insoluble in water, remain stable during storage and have easily controllable properties (first of all their stiffness) [32]. While the sound velocity and acoustic attenuation values of PVCP are slightly lower and higher, respectively, than those of biological soft tissues, these limitations are even greater for silicone and PDMS, with values close to $1,000\text{ m}\cdot\text{s}^{-1}$ [46] and over $9.8\text{ dB}\cdot\text{cm}^{-1}$ at 3 MHz [75], respectively. However, unlike silicone and PDMS gels, the preparation of PVCP gels requires curing at a given temperature range. In addition, access to the exact

chemical composition of PVCP (PVC concentration, additives ...) [32] is manufacturer-dependent, which means that any direct comparison between different studies on PVCP should be performed with care. Another oil-based material, SEBS has been shown to mimic adequately acoustic (speed of sound between 1,420 and 1,464 m.s⁻¹ and acoustic attenuation between 0.4 and 4 dB.cm⁻¹ at 3.5 MHz, depending of the speckle particle density [25, 76]) and mechanical properties of soft tissues. Similar to PVCP gels in this study, SEBS gels were shown to be suitable TMPs for both MRE and ultrasound elastography [23], stable over time. However, the use of PVCP TMP is characterized by the simplicity and speed of its preparation process.

CONCLUSION

This study provides indications about how to prepare a mechanically-stable phantom using plastisol in order to mimic biological soft tissues for MRE and/or sonoelastography. On the basis of their mechanical properties, PVCP phantoms are demonstrated to be realistic TMP with NMR relaxation times compatible with MRE. With the addition of cellulose particles, it is possible to develop a calibrated TMP for both MRE and sonoelastography measurements. Mechanical properties of the tested plastisol were found stable at room temperature after 2 weeks and at least until 6 weeks after gelation-fusion. For instance, a 60–70% mass ratio between resin and plasticizer $M_{\text{Res/Plast}}$ sample will mimic the linear elastic behavior of liver tissue correctly. In MRE, the T_2 values of the tested PVCP TMP are close to those of hepatic tissue, while with addition of 0.6% of cellulose particles, this gel becomes also a good liver-mimicking candidate in sonoelastography. Investigating the mechanical properties over a larger range of frequency using dynamic mechanical analysis [37, 69] would be a great extension for this study.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SC, EB and JV wrote the manuscript. The PVCP TMP were prepared by SC. The indentation tests were developed and performed by SC, AA, LM, BW and JV. The acoustic test bench was developed, and the acoustic properties were investigated by SC, BW and JV. The NMR properties of the TMP were investigated by SC, EB and CG. SC performed final approval of the manuscript to be published and agreed to be accountable for all aspects of the work related to the accuracy and integrity. All authors agree to be accountable for the content of the work.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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APPENDIX A

Measurement of the Acoustic Attenuation Coefficient

The acoustic attenuation coefficient is measured at 1 MHz for PVCP samples with varying mass ratio between cellulose and PVCP $M_{\text{Res/Plast}}$ using the set-up illustrated in **Figure 5**.

The acoustic attenuation of PVCP samples is investigated using the method proposed in Ref. 41. The Fourier transform of a single-frequency plane wave propagating over two different media (water over a distance z_w and a specimen over a depth z -height of the sample) can be modeled as:

$$U(f) = A(f)e^{-i\theta} = U_0(f)e^{-(\alpha_w + i\beta_w)z_w}e^{-(\alpha + i\beta)z}T(f) \quad (1)$$

where $U_0(f)$, f , β_w , α_w , β , and α are the Fourier transform of the initial generated pulse wave, its frequency, the propagation and the attenuation factors of water and the specimen, respectively.

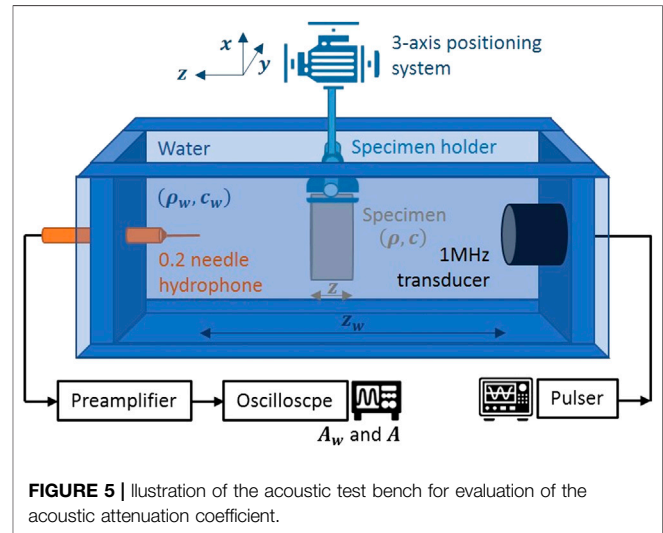
T is the overall water-specimen transmission factor defined by:

$$T(f) = \frac{4\rho c(f)\rho_w c_w}{(\rho c(f) + \rho_w c_w)^2} \quad (2)$$

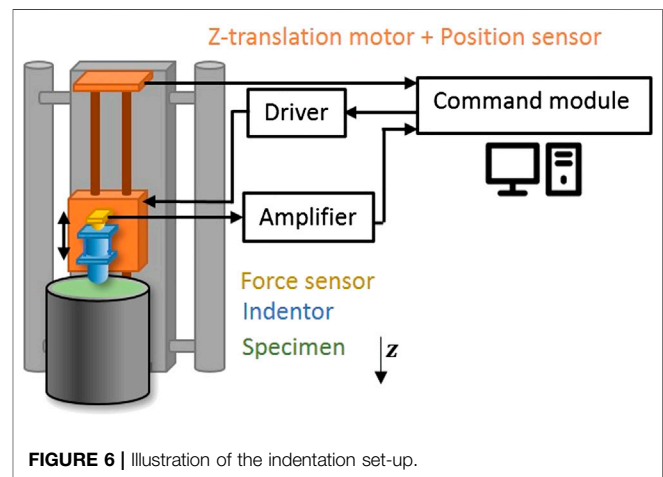
with ρ_w , c_w , ρ , and c density and speed of sound in water, the density of the specimen and the phase velocity, respectively. According to [41], the attenuation coefficient is deduced from the amplitude spectra of the transmitted pulses with (A) or without (A_w) the specimen:

$$\alpha \cong \frac{\ln(T)}{z} + \frac{1}{z} \ln\left(\frac{A_w}{A}\right) \quad (3)$$

The amplitude drop is mostly expressed in dB by defining: $\alpha_{dB} = z\{20\log_{10}(e^{\alpha z})\} = 8.6886\alpha$. The attenuation coefficient of a material is generally dependent on the frequency f of the ultrasound waves. A power law $\alpha_{dB} = af^\gamma$ can be assumed for this dependency [44, 77].



APPENDIX B



APPENDIX C

Mechanical and imaging properties of PVCP.

The main results obtained in this work on PVCP TMP are summarized in **Table 1** (mean and standard deviation values) for all tested properties (indentation, echographic, and MRI properties).

TABLE 1 | Mechanical, ultrasonic and MRI properties of the tested PVCP.

Mass ratio resin to plasticizer MRes/Plast [%]			40	50	60	70	80
Echography	Mass ratio cellulose to PVCP $M_{Cell/Plast}$ [%]		—	—	—	—	—
	α [-] at 1 MHz	0.00	0.146	0.381	0.203	0.326	-
		0.60	0.458	0.817	0.676	0.713	-
		0.80	0.665	1.251	1.142	1.127	-
		1.00	0.827	1.631	1.641	1.340	-
	ARFI	$M_{Cell/Plast}$ [%]	—	—	—	—	—
	Initial G [Pa]	0.6	602 ± 109	772 ± 155	1901 ± 458	2,913 ± 624	4,529 ± 831
MRI	Long-term G [Pa]	0.6	714 ± 221	1,173 ± 150	2,211 ± 288	3,797 ± 636	5,365 ± 593
	—	$M_{Cell/Plast}$ [%]	—	—	—	—	—
	T_1 [ms] at 1.5 T	0.00	263	253	234	223	-
		0.60	257	247	233	222	-
		0.80	255	247	235	223	-
		1.00	258	255	241	224	-
	—	Mean T_1 [ms]	258 ± 3	251 ± 4	236 ± 4	223 ± 1	-
	—	$M_{Cell/Plast}$ [%]	—	—	—	—	—
	T_2 [ms] at 1.5 T	0.00	46	43	42	40	-
		0.60	53	46	45	45	-
		0.80	54	45	46	46	-
		1.00	47	42	42	40	-
	—	Mean T_2 [ms]	50 ± 3	44 ± 2	44 ± 2	43 ± 3	-
	MRE at 60.1 Hz	$M_{Cell/Plast}$ [%]	—	—	—	—	—
	G [Pa] day #1	0.6	272 ± 28	796 ± 113	1,272 ± 120	2,549 ± 438	4,140 ± 266
	G [Pa] day #43	0.6	670 ± 84	890 ± 74	1810 ± 96	3,401 ± 66	5,240 ± 114
Indentation	—	$M_{Cell/Plast}$ [%]	—	—	—	—	—
	G [Pa] day #1	0.6	—	508 ± 109	985 ± 182	2,410 ± 148	3,836 ± 197
	G [Pa] day #43	0.6	—	640 ± 23	1803 ± 81	3,480 ± 299	5,155 ± 215



Cerebral Ultrasound Time-Harmonic Elastography Reveals Softening of the Human Brain Due to Dehydration

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Hydration influences blood volume, blood viscosity, and water content in soft tissues – variables that determine the biophysical properties of biological tissues including their stiffness. In the brain, the relationship between hydration and stiffness is largely unknown despite the increasing importance of stiffness as a quantitative imaging marker. In this study, we investigated cerebral stiffness (CS) in 12 healthy volunteers using ultrasound time-harmonic elastography (THE) in different hydration states: (i) during normal hydration, (ii) after overnight fasting, and (iii) within 1 h of drinking 12 ml of water per kg body weight. In addition, we correlated shear wave speed (SWS) with urine osmolality and hematocrit. SWS at normal hydration was 1.64 ± 0.02 m/s and decreased to 1.57 ± 0.04 m/s ($p < 0.001$) after overnight fasting. SWS increased again to 1.63 ± 0.01 m/s within 30 min of water drinking, returning to values measured during normal hydration ($p = 0.85$). Urine osmolality at normal hydration (324 ± 148 mOsm/kg) increased to 784 ± 107 mOsm/kg ($p < 0.001$) after fasting and returned to normal (288 ± 128 mOsm/kg, $p = 0.83$) after water drinking. SWS and urine osmolality correlated linearly ($r = -0.68$, $p < 0.001$), while SWS and hematocrit did not correlate ($p = 0.31$). Our results suggest that mild dehydration in the range of diurnal fluctuations is associated with significant softening of brain tissue, possibly due to reduced cerebral perfusion. To ensure consistency of results, it is important that cerebral elastography with a standardized protocol is performed during normal hydration.

Keywords: brain, elastography, hydration, ultrasound, time-harmonic elastography

INTRODUCTION

The body water content ranges between 55 and 60% in adults and varies with age, sex, and body constitution, while human brain tissue has a very high water content of about 75% (Mitchell et al., 1945), underlining the importance of adequate cerebral tissue hydration for normal brain function. The human organism can easily adapt to a water deficit of 2–3%; however, beyond this limit, dehydration can impair mental and physical coordination, eventually leading to a fatal breakdown of vital body functions (Ashcroft, 2001). Clinically, three types of dehydration are distinguished: (i) isotonic dehydration, that is loss of body water and salt usually occurring after excessive vomiting, diarrhea or bleeding, (ii) hypotonic dehydration, in which

salt loss outweighs water loss, is often associated with the intake of diuretic drugs or kidney damage, and (iii) hypertonic dehydration, often resulting from a lack of water due to fasting or dehydration (Spital, 2007; Bhavé and Neilson, 2011; Jablonski, 2012). The diagnosis of chronic dehydration remains challenging since clinical signs such as dry mucous membranes or dry skin are unspecific (Jequier and Constant, 2010) while blood or urine markers can vary markedly among individuals (Armstrong, 2005; Duning et al., 2005; Patterson et al., 2008).

The hydration state of soft tissues affects a variety of biophysical properties, which can be assessed *in vivo* by MRI or ultrasound. While perfusion MRI, flow MRI, and Doppler ultrasound are sensitive to volume and velocity of blood flow, magnetic resonance elastography (MRE) and ultrasound elastography can non-invasively measure stiffness (Sack and Schaeffter, 2018). Hydration-specific imaging markers are potentially important for the management of neurological diseases since dehydration of brain tissue is a frequent and dangerous condition in elderly patients with Alzheimer's disease or other types of dementia (Lauriola et al., 2018).

Studies using MRI and ultrasound markers show that dehydration reduces cerebral blood flow (Trangmar et al., 2014, 2015), decreases brain parenchymal volume, and increases ventricular volume (Duning et al., 2005; Streitburger et al., 2012). However, to date no studies have been published that investigated the effect of dehydration on cerebral stiffness (CS).

Our hypothesis is that CS changes with dehydration and water drinking similar to stiffness changes reported for other organs (Guo et al., 2018). For instance, it has been shown that liver stiffness increases with water ingestion and hepatic blood flow (Ipek-Ugay et al., 2016; Tzschatzsch et al., 2016b). The opposite effect, i.e., softening, has been observed in the pancreas and spleen (Dittmann et al., 2017) while kidney stiffness has been reported to change only slightly with increasing bladder filling (Gandhi et al., 2020) and hydration (Marticorena Garcia et al., 2018).

In this study, we investigate the effect of dehydration on CS using cerebral time-harmonic elastography (THE), which utilizes multifrequency vibrations induced in the brain by an external driver in combination with transtemporal ultrasound (Tzschatzsch et al., 2018). Cerebral THE has several advantages over MRE such as being available at the bedside and providing instantaneous feedback, which facilitates identification of rapid CS changes. We will exploit this real-time feedback capability of THE to study possible CS changes induced by dehydration of brain tissue after overnight fasting and water drinking. Overall, this study aims at providing insight into the sensitivity of CS to physiological changes in tissue hydration toward a clinical tool for monitoring brain mechanical properties at the bedside.

MATERIALS AND METHODS

Study Design

The study protocol conformed to the guidelines of the Declaration of Helsinki and was approved by the institutional

review board of Charité–Universitätsmedizin Berlin (EA1/242/18). All study participants gave their written consent to conduct the experiment as well as for the publication of any potentially identifiable images or data included in this article. Inclusion criteria were absence of any history of cerebral disease or trauma and no impairment of renal function. Insufficient acoustic windows due to high skull thickness cause relatively high dropout rates of around 10% in studies of transcranial ultrasound (Marinoni et al., 1997). To avoid such dropout rates, we checked the acoustic window previously to the measurements. Finally, a total of 12 volunteers (3/9 females/males; mean age of 33 ± 9 years, range: 22–50 years) with a sufficient transcranial bone window were included in the study, while one volunteer did not meet the inclusion criterion. Two volunteers (#5 and #12) were further excluded from statistical group analysis due to incomplete fasting. Volunteer demographic data, including sex, age, and body mass index (BMI) are summarized in **Table 1**.

Each participant was investigated on 2 days. On the first day, data were acquired in a normally hydrated (NH) state, defined as drinking 1.5 L water within 5 h prior to the examination. After 12-h overnight fasting, the second set of data (dehydrated, DH) was acquired. Then, the volunteers were asked to drink 12 ml/kg of water within 15 min (Duning et al., 2005). Immediately after drinking water, four sets of data (rehydrated, RH1–RH4) were acquired at 15-min intervals. Thus, a total of six sets of data were acquired for every volunteer. Each dataset consisted of THE, transcranial Doppler (TCD), and recordings of blood pressure and heart rate. Urine and blood were collected at hydration states 1 (NH), 2 (DH), and 6 (RH4) to determine urine osmolality and hematocrit. The time sequence of THE examinations is illustrated in **Figure 1**.

Cerebral THE

The setup of cerebral THE including (i) customized vibration bed with vibration plate mounted on a shaker (GAMPT, Merseburg, Germany), (ii) standard clinical ultrasound scanner

TABLE 1 | Demographic data, including sex, age, and BMI with group mean and standard deviation (SD) of all volunteers.

Subject #	Sex	Age in years	BMI in kg/m ²
1	m	25	23.2
2	m	50	20.1
3	f	40	21.5
4	f	27	31.6
5	m	26	20.2
6	m	35	19.9
7	m	46	25.8
8	f	28	20.7
9	m	37	26.2
10	m	22	24.0
11	m	22	24.5
12	m	43	23.6
Mean (SD)		33.4 (9.3)	23.4 (3.5)

Volunteers #5 and #12 were excluded from statistical analysis due to poor compliance with the study protocol.

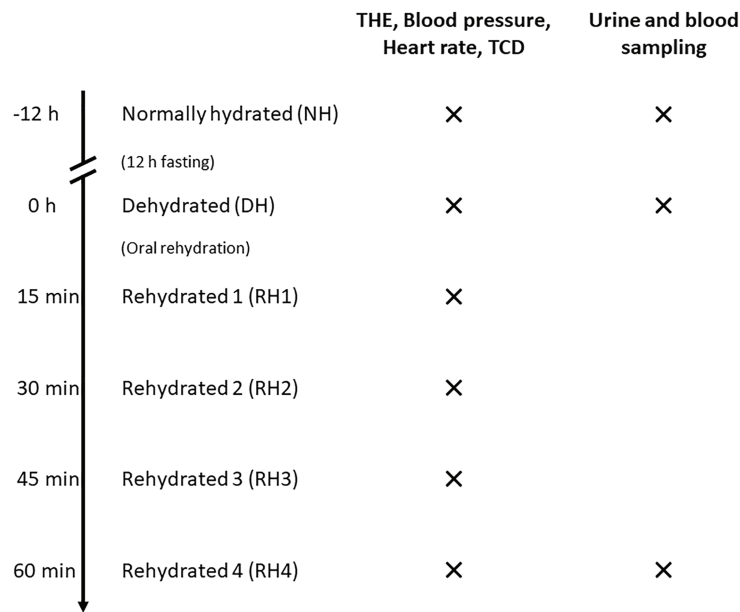


FIGURE 1 | Time sequence of THE examinations in volunteers. Volunteers were first examined in a normally hydrated state (NH). After a 12-h-fasting period, measurements were conducted in the dehydrated state (DH). Then, four sets of data in different hydration states were acquired after oral rehydration (RH1–RH4) at 15-min intervals. For every hydration state, time-harmonic elastography (THE), transcranial Doppler (TCD), and blood pressure and heart rate measurements were performed. Urine and blood for determination of urine osmolality and hematocrit were sampled at hydration states NH, DH, and RH4.

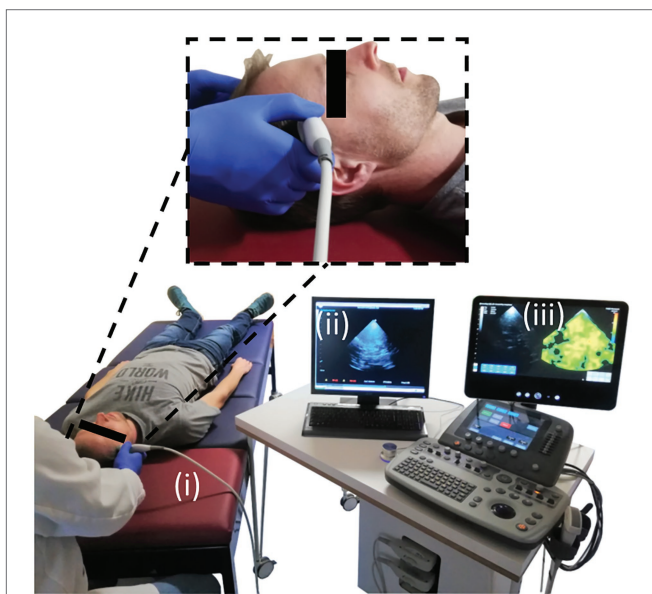


FIGURE 2 | Experimental setup for conduct of cerebral THE. The setup comprised of three main components: (i) the customized patient bed with the vibration plate (red), (ii) the clinical ultrasound scanner, and (iii) the elastography computer with the integrated post-processing software. The magnification shows the positioning of the ultrasound probe over the volunteer's temporal bone window for transcranial ultrasound.

(SonixMDP, UltraSonix, Scottsdale AZ, United States) equipped with a phased-array transducer (SA4-2/24), and (iii) elastography computer with the integrated post-processing pipeline is

illustrated in **Figure 2**. The volunteers were asked to lie in a supine position with the head on the vibration plate. According to the current guidelines for transcranial ultrasound (Brian D Coley et al., 2012), the probe was positioned for imaging through the temporal bone window, as shown in the magnification in **Figure 2**. Shear waves were induced in the head by applying a multifrequency waveform comprising six frequencies (27, 33, 39, 44, 50, and 56 Hz). Signal-to-noise-ratio (SNR) in transcranial ultrasound is known to be relatively low compared to abdominal ultrasound. The stability of shear wave speed (SWS) estimation was found to be stable at vibration amplitudes larger than 2 μm . Therefore, we ensured that vibration amplitudes were above this threshold in order to compensate for low SNR. For data acquisition, a basic preset for transcranial Doppler ultrasound was adapted from the UltraSonix-System (Phased Array, Vascular, TCD, 3.3 MHz). To further improve image SNR, number of pulse cycles was increased to two. In addition, the frame rate was adjusted to 80 Hz in order to fulfill the requirements of the post-processing pipeline. For elastography, ultrasound radiofrequency data were acquired over 1 s and transferred to the elastography computer, where the post-processing was performed.

To obtain the tissue displacement caused by multifrequency vibration, the axial phase shift between adjacent frames was calculated. Temporal Fourier transformation was used for the decomposition of the six superimposed frequencies. Thereby, three vibration frequencies were above the Nyquist limit of $\frac{1}{2}$ frame rate = 40 Hz (44, 50, and 56 Hz) and appeared at the aliased spectral positions (36, 30, and 24 Hz, respectively; Tzschatzsch et al., 2016b). Further post-processing was applied

for single frequencies. Noise and unwanted motion were suppressed by a spatial 2D Gaussian bandpass filter. The resulting complex valued shear wave field was directional filtered yielding eight single-directional (single wave number) wave images per frequency, which were converted into a single wave speed map by multifrequency phase gradient inversion and weighted averaging over frequency (Tzschatzsch et al., 2016a).

To exclude low SWS-values corresponding to noisy radiofrequency data, we applied a 1 m/s threshold to the elastogram, as explained by Kreft et al. (2020). To determine the average SWS in the temporal lobe parenchyma, a region of interest (ROI) was manually defined based on anatomical landmarks in the B-mode image delimiting that region from the midbrain, such as the butterfly-shaped hypoechoic mesencephalon and the surrounding hyperechoic basal cisterns. Areas in the elastogram that corresponded to B-mode artifacts such as reverberation artifacts from the skull were excluded from the ROI. **Figure 3** illustrates the drawn ROI in a representative volunteer. For further stabilization, measurements were repeated 10 times. Since motion was negligible, we applied the same ROI to all 10 elastograms for averaging the 10 intra-ROI SWS to one mean SWS-value. It should be noted that this mean SWS value efficiently averaged out the relatively large variability of SWS within a ROI and thus did not reflect intraregional standard deviations. The duration of a continuous examination was no longer than 2 min, which prevented heating of the skull by ultrasound energy absorption.

In order to reduce the expectation bias of the investigator who also analyzed the data, the order of THE acquisitions during all six hydration states was randomly permuted before the ROIs were drawn, and chronological order was restored just after the calculation of SWS.

Transcranial Doppler Ultrasound

A transcranial Doppler examination was performed with the ultrasound plane aligned for optimal visibility of the middle cerebral artery (MCA). One Doppler spectrum was acquired, which was used to measure mean MCA blood flow at each hydration state over a range of five heartbeats. As TCD is

highly dependent on the position and alignment of the MCA in the brain, blood flow measurement in the MCA was not accomplished in all volunteers. TCD was successful in six volunteers (#1, #6, and #8–11), while THE data could be evaluated in all 12 cases.

Statistical Analysis

All physiological parameters (SWS, cerebral blood flow velocity, blood pressure, heart rate, urine osmolality, and hematocrit) were measured once at three different hydration states (NH, DH, and RH4). Therefore, physiological parameters were considered as independent variables. Correlations between the 30 SWS values (hydration states NH, DH, and RH4 for all 10 volunteers included) and all other parameters were determined by calculating Pearson's linear correlation coefficients. Correlations between age or body mass index (BMI) and SWS were calculated separately only for NH, DH, and RH4 state. Differences in SWS variation between different hydration states were assessed using Bartlett's test for equality of variances. Statistical differences between all data measured at different hydration states were tested by one-way ANOVA. Standard deviations for every hydration state were calculated based on the mean values across all subjects. For all p -values lower than 0.05, the null hypothesis was rejected.

RESULTS

A typical B-mode image and corresponding SWS maps obtained through the temporal bone window by cerebral THE at different hydration states in one volunteer are shown in **Figure 3**. A blue line delimits the selected ROI, while white labels indicate typical anatomical landmarks, including the mesencephalon and basal cisterns.

We found a moderate linear correlation between SWS and urine osmolality ($r = -0.68$, $p < 0.001$), as shown in the scatter plot in **Figure 4**, while SWS did not correlate with any other physiological parameter. Blood pressure, heart rate, cerebral blood flow velocity, and hematocrit values did not change with hydration. In contrast, urine osmolality increased

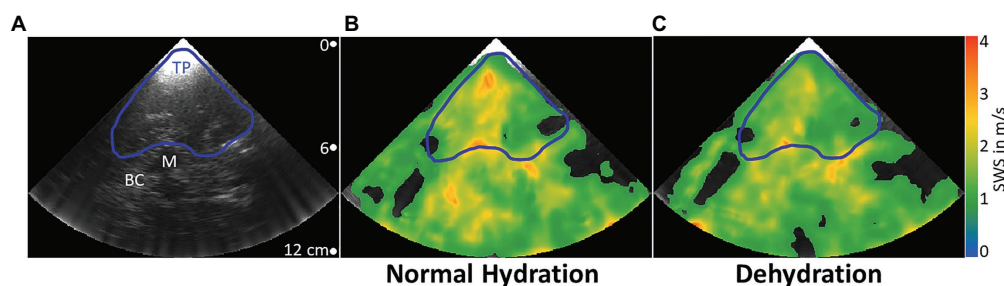


FIGURE 3 | Representative B-mode image and elastograms in normal hydrated and dehydrated states. Transcranial B-mode image (A). Typical landmarks are the temporal parenchyma (blue line, TP), where ROIs were drawn, the mesencephalon (white line, M), which was used as the most prominent landmark, and basal cisterns (BC), a highly vascularized area around the mesencephalon with high echogenicity. Elastograms are shown as color-coded shear wave speed (SWS) maps. In the normally hydrated state (B), mean SWS in the demarcated region of interest is higher than in the dehydrated state (C).

from a normal hydration value of 324 ± 148 mOsm/kg to 784 ± 107 mOsm/kg in the dehydrated state ($p < 0.001$), as illustrated in **Figure 5A**. In two subjects (#5 and #12), changes in urine osmolality deviated from those observed in the other volunteers, which was attributed to incomplete fasting. As the two volunteers later confirmed this, they were excluded from the statistical group analysis. After water drinking, urine osmolality decreased to 288 ± 128 mOsm/kg ($p < 0.001$), which corresponded to normal hydration values ($p = 0.83$). Inversely to urine osmolality, SWS was found to decrease with dehydration from 1.64 ± 0.02 m/s to 1.57 ± 0.04 m/s ($p < 0.001$; **Figure 5B**). Within 30 min of water ingestion, SWS increased again to 1.62 ± 0.02 m/s ($p < 0.001$) and remained unchanged for another 30 min, where SWS reached a plateau. The plateau value was similar to normal hydration values (all $p > 0.9$). The variability in SWS was not different between hydration states ($p = 0.14$). All data are summarized in **Table 2**.

DISCUSSION

This study shows for the first time that de- and rehydration of brain tissue influence brain stiffness *in vivo*.

In healthy subjects, urine osmolality increases with hypertonic dehydration and decreases with oral rehydration. Conversely, in patients with renal dysfunction or chronic kidney disease, when the kidneys' ability to concentrate urine is impaired, this parameter is less markedly affected by dehydration

(Roscoe, 1964; Tabibzadeh et al., 2019). In our study, two volunteers were identified as outliers not complying with the fasting protocol due to their urine osmolality. Notably, changes in SWS were still in line with the changes in urine osmolality observed in these two volunteers, providing further evidence for the high sensitivity of SWS to urine osmolality.

Unlike SWS and urine osmolality, other physiological markers such as blood pressure, heart rate, and hematocrit as well as cerebral blood flow velocity were not observed to be affected by dehydration. Other studies found a slight decrease in blood pressure and cerebral blood flow (Trangmar et al., 2014, 2015; Tsai et al., 2018; Watso and Farquhar, 2019) and an increase in heart rate variability (Castro-Sepulveda et al., 2014) upon mild hypertonic dehydration. However, these studies also measured vessel diameter, which is a critical parameter in assessing cerebral blood flow and could not be reliably assessed in this study. Additionally, the authors of these studies focused on dehydration during physical activity while our measurements were performed under resting conditions, which are characterized by relatively stable physiological parameters.

It is well-known that dehydration decreases cerebral blood volume and blood flow (Duning et al., 2005; Streitburger et al., 2012; Ryding, 2017; Sonig et al., 2020). Blood volume and blood flow in turn influence stiffness. Different studies have addressed the relationship between cerebral blood flow and CS (Guo et al., 2018). For example, studying regional variation of cerebral perfusion in deep gray matter using MRE, we found a direct and positive correlation between perfusion

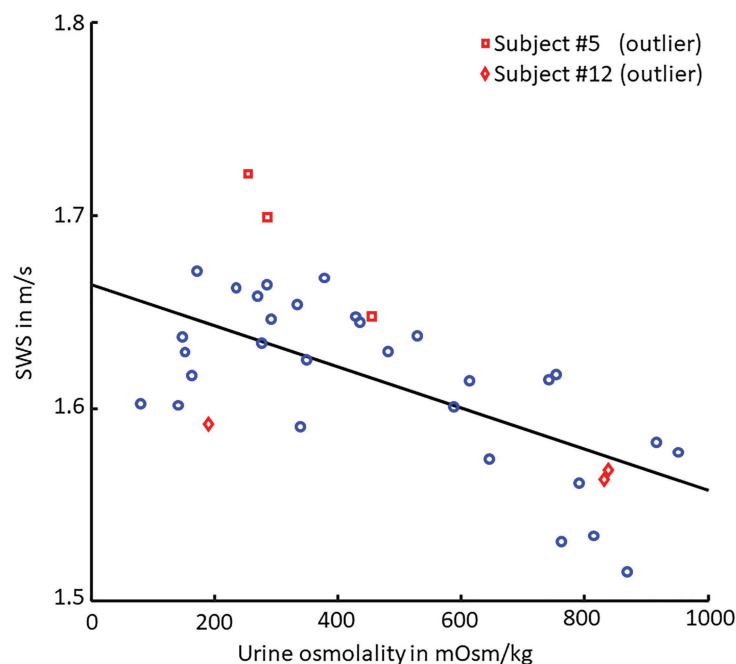


FIGURE 4 | Scatter plot of SWS vs. urine osmolality. Pearson's linear correlation between shear wave speed (SWS) and urine osmolality (30 values each, $p < 0.001$, $r = -0.68$). Linear regression is shown as black line. Volunteers #5 (□, red) and #12 (◇, red) were excluded from correlation analysis as they failed to comply with the fasting protocol.

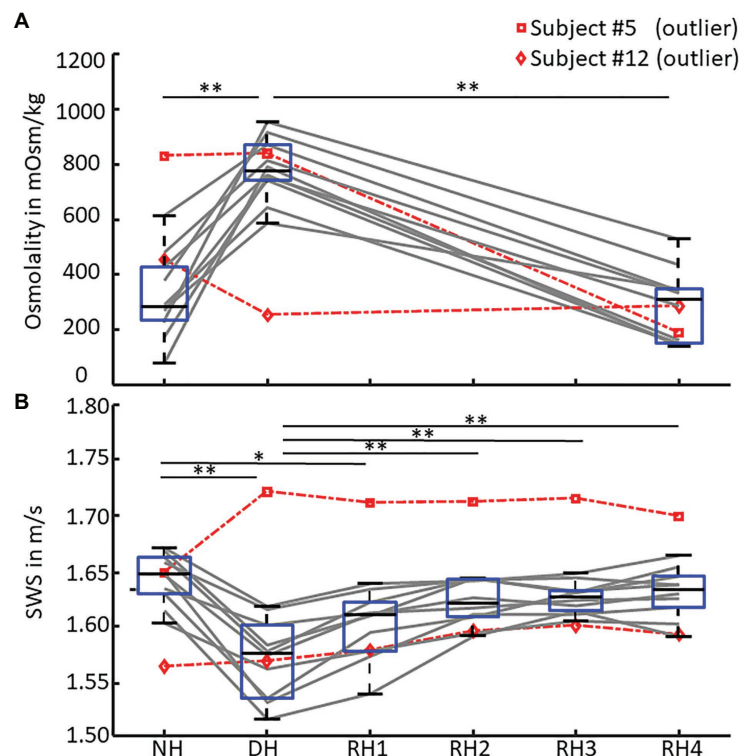


FIGURE 5 | SWS and urine osmolality changes during de- and rehydration. Boxplots of **(A)** urine osmolality for three hydration states – normal hydration (NH), dehydration (DH), and rehydration (RH4) and **(B)** mean shear wave speed (SWS) across all hydration states measured in each volunteer. Urine osmolality increased after dehydration and decreased to normal hydration values after 1 h of rehydration. Conversely, SWS significantly decreased after 12 h of fasting and returned to normal hydration values within 30 min of oral rehydration. Volunteers #5 (□, dashed red) and #12 (◇, dashed red) were excluded from statistical group analysis due to incomplete fasting, as reflected in urine osmolality and confirmed by them after the experiment. Significant differences between the groups are indicated by * $p < 0.05$ and ** $p < 0.001$.

TABLE 2 | Group mean values (standard deviation) across volunteers (#5 and #12 excluded) for all physical parameters obtained including shear wave speed (SWS), blood pressure, heart rate, cerebral blood flow velocity, and urine osmolality.

Parameters	NH	DH	RH1	RH2	RH3	RH4
SWS in m/s	1.64 (0.02)	1.57 (0.04)	1.60 (0.03)	1.62 (0.02)	1.63 (0.01)	1.63 (0.02)
Blood pressure in mmHg	131/79 (10/6)	127/75 (7/6)	123/73 (7/9)	123/74 (7/8)	125/77 (6/8)	125/76 (9/9)
Heart rate in bpm	68 (10)	67 (9)	60 (10)	63 (11)	62 (12)	60 (10)
Cerebral blood flow velocity in cm/s	68.3 (7.7)	71.7 (4.4)	68.3 (4.8)	69.4 (6.6)	65.2 (4.6)	68.5 (4.1)
Urine osmolality in mOsm/kg	324 (148)	784 (107)	-	-	-	288 (128)
Hematocrit in %	42.4 (3)	42.2 (2.8)	-	-	-	42.7 (3.3)

All parameters were measured for normal hydration (NH), dehydration (DH), and four times during rehydration (RH1–RH4).

pressure and CS (Tzschatzsch et al., 2016b; Hetzer et al., 2018). Similarly, experimentally induced hypercapnia was associated with a synchronous increase in cerebral blood flow and CS, as revealed by MRE (Hetzer et al., 2019) and THE (Kreft et al., 2020). Previous work also revealed that the Valsalva maneuver causes an increase in CS on a short time scale in the order of seconds (Tzschatzsch et al., 2018). Taken together, this previous evidence suggests that lower cerebral blood flow, as a result of dehydration, reduces CS, which is consistent with the observations made in our study. However, with $4.4 \pm 1.7\%$, the observed effect of dehydration is relatively

small, which may be attributable to two factors: first, tissue stiffness is only indirectly linked to blood perfusion through poroelastic interactions such as have been described for *in vivo* brain tissue (McGarry et al., 2015, 2019; Parker, 2017; Lilaj et al., 2020). Second, autoregulation of cerebral blood volume, perfusion pressure, and intracranial pressure only occurs across a small range of biophysical property changes and possibly compensates for minor changes in CS (Donnelly et al., 2015; Moerman and De Hert, 2019). Nevertheless, the observed decrease in SWS from normal hydration to dehydration is significant and potentially adds to the variability of CS

measured in patients. Therefore, we recommend that cerebral elastography be performed under normal hydration conditions.

Our study has limitations. Since we did not record any parameters to determine renal function, the urine osmolality we measured, although correlated with SWS, can only be considered as indirect measurements of body tissue dehydration. In TCD measurements, the exact transducer positioning relative to flow direction influences the measured blood velocity value. Therefore, variability of TCD is relatively high and operator dependent. Our experimental setup was optimized for THE leading to a relatively high drop-out rate of 6 from 12 and limited statistical power of our TCD data for the comparison to published values of cerebral blood flow during de- and rehydration (Trangmar et al., 2014, 2015). Additionally, our volunteers did not observe a standardized diet *before* fasting, which may have led to variability in our SWS data due to individual adaptation mechanisms. Further biological confounders of brain stiffness should be investigated by cerebral THE in a larger group of volunteers observing standardized conditions.

In summary, CS was measured by cerebral THE in a group of healthy volunteers who fasted overnight to induce mild hypertonic dehydration of brain tissue. We found dehydration to cause a slight decrease in CS on the order of 4.4%. CS increased to normal values within 1/2 h of drinking water. CS correlated with urine osmolality but not with hematocrit. Brain softening due to dehydration might be explained by reduced cerebral perfusion in agreement with prior findings of MRE and THE on the correlation between cerebral perfusion and CS. To minimize variability of CS values in a standardized protocol of cerebral elastography, examiners should make sure

that volunteers or patients are in normal hydration states during the examination.

DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusions drawn in the article have been presented in the manuscript. Additional data may be requested from the authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Charité - Universitätsmedizin Berlin. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study and formed the hypothesis. BK, HT, MS, and SH acquired the data. BK, HT, and LD analyzed the data. BK, HT, LD, and IS drafted the manuscript. All authors carried out critical revision of the manuscript equally.

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Reverberant Shear Wave Elastography: A Multi-Modal and Multi-Scale Approach to Measure the Viscoelasticity Properties of Soft Tissues

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There are a variety of approaches used to create elastography images. Techniques based on shear wave propagation have received significant attention. However, there remain some limitations and problems due to shear wave reflections, limited penetration in highly viscous media, requirements for prior knowledge of wave propagation direction, and complicated propagation in layers where surface acoustic waves and guided waves are dominant. To overcome these issues, reverberant shear wave elastography (RSWE) was proposed as an alternative method which applies the concept of a narrow-band diffuse field of shear waves within the tissue. Since 2017, the RSWE approach has been implemented in ultrasound (US) and optical coherence tomography (OCT). Specifically, this approach has been implemented in these imaging modalities because they are similar in image formation principles and both share several approaches to estimate the biomechanical properties in tissues. Moreover, they cover different spatial-scale and penetration depth characteristics. RSWE has shown promising results in the elastic and viscoelastic characterization of multiple tissues including liver, cornea, and breast. This review summarizes the 4-year progress of the RSWE method in US and OCT. Theoretical derivations, numerical simulations, and applications in *ex vivo* and *in vivo* tissues are shown. Finally, we emphasize the current challenges of RSWE in terms of excitation methods and estimation of biomechanical parameters for tissue-specific cases and discuss future pathways for the *in vivo* and *in situ* clinical implementations.

Keywords: reverberant fields, elastography, shear waves, OCE, ultrasound, tissue viscoelasticity, tissue stiffness

INTRODUCTION

The biomechanical tissue properties are strongly tied to pathological and physiological states involving alterations of elasticity, viscosity, and structure [1–5]. It has been shown that changes in tissue stiffness, occur in various diseases such as cancers [6], calcification associated with arteriosclerosis [6], fibrosis associated with liver cirrhosis [7], and various ocular diseases such as keratoconus and glaucoma [5]. Therefore, the evaluation and assessment of the tissue stiffness

could allow an early detection and treatment of numerous diseases and a better evaluation of the physiological conditions at different levels: cells, tissues, and organs.

Over the years, elastography as a medical imaging approach has been implemented in different imaging modalities including ultrasound (US) [8], magnetic resonance (MR) [9], and optical coherence (OC) tomography imaging [10], and new names were adopted: USE, MRE, OCE, respectively. USE and OCE are similar in their image formation principles and both share several approaches to estimate the biomechanical properties in tissues. However, they cover different spatial-scale and penetration depth characteristics. Each technique can be classified by spatial resolution, field-of-view limits, and displacement sensitivity. As an example, commercial USE can generate elasticity maps with resolution in the millimeter range at a depth penetration of approximately 1–7 cm, enabling the imaging of organ-size tissues. On the other hand, OCE offers spatial mechanical resolution in the micrometer range at a depth penetration of 1–10 mm, enabling the observation of diminutive tissues with higher resolution.

There are multiple approaches to the formation of elastography images. Those based on shear wave propagation, using continuous or transient waves, have been reported in multiple studies, but an inherent issue is the presence of reflected waves from organ boundaries and internal inhomogeneities [6, 11, 12]. The presence of reflected waves, propagating at the region of interest, may cause modal patterns applying continuous waves or backward traveling waves in transient wave experiments [13]. To overcome this problem, reverberant shear wave elastography (RSWE) was proposed as an alternative method which applies the concept of a narrow-band random isotropic field of shear waves within the tissue [14]. These multi-directional wave fields are naturally established (even unavoidable) in practical situations and can be reinforced by utilizing several shear sources near the tissues of interest. The reverberant shear wave field is produced by the interaction of multiple shear waves propagating in all possible random directions. This field promotes the propagation of shear waves in deeper areas (i.e., liver tissue in obese patients [15]), and at superficial areas (i.e., corneal tissue for layer characterization [16]) where surface acoustic waves could otherwise dominate. Moreover, this approach leads to a simple solution and implementation for the estimation of local tissue shear wavelength or shear wave speed (SWS). Since 2017, the reverberant shear wave approach has been implemented in USE and OCE with promising results in the elastic and viscoelastic characterization of a high variety of tissues including liver, cornea, and breast [13, 15–17]. For example, RSWE can evaluate the dispersion of shear wave speed, measuring the slope (change in SWS with change in frequency) or as a power law coefficient consistent with a more advanced framework of tissue rheology, by exciting the target organ with multiple vibration frequencies within a bandwidth [15, 17]. Thus, this approach is able to analyze the viscoelastic and lossy nature of the tissue under study.

In this work, we present the theory and application of the reverberant shear wave field method in USE and OCE. The

following sections cover the fundamental theoretical derivations, numerical simulations, penetration and layer characterization capabilities, and *ex vivo* and *in vivo* applications. We present and discuss reverberant elastography results in calibrated phantoms and *in vivo* deep liver tissues using USE, and thin layered phantom characterization with *ex vivo* porcine cornea elastography using OCE. Finally, we emphasize the current challenges of reverberant elastography and discuss future pathways using multi-scale approaches and possible clinical implementations.

THEORY

The reverberant shear wave field can be described as the superposition of plane shear waves propagating in random directions [18]. The theoretical description for plane shear waves traveling in different directions and, therefore, generating a reverberant field was first introduced by Parker et al. [14] for ultrasound shear wave elastography (SWE). Later, a more extensive description was presented by Zvietcovich et al. [16] for a 3-D reverberant field using OCE. In this Section, the analytical description is presented and summarized as follows (refer to Ref. 16 for further details): shear waves are transversal body-type waves, therefore, the particle velocity produced by these perturbations is perpendicular to the direction of propagation. For a given 3D point ϵ in the Cartesian system, three orthogonal vectors in the spherical coordinate system are found: $\hat{r}$, $\hat{\theta}$, and $\hat{\phi}$. Then, the corresponding particle velocity vector field $V(\epsilon, t)$, at position ϵ and time t , in a reverberant chamber produced by plane waves propagating with a wave number k and radial frequency ω_0 is modeled as:

$$V(\epsilon, t) = \sum_{q,l} \hat{n}_{ql} v_{ql} e^{i(k_q \cdot \epsilon - \omega_0 t)} \quad (1)$$

where the index q represents a realization of the random unit vector $\hat{n}_q$ describing the direction of wave propagation, and the index l represents a realization of the random unit vector $\hat{n}_{ql}$ describing the direction of particle velocity parallel to the disk formed by the basis vectors $\hat{\theta}$ and $\hat{\phi}$ defined within a realization of q . Then, $\hat{n}_q \cdot \hat{n}_{ql} = 0$. Finally, v_{ql} is an independent, identically distributed random variable describing the magnitude of particle velocity within a realization of q . The summation over q is understood to be taken over the 4π solid angle, and the summation over l is taken over a 2π angle within the disk.

If the particle velocity in Eq. 1 is measured along the z -axis, then $V_z(\epsilon, t) = V(\epsilon, t) \cdot \hat{e}_z$, where $\hat{e}_z$ is a unit vector in the z direction. Given the reverberant field $V_z(\epsilon, t_0)$, closed-form complex analytical solutions can be found for the spatial autocorrelation of such fields along parallel and orthogonal directions to the measurement axis $\hat{e}_z$. In the orthogonal case, the autocorrelation of the reverberant field along the x -axis (or y -axis) is given by [14, 16]:

$$B_{V_z V_z}(\Delta \epsilon_x) = \frac{\beta}{2} \left[j_0(k \Delta \epsilon_x) - \frac{j_1(k \Delta \epsilon_x)}{k \Delta \epsilon_x} \right] \quad (2)$$

where β is the expected value of squared particle velocity magnitude v_{ql}^2 over both q and l realizations; j_0 and j_1 are spherical Bessel functions of the first kind of zero and first order, respectively, and $\Delta\epsilon_x = \Delta\epsilon \cdot \hat{e}_x$, with $\hat{e}_x$ as a unit vector along the x -axis. It is important to note that $B_{V_z V_z}(\Delta\epsilon_x)$ and $B_{V_z V_z}(\Delta\epsilon_y)$ have the same solution since both correlation axes $\Delta\epsilon_x$ and $\Delta\epsilon_y$ are perpendicular to measurement axis $\hat{e}_z$. Therefore, $B_{V_z V_z}(\Delta\epsilon)$ is symmetric around the origin for any $\Delta\epsilon$ such that $\Delta\epsilon \cdot \hat{e}_z = 0$.

Finally, for the parallel case, the spatial autocorrelation of the reverberant field is taken along the z -axis and is given by [14, 16]:

$$B_{V_z V_z}(\Delta\epsilon_z) = \frac{\beta}{2} \left[\frac{j_1(k\Delta\epsilon_z)}{k\Delta\epsilon_z} \right] \quad (3)$$

The local wave number k^* is estimated by taking the 2D autocorrelation in a region of a reverberant field and fitting autocorrelation profiles to Eqs. 2 and 3 according to the plane case. Therefore, given the excitation frequency ω_0 , shear wave speed is calculated using $c_s = \omega_0/k^*$; and, assuming a homogeneous and isotropic material, shear modulus can be calculated as [3]:

$$G = c_s^2 \rho \quad (4)$$

where G is the shear modulus, and ρ is the density of the material. Furthermore, if a transverse isotropic model of the material is assumed, G corresponds to out-of-plane shear modulus. The mathematical framework of this method is designed for a single operational frequency (ω_0); however, in a reverberant field with multiple operational frequencies, the estimators in Eqs. 2 and 3 are still valid as long as a single operational frequency is isolated using a band-pass filter. This is possible since the interference of waves in this approach is represented as a summation in Eq. 1 and frequencies can be isolated in the Fourier domain as performed in the phase-speed analysis of single broad-band pulse wave propagation in transient elastography [19].

APPLIED METHODOLOGY IN RSWE

Scanners and Data Acquisition

The application of US-RSWE was performed using a Verasonics system (Vantage-128TM, Verasonics, Kirkland, WA, United States) connected to a convex ultrasound probe (model C4-2, ATL, Bothell, WA, United States) or a linear ultrasound probe (model L7-4, ATL, Bothell, WA, United States). This system was used to track the induced displacements using a Loupas estimator [20]. The linear probe was used for the breast phantom and breast tissue applications, whereas the convex probe was used for the viscoelastic phantom and liver tissue to scan at deeper areas [13, 15]. In Ref. 15, the center frequencies were 3 and 5 MHz for the convex and linear probes, respectively. The sampling frequency were 12 and 20 MHz for the convex and linear probes, respectively. The tracking pulse repetition frequency (PRF) was set to 3,600 Hz with a total acquisition time of 0.5 s. On the other hand, OCT-RSWE used a custom built spectral-domain phase-sensitive optical coherence tomography

(PhS-OCT) system for the motion detection of waves generated in the sample by a synchronized mechanical excitation system [16]. The capturing of spectral interference signals was synchronized with the scanning of the light beam on the sample to produce 2D, 3D, or 4D OCT datasets. The Doppler phase shift detection scheme was implemented to monitor the propagation pattern of vibrational waves [21].

Vibration Sources and Frequency Ranges

Figure 1 illustrates the different setups used to create the reverberant shear wave field. In Refs. 13 and 14, mechanical vibration sources (such as model 4810, Bruel and Kjaer, Naerum, Denmark), and miniature vibration sources (model NCM02-05-005-4 JB, H2W, Linear Actuator, Santa Clara, CA, United States) were used for initial experiments and proof of concept. Later, a custom-made portable trifold futon ($70 \times 60 \times 10 \text{ cm}^3$) including several embedded vibration sources (Quad Resonator Model EI718 TM, Elastance Imaging LLC, Columbus, OH, United States) was mounted to a clinical bed [15]. The active vibration sources generate strong displacements and shear waves into the body. The precise details of the active source configuration are proprietary to Elastance Imaging LLC. In Ref. 16, one of the ends of a piezoelectric bender (BA4510, PiezoDrive, Callaghan, NSW, Australia) was attached to a 3D printed ring containing eight vertical equidistant and circularly-distributed rods. The rods were slightly attached to the sample surface to produce the reverberant field after the piezoelectric bender was excited. The ring shape did not interfere with the OCT system to image the cornea, while the rods generate mechanical excitations.

Different vibration frequency ranges were used for the different applications. In US-RSWE, frequencies between 40 and 700 Hz were used for phantoms and *in vivo* tissue. In Ormachea et al. [15], a simultaneous multi-frequency range was applied in all vibration sources for each experiment to allow a rapid acquisition of shear waves propagating at different frequencies. In OCT-RSWE, Zvietcovich et al. [16] applied a higher vibration frequency, 2,000 Hz, for the phantoms and *ex vivo* cornea tissue. The selected excitation frequency was a balance to obtain smaller wavelengths with sufficient energy to avoid stronger wave attenuation below the sensitivity floor for motion detection using the PhS-OCT system.

Numerical Simulations

Numerical simulations were conducted to corroborate the theory of RSWE. In Ref. 14, a shell-element analysis and 3D solid finite element analysis in a breast shape tissue model were performed. Ormachea et al. [17] used a Monte Carlo analysis in models, exhibiting phase velocities behavior with different power law coefficients, to validate a 2D shear wave dispersion study on breast and liver tissues using multi-frequency US-RSWE. For OCT-RSWE, Zvietcovich et al. [16] performed a Monte Carlo analysis to validate Eqs. 2 and 3 in a uniform elastic medium ($c_s = 4 \text{ m/s}$) subjected to an ideal reverberant field. Here, we considered v_{ql} as a scalar and uniformly distributed random variable covering a range of $[-v_{\max}, v_{\max}]$, where $v_{\max}$ represents the maximum particle velocity in the analysis. Similarly, unit vectors $\hat{n}_{ql}$ and $\hat{n}_q$

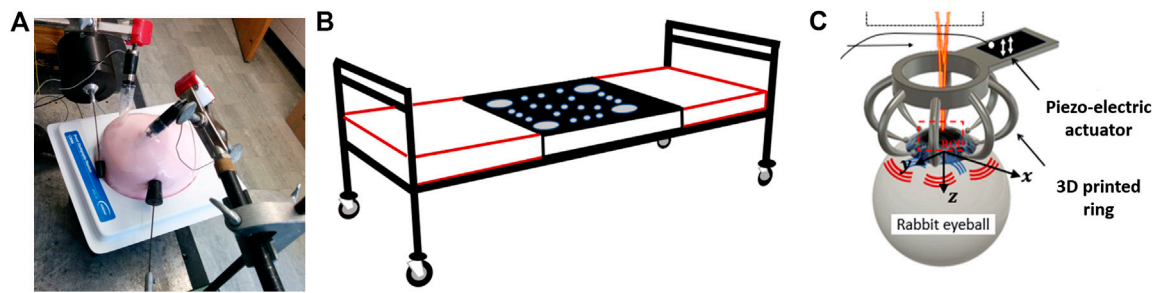


FIGURE 1 | (A) Initial schematic setup using four external vibration sources to generate a reverberant field inside the CIRS breast phantom. **(B)** Custom-made trifold vibration futon bed. The active array of sources is located in the black zone. The red lined outer sections consist of cushions for the head and lower body. ©Institute of Physics and Engineering in Medicine. Reproduced from Ref. 15 by permission of IOP Publishing. All rights reserved. **(C)** 3D printed ring. The output OCT beam goes through the objective lens toward the sample (porcine cornea). The cornea is excited by a piezoelectric actuator attached to an eight-head ring.

are uniformly distributed in the 3D (4π solid angle) and 2D (2π angle) space. In all of these simulations, a superposition of multiple random shear waves has been applying. The application of multiple shear waves is the analog procedure to apply multiple vibration sources located at different positions. **Figure 2** shows a simulated 3D volume of particle velocity along the z -axis produced by a 2 kHz reverberant field. Complex autocorrelation was analyzed at different planes: the xy -plane and the xz -plane. The autocorrelation curves, taken along perpendicular and parallel directions with respect to the motion axis (z -axis) were fitted to **Eqs. 2** and **3**, respectively, showing good agreement between theory and simulation. **Figure 3** shows a simulated 2D phase map in the xz -plane of $40 \times 40 \text{ mm}^2$ region in a uniform elastic material with a stiffer inclusion after conducting a Monte Carlo analysis of reverberant shear waves with frequency equal to 600 Hz. In order to explore an extreme case scenario, only 10 plane waves were used to create a reverberant shear wave field. In addition, white Gaussian noise

[signal-to-noise ratio (SNR) = 5 dB] was added to the particle velocity signal to simulate real data acquisition in *in vivo* cases. The SWS results for the background and inclusion agreed with the values used for the simulation. More recently, numerical simulations were performed in Ref. 22 to show that at least 60 incident plane waves were necessary to generate a reverberant shear wave field. It was found that as more incident waves propagate in the field, the coefficient of determination with respect to the theoretical models improved and the shear wave speed estimation error decreased.

Phantom Studies

Two calibrated phantoms were used to evaluate the US-RSWE method. The first was a CIRS breast phantom (Model 059, Computerized Imaging Reference Systems, Norfolk, VA, United States) with background (20 kPa nominal Young's modulus) and inclusion regions (at least two times stiffer than the background region per the manufacturer's datasheet). The

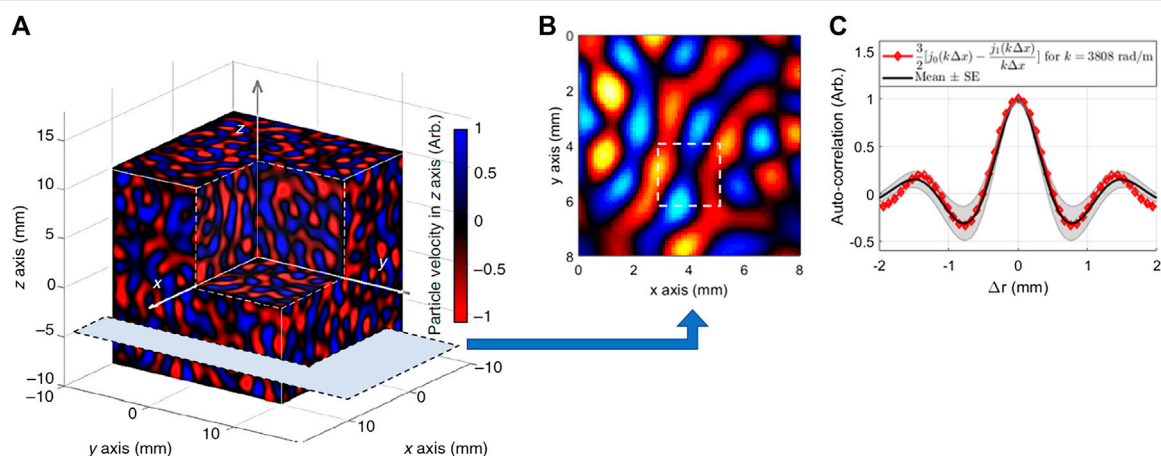
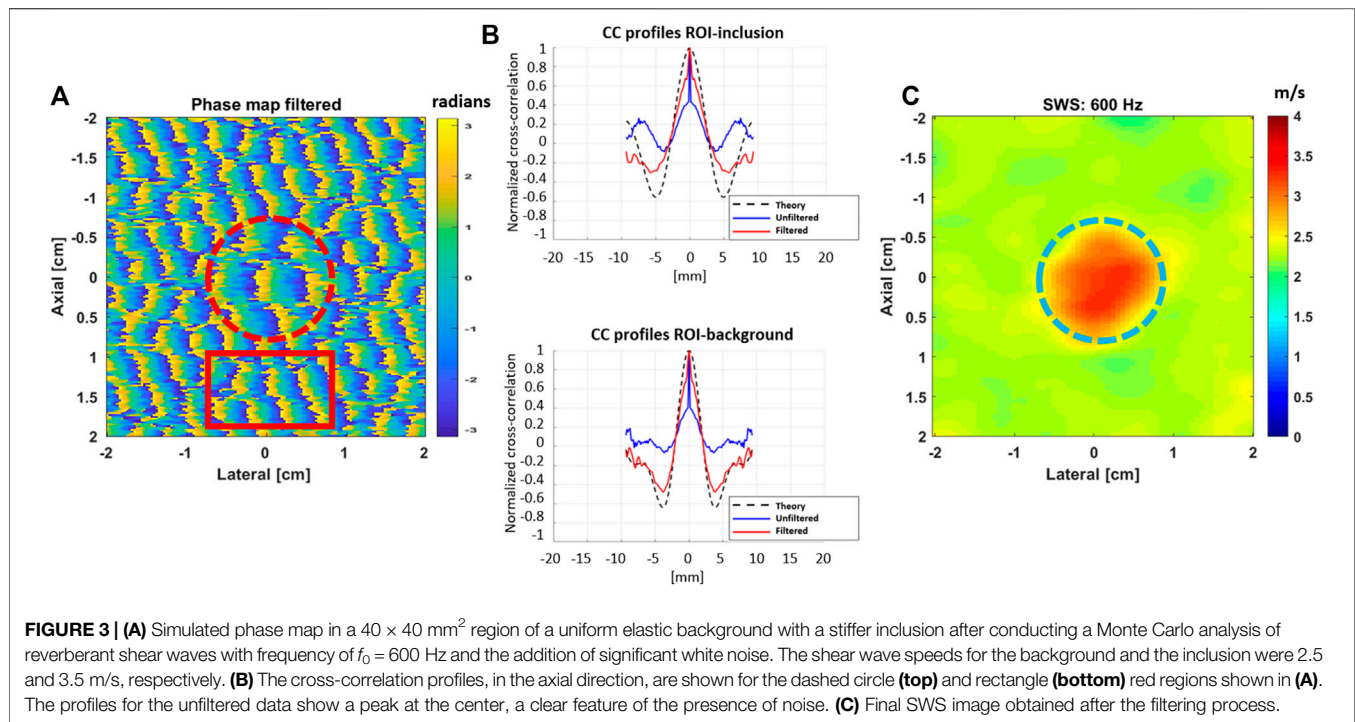


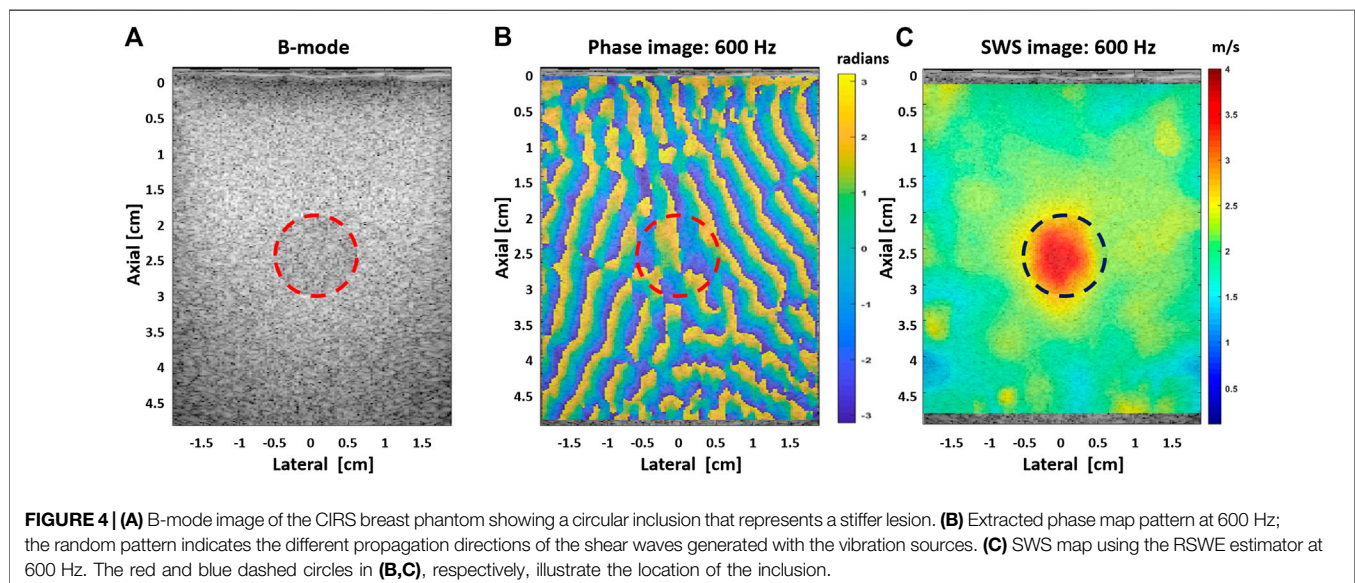
FIGURE 2 | (A) Particle velocity field along the z -axis after conducting a Monte Carlo analysis of reverberant shear waves at an arbitrary time t_0 . The cube dimensions are $20 \times 20 \times 20 \text{ mm}^3$. The simulated media is a uniform elastic material. The applied vibration was at frequency of $f_0 = 2 \text{ kHz}$, and a constant shear wave speed $c_s = 4 \text{ m/s}$. For this simulation, a total of 10,000 realizations of all random variables were performed. **(B)** Fitting of analytical **Eqs. 2** and **3** to autocorrelation profiles along the x -axis (red line) and the z -axis (blue line), respectively. **(C)** Fitting of analytical **Eq. 2** to autocorrelation profiles along the x -axis (red line) and y -axis (blue line). In all cases, wavenumber k^* can be estimated and converted into shear wave speed using $c_s = \omega_0/k^*$, where $\omega_0 = 2\pi \times 2000 \text{ rad/s}$.



other phantom was a custom made CIRS (Serial No. 2095.1- 1, Computerized Imaging Reference Systems) homogeneous viscoelastic phantom (6 kPa nominal Young's modulus). Both phantoms show different viscoelastic properties as shown by the shear wave dispersion analysis evaluated in Ref. 15.

The CIRS breast and viscoelastic phantoms were used to illustrate the ability of US-RSWE to differentiate the stiffer regions from the background material, see **Figure 4**. The viscoelastic properties of each CIRS phantom were evaluated by measuring the shear wave dispersion as a function of

frequency, see **Figure 5**. The initial experiments in Parker et al. [7] showed elastographic images using individual frequencies of 400 and 450 Hz; then, Ormachea et al. [10] used a broader set of individual frequencies from 60 to 450 Hz for the almost purely elastic breast phantom and 60–120 Hz for the viscoelastic phantom, respectively. The obtained mean SWS results were compared with another elastographic technique (single tracking location-acoustic radiation force) with good agreement at 220 Hz. The results in Ref. 10 also reported the feasibility of applying a multi-frequency vibration range. The



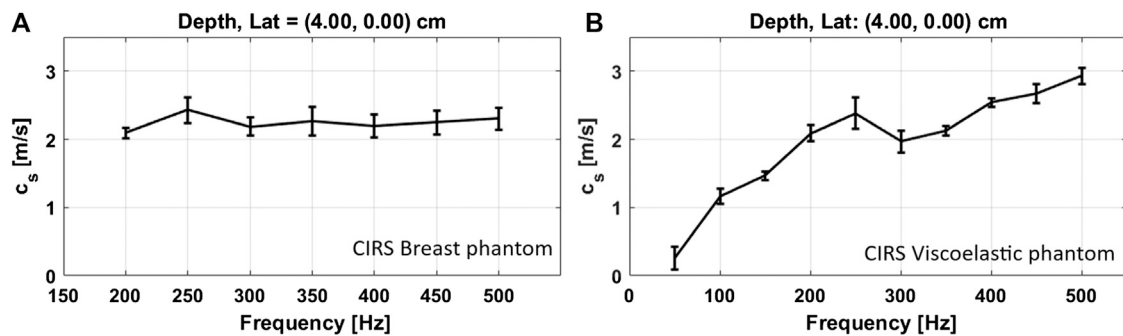


FIGURE 5 | Phase velocity dispersion curves. Estimated SWS vs. frequency plots for the CIRS breast (A) and viscoelastic phantoms (B). The center depth and lateral positions of a selected region of interest, to extract the SWS values, are indicated above each plot. As expected, a higher dispersion was obtained for the viscoelastic media. ©Institute of Physics and Engineering in Medicine. Reproduced from Ref. 15 by permission of IOP Publishing. All rights reserved.

application of multi-frequency tones allows a rapid collection of shear wave response to estimate and create 2D SWS and dispersion images at discrete frequencies. The results were similar to the ones obtained using individual frequencies and enabled a follow-up study to create not only 2D SWS images, but also 2D shear wave dispersion (SWD) images, since the method is able to obtain the SWS information at different frequencies and at different locations using the same collected data. The results were reported in Ref. 9, showing a clear difference between the phantoms with respect to their SWD values, and consequently, a better viscoelastic characterization for both materials. In Ref. 22, experiments using gelatin-based phantoms were performed to analyze the conditions to create a reverberant shear wave field. Similarly to the numerical simulations, reported in Ref. 22, the coefficient of determination with respect to the theoretical models improved and the shear wave speed estimation error decreased as more incident waves propagates into the field. Moreover, it was found that at least three vibration sources located at the top of the

phantom surface were necessary to measure an average SWS with an error less than 9%.

For OCT-RSWE, gelatin-based phantoms were created to evaluate the capability of the RSWE approach to detect elasticity gradients along depth. In Ref. 16, a reverberant field of 2 kHz was created in a horizontally-layered phantom by exciting the ring actuator with eight rods, as described in *Vibration Sources and Frequency Ranges* section. The phantom had a softer layer (thickness ~ 0.3 mm, 3% gelatin concentration) located on the top of a second stiffer layer (5% gelatin concentration). **Figure 6A** shows motion frames extracted at the top 3% layer (depth $z_0 = 0.21$ mm) and bottom 5% layer (depth $z_0 = 0.69$ mm), with their respective 2D autocorrelation calculated within a 0.8×0.8 mm² window. Then, using **Eq. 2**, the average speed transition along depth is calculated in **Figure 6B**. The speed transition is fitted to a sigmoid function and an axial elastography resolution of $\delta_z = 55.5 \mu\text{m} \pm 17.3 \mu\text{m}$ was found. Finally, experimental results were compared to material ground

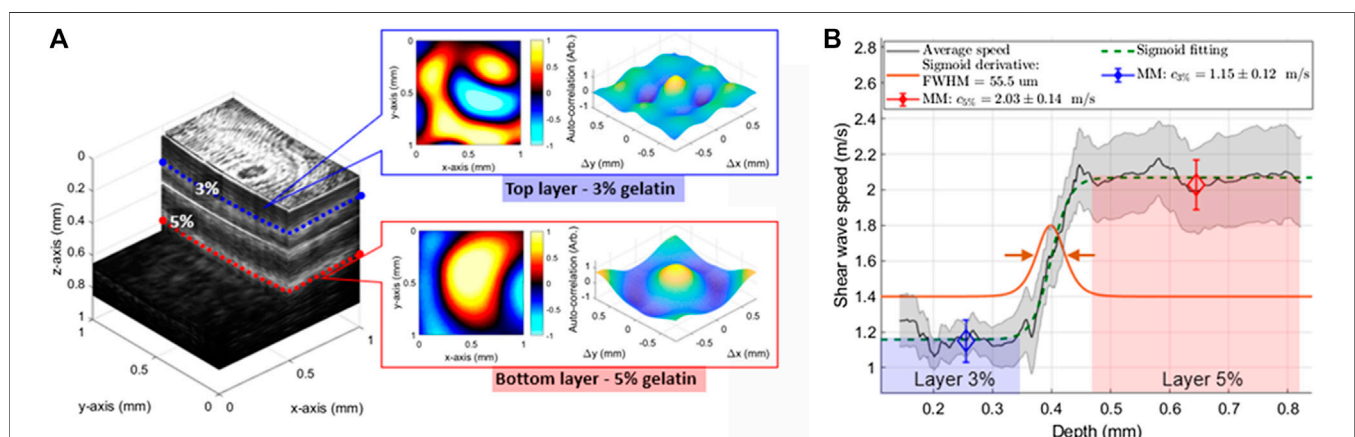


FIGURE 6 | (A) Horizontally distributed layered phantom ($1 \times 1 \times 1$ mm³) showing a stiffer bottom layer (gelatin concentration of 5%) and softer top layer (gelatin concentration of 3%) obtained using OCT. Motion frames (particle velocity) and 2D autocorrelation plots are calculated within each layer with notable wavelength and main-lobe spreading differentiation, respectively. **(B)** Average speed transition along depth from the 3–5% concentration layers of the phantom. Mean $\pm$ standard deviation ($N = 5$ measurements). For elastography resolution characterization, a sigmoid function was fitted to the speed profile). The average full-width half-maximum layer transition was found to be $55.5 \mu\text{m} \pm 17.3 \mu\text{m}$. The depth-dependent speed profile was compared against mechanical testing results ($N = 3$ samples per gelatin concentration) showing consistency of results in each layer. Reprinted by permission from Springer Nature from Ref. 16.

truth mechanical measurements, showing accuracy errors of 1.74 and 6.89% for the 3 and 5% gelatin concentration layers, respectively.

APPLICATION IN *EX VIVO* AND *IN VIVO* TISSUES

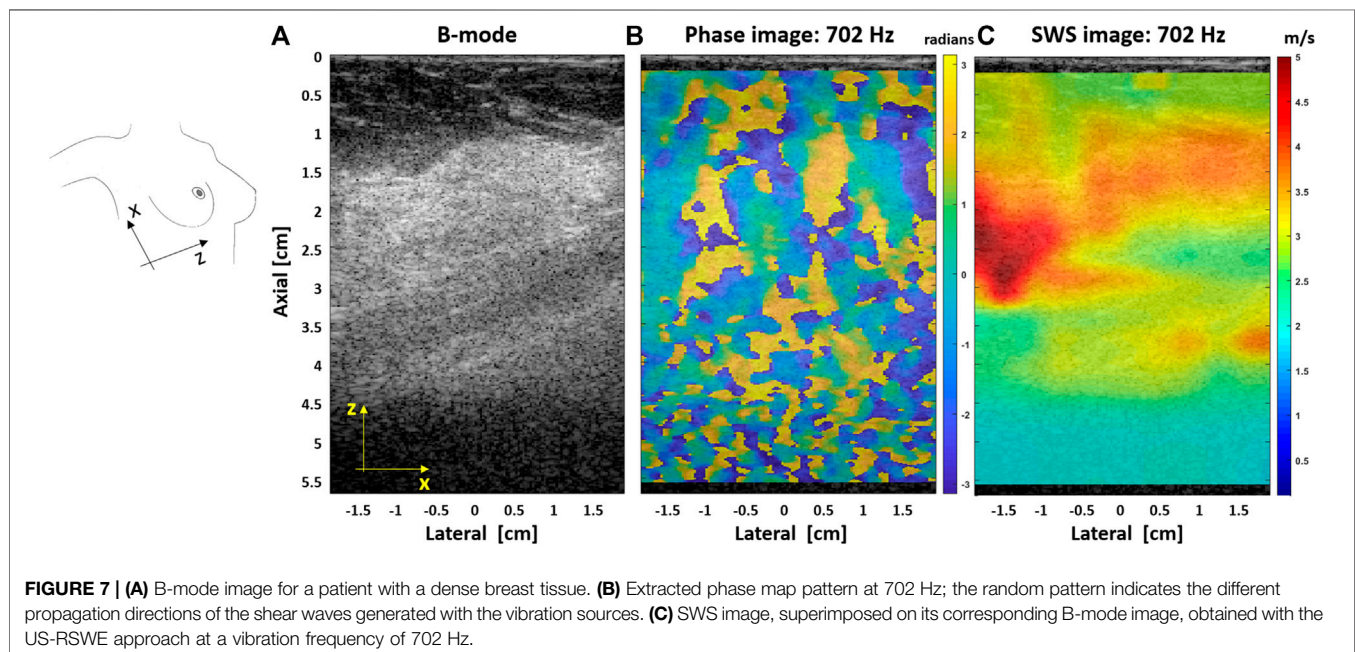
In Ref. 15, the authors demonstrated that reverberant shear wave fields can be produced in deep tissues from external sources, applying vibration frequencies up to 400 Hz in obese patients' livers and over 700 Hz in breast tissue. Moreover, SWD images were obtained by analyzing the linear slope or the power law coefficient. These additional parameters may provide a better image contrast and information about the scanned media. An altered dispersion parameter, compared with normal tissue, would show a different viscoelastic response for lesions or pathologies cases. **Figure 7** shows a SWS image at 702 Hz for the dense breast tissue experiment. The elastographic image clearly shows the dense breast region as stiffer tissue than the surrounding area.

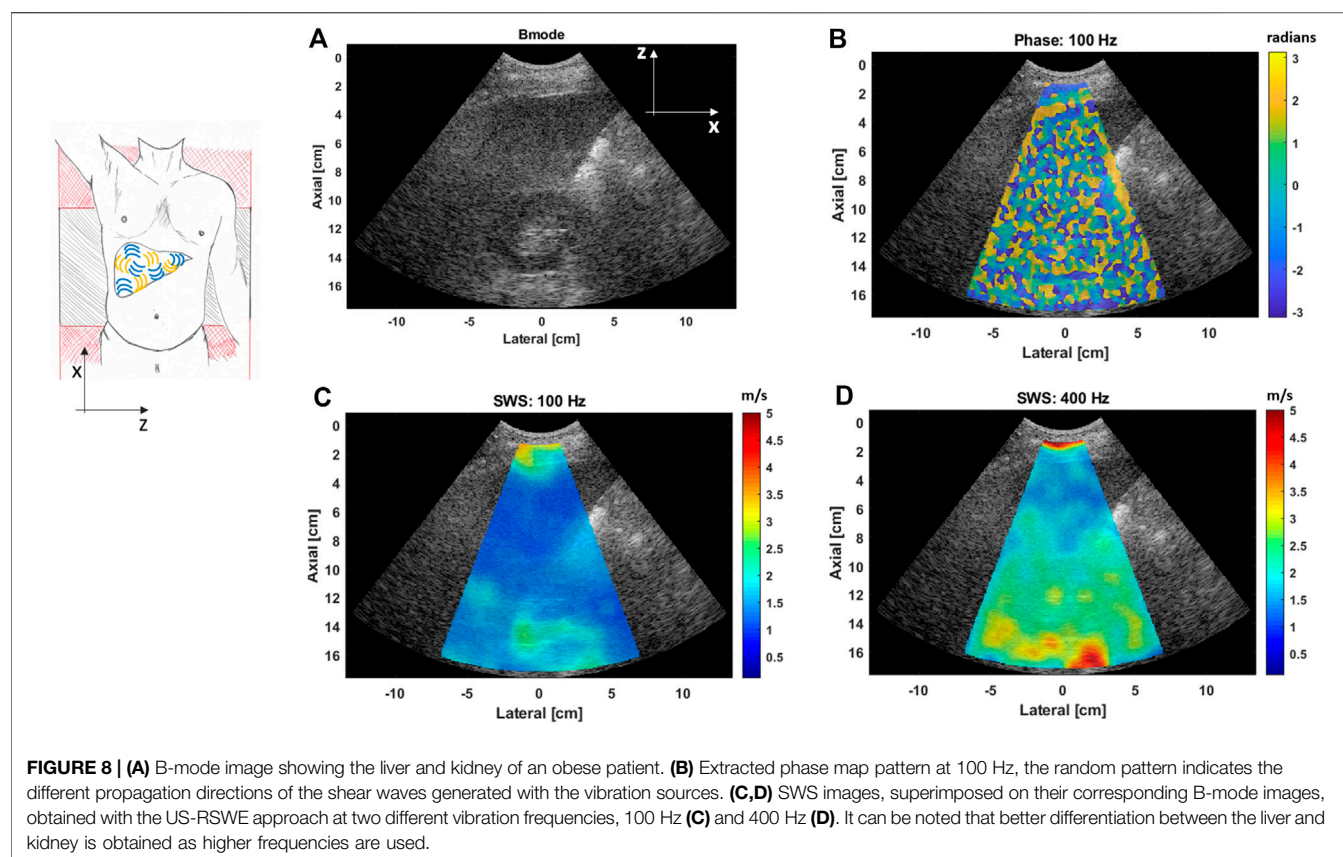
Figure 8 shows liver SWS images at two different vibration frequencies, 100 and 400 Hz, for an obese patient. The liver is located between 4 and 10 cm depth in this case. Thus, US-RSWE was able to measure liver viscoelasticity, in terms of SWS and SWD, in obese patients at deep regions. For this case, a simple observation shows the stiffness difference of the kidney region and the liver tissue as higher frequencies are used. Although this suggests that US-RSWE may be able to measure the kidney tissue biomechanical properties, further research is needed with more focus in this organ. **Table 1** shows a summary of SWS values obtained from a region of interest from 2D images of CIRS phantoms and *in vivo* liver obtained with US-RSWE [15].

In Ref. 16, OCT-RSWE was demonstrated to characterize the elasticity of single layers in *ex vivo* porcine cornea with unprecedented contrast in the dynamic wave-based OCE field. An *ex vivo* porcine cornea was subjected to a 2 kHz reverberant shear wave field. After the acquisition, a 3D structural volume (**Figure 9A**-left) and the reverberant complex 3D motion field (**Figure 9A**-right) were obtained. The curvature of the cornea was taken into account for the compensation of particle velocity along depth. The average depth-dependent shear wave speed plot of the cornea was obtained using **Eq. 2** for the estimation of local wavenumber (k^*) and $c_s = \omega_0/k^*$ using $\omega_0 = 2\pi \times 2000$ rad/s and shown in **Figure 9B**. Here, the speed profile correlates with the structural information from the B-mode intensity image, and the anatomical description of the corneal layers. Finally, **Figure 9C** shows shear wave speed volumes of cornea subjected to four intraocular pressure (IOP) levels: 10.24, 15.80, 20.50, and 25.25 mmHg. The tendency of SWS to increase in the reverberant patterns when the IOP level increased confirmed the non-linear elastic nature of corneal tissue.

Shear Wave Dispersion Measurements

Shear wave dispersion was measured in terms of the linear slope and the power law coefficient, in CIRS phantoms, *in vivo* breast, and *in vivo* liver tissues over an applied vibration frequency range. First, SWD was obtained over a region of interest of the obtained SWS images in Ref. 13. Later in Ref. 15, it was also possible to create SWD images to add information and contrast of the tissue viscoelastic properties. However, the practical upper limits to shear wave frequencies is still unknown for each specific soft tissue. The obtained results are still preliminary, further clinical studies are needed to obtain consensus about what is the expected SWD in normal and healthy populations, for example. Although the initial results only reflect few cases, it is encouraging that SWD shows better differentiation than SWS values for the obese





liver cases with respect to the thin liver patient case, and all of these values are in a similar range with respect to other independent studies that also measured this parameter. For example, the linear dispersion results reported in Ref. 15 agreed with the ones reported in Ref. 23, 0.1 and 0.6 m/s/100 Hz using a frequency range of 0–400 Hz, or the ones reported in Refs. 19 and 24, 0.36 m/s/100 Hz in *in vivo* healthy human volunteers using frequencies ranges of 40–450 Hz and 60–390 Hz, respectively. **Table 1** shows a summary of SWD obtained from a region of interest from 2D images of CIRS phantoms and *in vivo* liver obtained with US-RSWE [15].

DISCUSSION

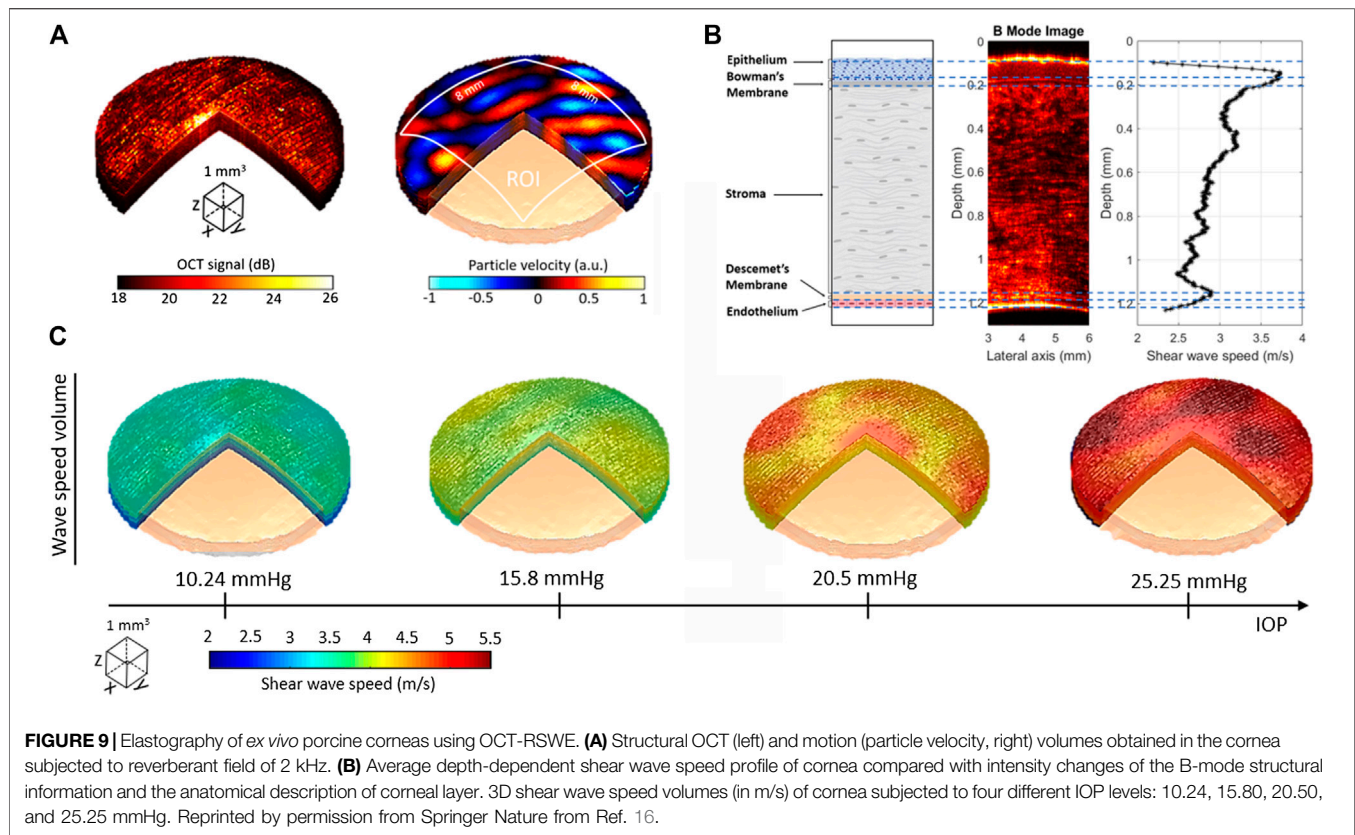
The RSWE approach, based on continuous sinusoidal excitation at specific frequencies, promotes the propagation of a higher quantity

of shear waves in tissues vs. other wave propagation methods. This is of particular interest in OCE since surface acoustic waves are typically dominant near boundary conditions during transient excitation (generated by an acoustic radiation force in SWE methods or external short vibration tone bursts), complicating the conversion of wave speed into shear or Young's moduli. Moreover, this new method establishes a profusion of shear waves propagating in different directions, incorporating shear wave reflections from boundaries and inhomogeneities. This limiting 3D shear wave distribution leads to a mathematical model with a simple local estimator of shear wave speed and stiffness covering a full field of view.

The application of numerical simulation has demonstrated to be an important tool to validate the mathematical framework of the RSWE approach, the different studies have reported simulations including the superposition of multiple random shear waves. These simulations demonstrate the capabilities of RSWE for inclusion

TABLE 1 | SWS and SWD estimations in phantoms and *in vivo* liver obtained with US-RSWE [15].

Scanned material or tissue	SWS [m/s]	SWD [m/s/100 Hz]	Frequency range [Hz]
Breast phantom (background)	2.15 ± 0.11	0.10 ± 0.02	200–500
Viscoelastic phantom	1.88 ± 0.38	0.42 ± 0.02	80–320
Patient #1, thin liver patient	1.99 ± 0.19	0.28 ± 0.14	80–320
Patient #2, obese liver patient	2.29 ± 0.37	0.49 ± 0.17	80–320
Patient #3, obese liver patient	2.38 ± 0.20	0.54 ± 0.19	80–320



and layer detections for two different clinical applications, breast and cornea tissue, respectively. However, the models to represent the media where shear waves propagate are still simple: isotropic, homogeneous, lossless. Further analysis including more realistic geometrics and inhomogeneities, attenuation of shear waves, and dispersion behavior are needed to evaluate the reverberant shear wave approach and its limitations.

As observed, one advantage of using RSWE is that it produces stronger shear waves at deeper tissue regions of interest, and it can provide additional parameters such as the evolution of SWS as a function of frequency (dispersion). Our preliminary *in vivo* liver and breast results indicate that SWD values could add information for better tissue characterization. A disadvantage of RSWE is that the spatial resolution of the final elastograms is limited by the tissue stiffness and the applied frequency. The approach also requires additional hardware in the form of external vibration sources in order to produce shear waves.

The RSWE approach presents some differences with respect to other methods that use external vibration sources. In Refs. [25–31], only one loudspeaker is attached to the clinical bed, RSWE uses multiple vibration sources. Zhao et al. [25, 26] used directional filters and applied a SWS estimator based on time delays between the temporal signals. RSWE does not use directional filters and uses all the generated shear waves in the field. Although Tzschatzsch et al. [29] also applied multi-frequency ranges and measured SWD similar to US-RSWE, the frequency ranges are different. In Ref. 29, a set of frequencies ranging from 30 to 60 Hz was used for the liver application, whereas US-RSWE applied 40 to 400 Hz. Another

modality with external sources is passive elastography. There are major differences between RSWE and passive elastography: the former is a narrowband, spatial domain autocorrelation method derived from classical acoustics which uses active sources vibrating at specific frequencies; the latter is a broad-band random signal, temporal correlation *via* time reversal, derived from geophysics and originally relying on natural shear wave sources.

Our preliminary results, using US-RSWE, show its capability to obtain SWS and SWD images. As reported in Ref. 15, US-RSWE was able to generate images in deep areas (~16 cm depth) for liver application in obese patients. Thus, this method may be able to overcome one of the main liver clinical limitations: the ability to obtain elastographic images in obese cases [7]. For breast tissue, US-RSWE applied higher vibration frequencies than in the liver. Our preliminary experiments showed the possibility to obtain shear waves propagating through the field using frequencies up to 700 Hz, despite the loss due to attenuation. The higher frequencies also allow us to generate shorter shear wavelengths and a possible improvement in the spatial resolution to detect lesions. However, since the spatial resolution also depends on the tissue stiffness, more experiments are needed to determine ‘low’ and ‘high’ thresholds for vibration frequencies to define which are appropriate for this specific tissue.

The work done in OCT-RSWE shows the advantage of generating elastograms with axial elastography resolution of $55.5\ \mu\text{m}$ [16], which is a promising tool for optimal fine-scale medical imaging. Furthermore, OCT-RSWE is able to detect

elasticity changes along the depth axis, which is very important for characterizing corneal layers stiffness. In particular, the properties and boundary conditions of cornea (layer distribution, surfaces, and stiffness heterogeneities) help to produce multiple reflections and aberrations of shear waves. These properties are optimal to estimate the SWS using RSWE, a clear advantage compared to other peak tracking-based methods where these conditions will diminish the effectivity of their SWS estimators. In OCT-RSWE, a multi-frequency range has not yet been applied. However, the system can be modified to send multi-harmonic vibrations at the same time. The simultaneously captured data will give information about the depth dependent SWD and its relationship with the viscoelastic parameters. A procedure similar to US-RSWE can be performed to obtain SWD plots and 2D images. This procedure shows an example that OCT and US, using the reverberant shear wave elastography approach, may be integrated to generate a multi-modal approach where each can add important information from the micro and macro level structure of the tissues.

Since the RSWE approach has been studied the last 4 years and results are preliminary, a number of issues remain to be evaluated. One practical problem may occur when the field fails to approach the diffuse or reverberant ideal or a partial reverberant field is created with dominant waves propagates at specific regions. A more comprehensive determination of the degradation or deviation from the model remains to be quantified including the quantification of the minimum amount of vibrations sources in more realistic tissue models and the attenuation of waves when using high frequencies. Moreover, the assumption of local homogeneity and constant density remains true in RSWE as in most of the reported elastography techniques [3]. The plane wave assumption has a different impact in RSWE since Eq. 1 represents the limiting case in which statistical summation of plane waves traveling at different direction converges to expressions of Eqs. 2 and 3. We believe this statement holds true if the summation is performed using spherical (or cylindrical) waves since these waves contain a variety of propagation directions in a single wavefront that reinforces the statistical summation along the 4π solid angle. Future work may focus in the impact of the use on non-plane waves in the accuracy of the estimators proposed in *Theory* section. As mentioned in Ref. 16, a future implementation of OCE-RSWE for *in vivo*, *in situ*, and a fully non-contact method for 3D elasticity characterization of corneal layers, requires some modifications to the current system. For instance, the 3D printed ring and the attached actuator can be replaced by a circular array of air-coupled focused ultrasonic transducers. Finally, up to date, RSWE has not been implemented in MRE; however, we believe MRE-RSWE is possible for excitation frequencies in the range of

20–200 Hz which generate small enough shear wavelength (2–8 cm) in large fields of view (30–50 cm) in order to produce speed maps.

CONCLUSION

RSWE is an approach that overcomes some major limitations of current elastography: by incorporating reflections and deep penetration of shear waves, by avoiding the need for prior knowledge of wave propagation direction, and by minimizing the effects of surface acoustic waves. We have conducted a series of studies using numerical simulations and tissue-mimicking phantoms to understand the nature, properties, and capabilities of the RSWE approach. This new method has estimated the viscoelastic properties, in terms of SWS and SWD, in phantom materials, *ex vivo* porcine cornea tissue, and *in vivo* human tissue. The reverberant field can be produced in deep tissues by external sources, up to 400 Hz in obese patients' livers, over 700 Hz in breast tissue, and 2 kHz for cornea tissue. We have demonstrated that RSWE could be an excellent method to study different tissues at different scales, including different boundary conditions (composite plate-shaped media such as mouse *in situ* brain tissue, etc.) and diverse mechanical properties (viscoelastic, and heterogeneous). The results are robust across platforms and can be extended further to magnetic resonance imaging and other imaging systems.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Poroelasticity as a Model of Soft Tissue Structure: Hydraulic Permeability Reconstruction for Magnetic Resonance Elastography in Silico

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Magnetic Resonance Elastography allows noninvasive visualization of tissue mechanical properties by measuring the displacements resulting from applied stresses, and fitting a mechanical model. Poroelasticity naturally lends itself to describing tissue - a biphasic medium, consisting of both solid and fluid components. This article reviews the theory of poroelasticity, and shows that the spatial distribution of hydraulic permeability, the ease with which the solid matrix permits the flow of fluid under a pressure gradient, can be faithfully reconstructed without spatial priors in simulated environments. The paper describes an in-house MRE computational platform - a multi-mesh, finite element poroelastic solver coupled to an artificial epistemic agent capable of running Bayesian inference to reconstruct inhomogeneous model mechanical property images from measured displacement fields. Building on prior work, the domain of convergence for inference is explored, showing that hydraulic permeabilities over several orders of magnitude can be reconstructed given very little prior knowledge of the true spatial distribution.

Keywords: Elastography, MRI, Biophysics, Biomechanics, Bayesian Inference, Effective Field Theory, Continuum Mechanics, Porous Materials

1 INTRODUCTION

In the past two decades magnetic resonance elastography (MRE) has emerged as an imaging modality that extends the capabilities of standard MRI, giving clinicians and researchers unprecedented access to tissue properties *in vivo*. As it develops rapidly, MRE promises clinicians information akin to the data surgeons acquire through manual palpation - material properties such as stiffness - but noninvasively and quantitatively, with applications in diagnosis and monitoring of diseases. Connecting macroscopic mechanical response to microscopic cellular organization of tissue, MRE opens up new avenues for assessing the etiology of tissue pathology. The modality has been implemented to varying degrees of success on skeletal muscle [1–9], breast [10–17], liver [18–24], and brain [25, 26].

Elastography dates back to Gierke et al's 1952 paper, *Physics of Vibrations in Living Tissue* - one can discern tissue properties by observing a tissue's response to vibration [27]. MRE is multidisciplinary at its core, requiring expertise in magnetic resonance imaging, biophysical modelling/theoretical mechanics, and numerical simulations/machine learning. Several reviews are available [28–33].

The purpose of the current paper is to explore a poroelastic model of tissue, and convey novel results on the simultaneous imaging of both shear stiffness and hydraulic permeability in silico. These results extend prior studies on poroelastic parameter estimation [34–37], and quantify the domain of valid inference. We show that convergence to the correct in-silico property values is possible even when prior knowledge is off by several orders of magnitude. These results are a foundation for ongoing *in vitro* and *in vivo* work, giving reasonable bounds for noiseless data.

This paper is organized as follows: **Section 2** covers the theoretical details of our tissue model and how inference is accomplished. It also details some of the numerical considerations that have been implemented in our in-house MRE computational platform. **Section 3** details the setup of our simulations - inference of one and two properties from in-silico data generated by solving Newton's Laws for a poroelastic medium. Single and dual property contrast conditions necessary for fidelitous inference are examined. In **Section 4** we discuss the results from our simulations and conclude with planned future directions for *in vitro* and *in vivo* application.

1.1 Notational Conventions

Throughout, we use coordinate independent tensor notation to emphasize the physical underpinnings of expressions, but, when required to use coordinates, follow the *Einstein summation convention* where repeated indices in terms are summed over. Indices are drawn from the middle of the Latin alphabet, $i, j, k, \dots$, and run from 1 to 3. Scalar quantities are written in standard font with no typographical emphasis. Vectors and second rank tensors appear in lower case bolded font, the former in Latin and latter in Greek. Fourth rank tensors are bolded, and in capitalized Latin. When discussing either solid or fluid phases similar symbols are used to denote physical quantities, however the addition of a subscript s or f , respectively, is added to ensure physical clarity.

We denote the standard tensor product with a $\otimes$. An orthonormal vector basis is denoted $\{\hat{\mathbf{e}}_i\}_{i=1}^3$, with $\hat{\mathbf{e}}_i \cdot \hat{\mathbf{e}}_j = \delta_{ij}$ where the RHS is the Kronecker delta. At times we use the Cartesian representation of the basis, $\hat{\mathbf{e}}_1 = \hat{\mathbf{x}}, \hat{\mathbf{e}}_2 = \hat{\mathbf{y}}$, and $\hat{\mathbf{e}}_3 = \hat{\mathbf{z}}$. We construct the basis for second and fourth rank tensors from these, $\{\hat{\mathbf{e}}_{ij} = \hat{\mathbf{e}}_i \otimes \hat{\mathbf{e}}_j\}_{i,j=1}^3$ and $\{\hat{\mathbf{e}}_{ijkl} = \hat{\mathbf{e}}_i \otimes \hat{\mathbf{e}}_j \otimes \hat{\mathbf{e}}_k \otimes \hat{\mathbf{e}}_l\}_{i,j,k,l=1}^3$ respectively. The second rank identity tensor is denoted $\mathbb{1} = \hat{\mathbf{e}}_{ii}$. The dot product, $\cdot$, and double dot product, $:$, act on neighboring basis tensors via $\hat{\mathbf{e}}_i \cdot \hat{\mathbf{e}}_j = \delta_{ij}$ and $\hat{\mathbf{e}}_{ij} : \hat{\mathbf{e}}_{kl} = \delta_{ik} \delta_{jl}$, implying contraction on the component indices. For example, given an arbitrary vector, $\mathbf{a}$, second rank tensor, $\boldsymbol{\beta}$, and fourth rank tensor, $\mathbf{C}$, we can construct a vector as follows:

$$\begin{aligned} \mathbf{a} \cdot \mathbf{C} : \boldsymbol{\beta} &= (a_i \hat{\mathbf{e}}_i) \cdot (C_{jk\ell m} \hat{\mathbf{e}}_{jk\ell m}) : (\beta_{no} \hat{\mathbf{e}}_{no}) \\ &= a_i C_{jk\ell m} \beta_{no} \hat{\mathbf{e}}_i \cdot \hat{\mathbf{e}}_j \otimes \hat{\mathbf{e}}_k \otimes \hat{\mathbf{e}}_\ell \otimes \hat{\mathbf{e}}_m : \hat{\mathbf{e}}_n \otimes \hat{\mathbf{e}}_o \\ &= a_i C_{jk\ell m} \beta_{no} \delta_{ij} \hat{\mathbf{e}}_k \delta_{\ell n} \delta_{mo} \\ &= a_i C_{ik\ell m} \beta_{\ell m} \hat{\mathbf{e}}_k \end{aligned}$$

The index gymnastics in evaluating such expressions can get pretty messy, which is why we stick to coordinate independent notation as much as possible. The transpose acts on second rank basis tensors as $\hat{\mathbf{e}}_{ij}^T = \hat{\mathbf{e}}_{ji}$. The gradient operator is symbolized by a nabla, ∇ . Temporal Fourier transforms of quantities appear with the same typographical emphasis as above, having the added decor of a tilde. For temporal partial derivatives we employ fluxion notation, so that $\dot{f} \equiv \frac{\partial f}{\partial t}$, $\ddot{f} \equiv \frac{\partial^2 f}{\partial t^2}$, etc.

2 MODELLING TISSUE

Biological tissue is a complex medium, the result of almost four billion years of continual evolutionary pressure and the whims of chance. At the microscopic level, the variety of cellular structures in animals would require a vastly more complicated set of modelling assumptions than continuum mechanics can provide. However, tracing over these microscopic degrees of freedom, an effective theory of tissue should have more in common with a coupled solid-fluid description than one consisting of a single continuum. Such a model already exists, developed extensively during the last century by the geophysics community - poroelasticity.

2.1 Poroelasticity

Poroelasticity is an effective mechanical model describing the continuum as a coarse grained biphasic medium consisting of an *elastic matrix* coupled to a *fluid*. The former is not simply-connected, but has a connected pore space with a tortuous topology - it is this pore space that the fluid moves through. The coupled dynamics of solid and fluid result in a much richer phenomenology than single phase continuum models. **Figure 1** shows a schematic of a porous structure.

The theory of poroelasticity has its roots in the empirical work of Henry Philibert Gaspard Darcy, a water engineer tasked in 1856 with evaluating the many elaborate public fountains within the city of Dijon, France. Darcy, who had been working as a water engineer for nearly three decades, was pivoting towards pure research, and used this as an opportunity to write his 600 page magnum opus *Les Fontaines Publiques de la Ville de Dijon*, wherein he describes the sand column experiments pictured in **Figure 2** [38]. By examining the flow of water through sand in cylindrical pipes, Darcy discovered a linear proportionality between the rate of fluid volume leaving the bottom of a pipe, $\Delta V_f / \Delta t$, and the difference in piezometric head at the ends of the pipe, Δh ,

$$\frac{1}{A} \frac{\Delta V_f}{\Delta t} = K \frac{\Delta h}{L}, \quad (1)$$

where L is the length of the pipe, and A its cross-sectional area. *Hydraulic conductivity* is the name given to the constant of proportionality, K , and for water flowing through sand was measured to range from $2.07 - 3.22 \times 10^{-4}$ m/s [39]. This name is kept alive today by geologists, though we will see shortly that other fields' naming conventions have created an aura of ambiguity. Note that K depends on the properties of the fluid used in the piezometers, as well as the porous medium and the fluid flowing through said medium - since geology is primarily concerned with the filtration of water through soils, rocks, and clays, they think of it as intrinsic to the medium. For us, biologically relevant fluids can differ substantially in density and viscosity, so we mustn't fall into the same mindset. The

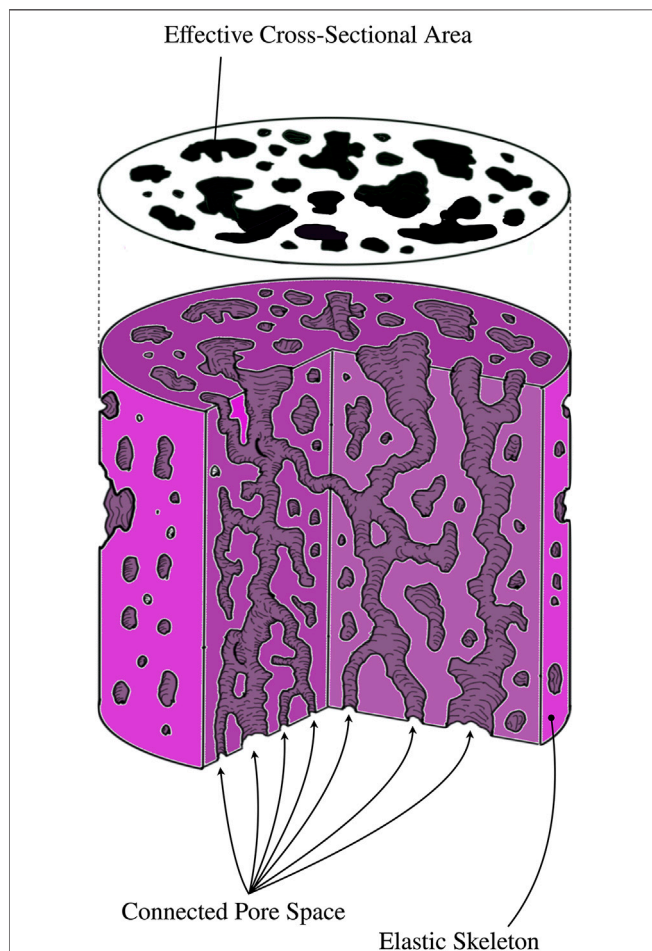


FIGURE 1 | The model of a porous material consists of an elastic matrix - the solid - and a connected pore space. Pores whose connected boundaries are homeomorphic (topologically identical) to spheres are not a part of the connected pore space, as nothing can flow through them. The connected pore space is filled with a fluid, whose interactions with the walls of the elastic skeleton provide a much richer phenomenology than elastic/viscoelastic models of materials. When a porous substance is intersected with a plane, the cross section, whose area is A , will have holes in it. The effective cross-sectional area, A_f , is the area of all of these holes. For an isotropic material, the orientation of the intersecting plane does not change the ratio A_f/A . The Delesse-Rosiwal law states that this ratio is equal to the ratio of the pore space volume to the total volume i.e. the porosity [40].

empirical formula **Eq. (1)** came to be known as *Darcy's Law*, a starting point for understanding how the flow of a fluid is affected by its coupling to a porous material.

A modern view of **Eq. (1)** utilizes the instantaneous rate of fluid volume moving through a cylinder at constant velocity, v_f , replacing $\Delta V_f/\Delta t \rightarrow v_f A_f$, where A_f is the effective area seen by

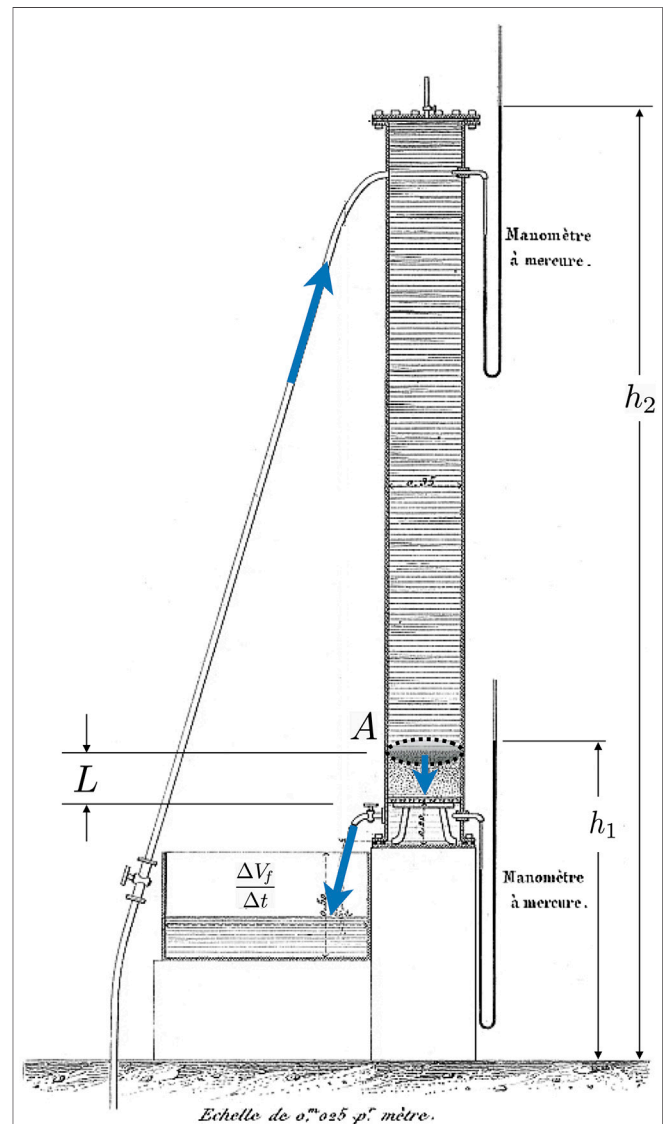


FIGURE 2 | The setup of one of the sand column experiments, from plate 24, **Figure 3** of Darcy's 1856 *Les Fontaines Publiques de la Ville de Dijon* [38]. Annotations have been added to clarify the origin of the physical quantities appearing in **Eq. (1)**. The piezometric heads h_1 and h_2 in the two piezometers on the right are measured using mercury. L is the length of the sand column, and A its cross-sectional area. In this setup the diameter of the column is 0.35 m, and the height of the column is 3.5 m. $\Delta V_f/\Delta t$ is the rate at which fluid volume is leaving the column, measured by observing the rise of the water level in the container. One can quickly see that this particular setup is problematic for applying **Eq. (1)** directly unless the sand column reaches up to near the top piezometer. If not, then the piezometric head must be replaced by the total head so as to includes the pressure head coming from the column of water.

the fluid due to the microscopic geometry of the porous medium. The effective area is related to the *porosity* (volume fraction of fluid, assuming saturation) by the Delesse-Rosiwal law (second equality) [40], $\phi = V_f/V = A_f/A$. Identifying the pressure differential between the ends of the pipe with the drop in head via $\Delta P = \rho_f g \Delta h$ (ρ_f is the fluid density and g the gravitational acceleration), shrinking the pipe to infinitesimal length, $L \rightarrow 0$, and restoring the tensorial nature of the physical quantities, we find

$$\mathbf{q} = -\frac{1}{\eta} \boldsymbol{\kappa} \cdot \nabla P \quad (2)$$

where two clarifications are in order. (1) The LHS is the *Darcy velocity* (or *filtration velocity*), $\mathbf{q} = \phi \mathbf{v}_f$, the magnitude of which is called the *specific discharge*. (2) The RHS is rewritten using the *hydraulic permeability tensor*, $\boldsymbol{\kappa}$ - a property belonging solely to the porous medium - and η - the dynamic viscosity of the fluid. The full justification of this separation is possible through a microscopic treatment using homogenization theory [41–44], extending the validity of Darcy's Law to pressure gradients not necessarily generated by gravitational forces.

For an isotropic medium, such as Darcy's sand column, $\boldsymbol{\kappa} = k\mathbb{I}$, and we call k the *hydraulic permeability*. Unfortunately, as mentioned earlier, there is a bit of ambiguity within the literature depending on the scientific field one is enamoured with - geologists measure piezometric head and refer to Darcy's original K [LT^{-1}] as hydraulic conductivity, while most other fields measure pressure gradients and refer to $\bar{K} = K/\rho_f g$ [L^3TM^{-1}] as such. Furthermore, the medical and bio-engineering literature has, on occasion, used the terms hydraulic conductivity and permeability interchangeably; it is best to always check units to make sure that you and the author are on the same page. Given the dynamic viscosity of water is $\sim 10^{-3}$ Pa s, this implies Darcy's original measurements of sand's hydraulic permeability on the order of $\sim 10^{-11}$ m². In honor of this, hydraulic permeability is now measured in units of Darcys, D, so that clean sand in aquifers comes out as $\sim 10^1$ D. The unit is defined so that 1D permits a filtration velocity of a cm/s for a fluid with dynamic viscosity 10^{-3} Pa s under a pressure gradient of 1 atm/cm, or in SI, $1\text{D} = 9.86923 \times 10^{-13}$ m². The strangeness of this value comes from there being 101,325 Pa in 1atm, rather than a round 10^5 , but who are we to quibble with history?

Karl von Terzaghi, a mechanical engineer, laid out the dynamics of structure consolidation on soil in a series of papers [45–47] between 1922 and 1925, extending Darcy's insights to become the *father of soil mechanics*. He incorporated the dynamics of soil in response to the fluid by introducing an effective stress tensor, $\boldsymbol{\sigma}$, to describe the bulk medium,

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}_s - P\mathbb{I}. \quad (3)$$

Here $\boldsymbol{\sigma}_s$ is the stress tensor of the solid, and P is the *pore pressure*. The effective stress describes the net stress on the solid skeleton, and its definition is referred to as *Terzaghi's Principle* - the total stress on the elastic matrix is lessened by the pore pressure. Terzaghi's analysis describes a static, highly

symmetric, and fully saturated system in which an incompressible fluid is confined to a one dimensional column of incompressible solid grains. Rearrangements of these grains drive deformation of this *soil* when a load is applied. The decomposition of the stress into an effective bulk and fluid is a bit like looking at the soil through poor lenses that smear the distinction between microscopic grains and pore fluid - a technique central to homogenization theory in engineering, and coarse graining in physics and chemistry [48–50]. One should be a tad careful in their mind's eye in all that follows - in going to this effective picture we now imagine solid and fluid components existing simultaneously at every spatial point, their dynamics coupled as a result of coarse graining the microscopic structure.

Though revolutionary in application, Terzaghi's assumptions were ill suited for generality. Thermodynamically speaking, his model contains the pair of dynamical variables $\{\boldsymbol{\sigma}, P\}$, the effective stress and the pore pressure, respectively; the elastic matrix strain,

$$\boldsymbol{\varepsilon} = \frac{1}{2} (\nabla \otimes \mathbf{u}_s + (\nabla \otimes \mathbf{u}_s)^T), \quad (4)$$

is conjugate to the former, where $\mathbf{u}_s$ is the displacement field of the solid matrix. A good guess for the kinematic variable corresponding to the conjugate of the pressure would seemingly be the fluid strain rate, which for a perfect fluid with velocity field $\mathbf{v}_f = \dot{\mathbf{u}}_f$ is the single degree of freedom $\text{tr}(\dot{\boldsymbol{\varepsilon}}_f) = \nabla \cdot \mathbf{v}_f$. Though this works to describe a fluid alone and results in the Navier-Stokes equations, this term cannot be coupled to the strain in a linear theory, nor to the solid displacement as that ruins translational invariance. The only option to preserve these necessary ingredients would be the fluid strain, $\nabla \cdot \mathbf{u}_f$. It turns out that this choice almost works; unfortunately it couples the solid and fluid a tad too weakly by predicting a vanishing pore pressure when the fluid moves in sync with the solid. With the obvious choices for a kinematic conjugate used up, it appears as if we must continue our search in darkness.

Maurice Antony Biot, an applied physicist, shed light on the correct coupling in 1941 by introducing [51] the *variation in relative fluid content*,

$$\zeta = -\nabla \cdot \phi(\mathbf{u}_f - \mathbf{u}_s) + \Gamma, \quad (5)$$

where $\gamma = \dot{\Gamma}$ is referred to as a *fluid source*. His treatment generalized the Darcy velocity to a relative motion of the fluid with respect to the solid, $\mathbf{q} = \phi(\mathbf{v}_f - \mathbf{v}_s)$, thus allowing for an analysis of situations in which both the fluid and porous medium are in motion. Note that the relative fluid content decreases when net flow out of a dilating volume of the solid matrix occurs, and increases if the flow is reversed - together with the filtration velocity this is summarized by a continuity equation

$$\dot{\zeta} + \nabla \cdot \mathbf{q} = \gamma. \quad (6)$$

Here, we see the fluid source, $\gamma = \dot{\Gamma}$, in action either creating fluid content, $\gamma > 0$, or annihilating it, $\gamma < 0$ - a rather useful quantity when considering tissue near veins and arteries.

In Terzaghi's analysis, bulk volume change is driven by rearrangements of the pore space and flow of the fluid. Biot realized that this physics may be true for nearly incompressible sand grains, but for a softer elastic medium one should also take into account the contribution of solid compressibility to the bulk volume change. An applied isotropic load can now change the volume of the bulk by altering the volume of the solid skeleton due to the fluid pushing back - for reasonably small volume changes the effective support of the fluid with regards to the bulk is then less than the fluid pressure. Biot argued that the correct effective stress should be a modification of Eq. (3),

$$\sigma = \sigma_s - \alpha P \mathbb{1} \quad (7)$$

with the *Biot effective stress coefficient*, α , a function of the drained effective bulk modulus and theunjacketed solid bulk modulus, K and K_s respectively [52],

$$\alpha = 1 - \frac{K}{K_s}. \quad (8)$$

To clarify, experimentally the unjacketed bulk modulus is measured in undrained conditions wherein a sample is submerged in fluid; the fluid pressure is then allowed to vary and the volume change of the sample is measured. A jacketed test, on the other hand, is done under drained conditions wherein a dry sample is wrapped in a membrane and connected to the atmosphere via a small hole; a load is then applied under constant atmospheric pressure, and the volume change measured. In aerated soil $K/K_s \ll 1$, validating Terzaghi's treatment [53, 54]. The theory predicts a second type of longitudinal wave within a porous medium, which was observed in 1979 [55]. Biot refined his model by incorporating anisotropy, analyzing dispersion in wave propagation, and examining non-linear extensions of the constitutive relations, earning him the title of *father of poroelasticity* [56–59].

Examining the effective potential can further illuminate the role of α - it is the dimensionless coupling between the volumetric strain of the skeleton and the variation in relative fluid content. Since it and ζ are both dimensionless, the *Biot modulus*, M , must be introduced to describe the energy density coming from the relative fluid content and its coupling to the elastic matrix, allowing the quadratic potential energy density for a linear poroelastic medium to be written as

$$U = \frac{1}{2} \boldsymbol{\varepsilon} : \mathbf{E} : \boldsymbol{\varepsilon} + \frac{1}{2} M \zeta^2 - \alpha M \zeta \text{tr}(\boldsymbol{\varepsilon}) \quad (9)$$

where $\mathbf{E} = \mathbf{E}_0 + \alpha^2 M \mathbb{1} \otimes \mathbb{1}$ is an effective elastic modulus, a fourth rank tensor describing the effective coupling between the components of the strain. For an isotropic Hookean material described by Eq. (20), note that the Biot effective stress coefficient and modulus serve to renormalize the second Lamé parameter, λ . The conjugate variables can now be computed from this, giving the *poroelastic constitutive relations*

$$\sigma = \frac{\partial U}{\partial \boldsymbol{\varepsilon}} = \mathbf{E} : \boldsymbol{\varepsilon} - \alpha M \zeta \mathbb{1} \quad (10)$$

$$P = \frac{\partial U}{\partial \zeta} = -\alpha M \text{tr}(\boldsymbol{\varepsilon}) + M \zeta \quad (11)$$

Combining Eq. (10) and Eq. (11) recovers the Biot decomposition, Eq. (7). These expressions clarify the interpretation of M - it is the change in pore pressure caused by a variation in water content under the constraint of no solid dilation, $M = \partial P / \partial \zeta|_{\text{tr}(\boldsymbol{\varepsilon})=0}$. It can be written in terms of the Biot effective stress coefficient, as well as the fluid and jacketed solid moduli, K_f and K_s respectively [44, 52],

$$\frac{1}{M} = \frac{1}{K} \left(\alpha(1 - \alpha) - \phi \frac{K}{K_s} + \phi \frac{K}{K_f} \right). \quad (12)$$

Biot, and others, explored the physical meaning of both α and M in a number of manuscripts [53, 60, 61]. The effective potential, Eq. (9), can also be constructed beginning at microscopic scales to justify the expression through homogenization theory, coarse graining, or mixed micro-macro formulations [41–43, 62–64].

We now use the effective stress and pressure to write the coupled equations of motion for the effective medium and relative fluid flow components in the absence of external stresses. The equations of motion can be derived via the principle of least action [42, 44, 62], however that would take us too far afield, so we introduce them qualitatively. Momentum conservation for the effective medium reads

$$(1 - \phi) \rho_s \ddot{\mathbf{u}}_s + \phi \rho_f \ddot{\mathbf{u}}_f = \nabla \cdot \boldsymbol{\sigma} + (1 - \phi) \rho_s \mathbf{g} + \phi \rho_f \mathbf{g}, \quad (13)$$

where the LHS is the total change in inertia of both solid and fluid. If we define the effective density as $\rho = (1 - \phi) \rho_s + \phi \rho_f$, the LHS can be written in a more illuminating form as $\rho \ddot{\mathbf{u}}_s + \rho_f \phi (\ddot{\mathbf{u}}_f - \ddot{\mathbf{u}}_s)$ - the first term is the effective inertia following the acceleration of the solid component, while the second term is the relative acceleration of the fluid with respect to the solid. The RHS is simple to interpret - the divergence of the bulk stress tensor describes the internal forces, while the external forces consist of a gravitational field.

Similarly, momentum conservation for the fluid reads

$$\phi \rho_f \ddot{\mathbf{u}}_f + \rho_a (\ddot{\mathbf{u}}_f - \ddot{\mathbf{u}}_s) = -\phi \nabla P - \phi^2 \frac{\eta}{k} (\dot{\mathbf{u}}_f - \dot{\mathbf{u}}_s) + \phi \rho_f \mathbf{g}. \quad (14)$$

The LHS here is the change in fluid inertia; however a mass density, ρ_a , is added for the fluid moving relative to the solid. This addition can be derived from a micro-macro approach, and captures the effect of the fluid being slowed down through interactions with the structure of the pores [43, 44, 65]. It is typically written as $\rho_a = (a - 1) \phi \rho_f$, with a for packed spheres being related to the porosity as $a = \frac{1+\phi}{2\phi}$ - for glass beads at low frequencies it has been measured as $a \approx 1.66 \pm 0.13$, or $\phi \approx 0.43 \pm 0.05$, which coincides nicely with the porosity of loosely packed spheres, 0.41 ± 0.04 [66, 67]. As in Eq. (13), the LHS of Eq. (14) is rewritten using the relative acceleration of the fluid to the solid as $\phi \rho_f \ddot{\mathbf{u}}_s + (\phi \rho_f + \rho_a) (\ddot{\mathbf{u}}_f - \ddot{\mathbf{u}}_s)$. The RHS has the pressure gradient driving the fluid acceleration, but also

includes a viscous dissipation term coming from the relative motion of the fluid with respect to the solid, serving to synchronize the former to the latter. The porosity factor for the former is due to the fact that the pore pressure is acting on the effective area seen by the fluid. The ϕ^2 factor on the latter can be broken down as follows - one factor comes from the dynamic viscosity, which is proportional to the fluid mass, while the second comes from the hydraulic permeability, which is proportional to the effective area seen by the fluid mass.

Note the inverse dependence on hydraulic permeability - the more easily the solid permits fluid flow the less dissipation occurs. Using the definition of the filtration velocity, **Eq. (14)**, can be written as a generalization of Darcy's Law, **Eq. (2)**,

$$\mathbf{q} = -\frac{k}{\eta} \left(\nabla P + \rho_f \ddot{\mathbf{u}}_s + \frac{\rho_a + \phi \rho_f}{\phi^2} \dot{\mathbf{q}} - \rho_f \mathbf{g} \right), \quad (15)$$

which now includes the inertial forces coming from the motion of the solid skeleton, as well as the fluid's interactions with the pore space. Accordingly, we drop the subscript on the solid displacement, $\mathbf{u} = \mathbf{u}_s$.

The system of **Eqs. (6, 13, 14)** together with the constitutive relations **Eqs. 10, 11** are a more suitable continuum model of tissues containing a mobile interstitial fluid component than standard viscoelastic continua. Since MRE subjects tissue to oscillatory driving conditions at constant frequency, the system can be simplified by Fourier transforming into frequency space - linearity assures us that distinct temporal modes evolve independently. The driving frequency, ω , and all of its harmonics, $\omega_n = n\omega \forall n \in \mathbb{Z}^+$, contribute to the spatial signal. For each of the d.o.f./hyperparameters $x \in (\mathbf{u}, \zeta, \mathbf{q}, \sigma, P; \mathbf{g}, \gamma)$, the Fourier decomposition and its inverse read

$$\begin{aligned} x(\mathbf{r}, t) &= \sum_{n \in \mathbb{Z}} \tilde{x}_n(\mathbf{r}) e^{-i\omega_n t} \\ \tilde{x}_n(\mathbf{r}) &= \frac{1}{T} \int_0^T dt x(\mathbf{r}, t) e^{i\omega_n t}. \end{aligned}$$

The decomposition acts formally by replacing $x \rightarrow \tilde{x}_n$ and $\partial_t \rightarrow -i\omega_n$. Decomposing each d.o.f. in the equations of motion, we find the mechanical response at each frequency ω_n is described by

$$i\omega_n \tilde{\zeta}_n = \nabla \cdot \tilde{\mathbf{q}}_n - \tilde{\gamma}_n \quad (16)$$

$$-\omega_n^2 \rho \tilde{\mathbf{u}}_n - i\omega_n \rho_f \tilde{\mathbf{q}}_n = \nabla \cdot \tilde{\boldsymbol{\sigma}}_n + \rho \tilde{\mathbf{g}}_n \quad (17)$$

$$-\omega_n^2 \rho_f \tilde{\mathbf{u}}_n - i\omega_n \rho_f \beta_n^{-1} \tilde{\mathbf{q}}_n = -\nabla \cdot \tilde{\mathbf{P}}_n + \rho_f \tilde{\mathbf{g}}_n \quad (18)$$

$$\text{where } \beta_n^{-1} = \frac{\rho_a + \phi \rho_f}{\rho_f \phi^2} + i \frac{\eta}{\omega_n \rho_f k}. \quad (19)$$

together with the transformed constitutive relations. Here, we have introduced the poroelastic β factor, a key ingredient in differentiating poroelastic and viscoelastic dynamics. In a static and constant gravitational field we of course have that $\tilde{\mathbf{g}}_n = -g\delta_{0n}\hat{\mathbf{z}}$.

We want to connect the MR images of tissue to this model, so we assume that the MRI phase contrast displacement measurements acquired by MRE trace the motion of the

elastic skeleton. Since fluid gives a strong MR signal, this assumption relies on there being significant amounts of fluid in the disconnected pore space, acting as part of the bulk. Since fluid pressure within tissue is critical for homeostasis, we should reduce our system of equations further by eliminating all variables except the pair $(\tilde{\mathbf{u}}, \tilde{P})$. For analytical simplicity, we choose an isotropic elastic stress tensor parameterized by the standard Lamé parameters μ (shear modulus) and λ ,

$$\mathbf{E}_0 = \mu \hat{\mathbf{e}}_{ij} \otimes \hat{\mathbf{e}}_{ij} + \mu \hat{\mathbf{e}}_{ij} \otimes \hat{\mathbf{e}}_{ji} + \lambda \mathbb{1} \otimes \mathbb{1} \quad (20)$$

After some lengthy algebra, one finds a vector and scalar family of equations for each harmonic,

$$\begin{aligned} -\omega_n^2 (\rho - \beta_n \rho_f) \tilde{\mathbf{u}}_n &= \nabla \cdot [\mu \nabla \otimes \tilde{\mathbf{u}}_n + \mu (\nabla \otimes \tilde{\mathbf{u}}_n)^T] \\ &\quad + \nabla (\lambda \nabla \cdot \tilde{\mathbf{u}}_n - \alpha \tilde{P}_n) + \beta_n \nabla \tilde{P}_n \\ &\quad + (\rho - \beta_n \rho_f) \tilde{\mathbf{g}}_n \end{aligned} \quad (21)$$

$$\begin{aligned} \alpha \nabla \cdot \tilde{\mathbf{u}}_n - \nabla \cdot [\beta_n \tilde{\mathbf{u}}_n] &= -\frac{1}{\rho_f \omega_n^2} \nabla \cdot [\beta_n \nabla \tilde{P}_n] - \frac{1}{M} \tilde{P}_n \\ &\quad + \frac{1}{\omega_n^2} \nabla \cdot [\beta_n \tilde{\mathbf{g}}_n] + \frac{i}{\omega_n} \tilde{\gamma}_n. \end{aligned} \quad (22)$$

Up to this point we have not imposed assumptions on the spatial distribution of the elastic moduli in the constitutive relations, or the hydraulic permeability. We do so now by assuming that the underlying solid and fluid are nearly incompressible relative to the bulk, namely that $K/K'_s \ll 1$, $K/K'_f \ll 1$, and $K/K_f \ll 1$ [68]. Plugging the first of these in **Eq. (8)** shows that in this regime $\alpha \rightarrow 1$, while using all three in **Eq. (12)** shows that $M^{-1} \rightarrow 0$. In this physical regime, the above reduce to [52]

$$\begin{aligned} -\omega_n^2 (\rho - \beta_n \rho_f) \tilde{\mathbf{u}}_n &= \nabla \cdot [\mu \nabla \otimes \tilde{\mathbf{u}}_n + \mu (\nabla \otimes \tilde{\mathbf{u}}_n)^T] + \nabla (\lambda \nabla \cdot \tilde{\mathbf{u}}_n) \\ &\quad - (1 - \beta_n) \nabla \tilde{P}_n + (\rho - \beta_n \rho_f) \tilde{\mathbf{g}}_n \end{aligned} \quad (23)$$

$$i\omega_n \rho_f \tilde{\gamma}_n = \nabla \cdot [(1 - \beta_n) \rho_f \omega_n^2 \tilde{\mathbf{u}}_n + \beta_n \nabla \tilde{P}_n - \beta_n \rho_f \tilde{\mathbf{g}}_n]. \quad (24)$$

The vector equation shares some resemblance to viscoelasticity - the main differences being the presence of the complex β_n and displacement driving pressure gradient. Both **Eq. (13)** and **Eq. (14)** can be rewritten using the filtration velocity, getting rid of all mentions of the fluid displacement. The scalar equation is a divergence condition, implying that the given combination of solid displacement and pressure gradient can only contribute a net curl to the system unless a fluid source is present. Solving this system with given boundary conditions is referred to as the *forward problem*.

2.1.1 Numerical Considerations I

Analytical solutions of the equations of motion exist for highly idealized geometries [41, 44, 62]; in the context of classical clinical MRE such idealizations do not exist, hence computational methods are required to find approximate numerical solutions [69]. We use a finite element forward solver, described in detail elsewhere [35, 37]. It exploits multiple meshes - displacement fields can be supported on either tetrahedral or hexahedral

element meshes, while material properties, such as the shear modulus or hydraulic permeability, are supported on separate hexahedral meshes [70]. Supporting each property on its own mesh provides versatility. It decouples the resolution of the unknown properties being inferred from the mesh resolution of the $(\mathbf{u}, P)$ solutions generated by the forward problem, which balances discretization error and computational load without impacting the spatial scale of parameter inference. As we move forward into work on phantoms and human subjects, this versatility will allow for mitigating the effects of noise through coarser property meshes.

2.2 Inference

In MRE we observe the displacement field of the tissue being examined with little or no knowledge of the underlying mechanical properties of the tissue. This *inverse problem* of using the observation to infer the properties, is ideal for the application of a powerful epistemological tool known as Bayesian inference.

The Bayesian interpretation of probability brings with it many tools, some of which have started to be used by the MRE community [71, 72]. The fundamental idea behind it is simple and intuitive - observation carries with it information, and information leads to a reduction in uncertainty [73–77]. Beliefs evolve in order to reduce uncertainty, thereby maximizing the fitness of a cognizant being within an environment that is sufficiently complex to be indistinguishable from one behaving pseudorandomly. Note that, in a Bayesian setting, belief/plausibility are synonymous with probability, and in what follows the terms will be used interchangeably [78, 79].

Applying these ideas to MRE, let us fix a particular mechanical model of tissue so that the forward problem is specified. The space of all possible material property fields is denoted $\mathcal{M} = \{\theta\}$. An epistemic agent has a preexisting, or *prior*, belief distribution over $\mathcal{M}$, namely $p(\theta)$ [80]. If they know absolutely nothing about what to expect then the prior is uniform over all possible property fields - a uniform distribution is maximally uncertain [81]. Of course, if they have information that may reduce uncertainty in some way, the prior could be peaked over a region of $\mathcal{M}$ that is deemed more likely to occur. The space of all possible observations of the tissue displacement field is denoted $\mathcal{D} = \{\mathbf{u}\}$. Finally, the *posterior* over $\mathcal{M}$ is the informed belief distribution that the epistemic agent has after observing the data, denoted $p(\theta|\mathbf{u})$. The information content of a particular belief is defined uniquely [73, 82] in order to satisfy a natural sub-additive constraint on dependent beliefs, $I[\theta] = -\log p(\theta)$. Note that strong beliefs entail little information content, while implausible beliefs the opposite. Observing a rare event endows one with more information than observing a common event. It is for this reason that sometimes information content is referred to as *surprise* - we will be

content in interpreting information content as a measure of the implausibility of a belief.

The posterior and prior are connected via bayes' theorem

$$\begin{aligned} p(\theta|\mathbf{u}) &= \frac{p(\mathbf{u}|\theta)}{p(\mathbf{u})} p(\theta) \\ &\equiv \mathcal{L}(\mathbf{u}, \theta) p(\theta) \end{aligned} \quad (25)$$

where the quantity multiplying the prior is the *likelihood* of the observation given a model. Note that observations that are more likely in the context of a set of material properties, θ , have $\mathcal{L}(\mathbf{u}, \theta) > 1$ and enhance the posterior over the prior, whereas unlikely observations, $\mathcal{L}(\mathbf{u}, \theta) < 1$, reduce the belief in that particular θ .

Specifying a poroelastic model with parameters $\theta = \{\mu(\mathbf{r}), k(\mathbf{r})\}$, along with the hyperparameters $\{\phi, \rho_s, \rho_f, \rho_a, \eta, \lambda\}$ and boundary conditions, one solves the equations of motion Eqs. (23, 24) for $\tilde{\mathbf{u}}_n(\mathbf{r})$. As will be explained shortly, we need only do this for the fundamental frequency, so we define the *model displacement* as $\mathbf{u}^M(\mathbf{r}, \theta) = \tilde{\mathbf{u}}_1(\mathbf{r})$. We have left the model as an argument here to remind us that the solution for the fundamental mode depends on the parameters that have been chosen.

MRE data acquisition images steady state vibration fields using phase sensitive MRI sequences [28, 83–87]. Displacements are measured at a number of times across the harmonic motion cycle, which are processed into a sequence of displacements at each voxel and the fundamental mode of the time series is computed to give $\mathbf{u}^D(\mathbf{r})e^{i\omega t}$ for each voxel [88, 89]. Vibrations are often in the range of 25 – 100 Hz and supplied using an external actuator, or alternatively, our intrinsic actuation MRE uses retrospective gating to measure cardiac induced motions at ~ 1 Hz without the need for actuation hardware [90].

With $\mathbf{u}^D$ and $\mathbf{u}^M$ in hand, we are much closer to understanding the likelihood. The latter are encoding the material property degrees of freedom, while the former provide constraints. Both are discretized so one must always make sure to interpolate to the same voxel domain, taking into account that there are enough constraints to fully fix the degrees of freedom. Once we have fixed the voxel domain, we can treat the scalar difference between the two as a random variable $\Delta u_i(\theta) = |\mathbf{u}^D(\mathbf{r}_i) - \mathbf{u}^M(\mathbf{r}_i, \theta)|$. The differences are independent across voxels, though this assumption can be relaxed when the noise structure of the data collection can be properly described. The Δu_i are at the voxel level, so each one is actually the mean of all the degrees of freedom at the subvoxel level, of which there are a great many. Invoking the Central Limit Theorem allows us to claim that the distribution of Δu_i is Gaussian for each voxel such that

$$\mathcal{L}(\mathbf{u}, \theta) \propto p(\mathbf{u}|\theta) = \prod_i p(\Delta u_i) \propto \exp\left(-\frac{1}{2} \sum_i \frac{\Delta u_i^2}{\sigma_i^2}\right), \quad (26)$$

with the σ_i quantifying the root mean square fluctuations in the subvoxel degrees of freedom of the i^{th} voxel. Since no estimate is available for these values, they are set to unity. Using Bayes' Rule, Eq. (25)

$$I[\theta|\tilde{\mathbf{u}}] = -\log\mathcal{L}(\tilde{\mathbf{u}}|\theta) + I[\theta] \\ \approx \frac{1}{2} \sum_i |\mathbf{u}^D(\mathbf{r}_i) - \mathbf{u}^M(\mathbf{r}_i, \theta)|^2 + I[\theta], \quad (27)$$

where the $\approx$ in the second line means equality modulo a constant independent of θ , leads to Eq. (27) as a *cost function*; the first term on the RHS is an *error function*, while the second term, is a *regularizer*. Finding the set θ that minimizes cost is equivalent to having found the most believable set of parameters - an epistemological interpretation grounded in the tools of information theory.

2.2.1 Numerical Considerations II

There are, of course, multiple ways to attack this particular question, but they all have in common the theme of sliding down the cost surface. If the cost surface is convex, such a slide will eventually get us to the most plausible set of parameters. The idea can be broken down as follows: Let's say that we are at some arbitrary model $\theta \in \mathcal{M}$. Our goal will be to move to a new model $\theta + \delta\theta$ so that the information content at this new model is: $I[\theta + \delta\theta|\mathbf{u}] \leq I[\theta|\mathbf{u}]$. We are content to slide either down or horizontally, but never back up the error surface. By an appropriate sequence of choices for the $\delta\theta$'s one has an iterative procedure for finding the minimum. Different methods rely on expanding the above information content inequality to different orders in $\delta\theta$, and then making appropriate approximations with the resulting gradients.

Our MRE platform has several of these methods implemented - descent direction can be computed via Gauss-Newton, Conjugate Gradient, or Quasi-Newton updates [37]. Travel distance is modulated by a binary line search once the descent direction is found. Computation of gradients depends not only on the log likelihood, but also on prior information content - the regularizer. Multiple regularization terms have been implemented to take into account prior knowledge on material property limits, their spatial distribution, and smoothness [35, 91, 92].

To manage the computational resources required for multiple solutions of the 3D finite element forward problem required for inference, an efficient zoning strategy has been developed [93, 94] that breaks up the domain of interest into smaller overlapping zones. Measured displacements on the boundary voxels of zones provide Dirichlet boundary conditions, leading to a similar inference problem on a smaller scale. Computational complexity in our MRE-suite for the finite element solution of Eqs. (23) and (24) on a cubic zone of side length L scales as $\mathcal{O}(L^{4.6})$, which is less than the $\mathcal{O}(L^6)$ complexity of LU factorization due to the sparsity resulting from using local operators [95]. Each zone is treated in parallel, computing as many gradient descent

iterations as desired. Property solutions from each zone are collected and stitched together to complete a global iteration [70]. Each global iteration begins with a reseeding of the zone structure to limit propagation of bias or boundary noise inherent in a particular zone throughout the inference.

Convergence of any combination of the above methods is tricky to gauge due to the immense dimensionality of $\mathcal{M}$. Furthermore, the use of zoning means each global iteration is performing inference on a slightly different cost surface. To understand convergence we have therefore opted towards a coarse grained approach, projecting the inferred d.o.f. to a lower dimensional subspace consisting of the mean value(s) of the inferred property descriptors. Inference is judged by cost function descent on this subspace. A geometric interpretation of the method is given in Figure 3.

3 SIMULATION

We use our MRE computational platform - a finite element code capable of simulating displacement fields in poroelastic materials, and an artificial epistemic agent running Bayesian inference on

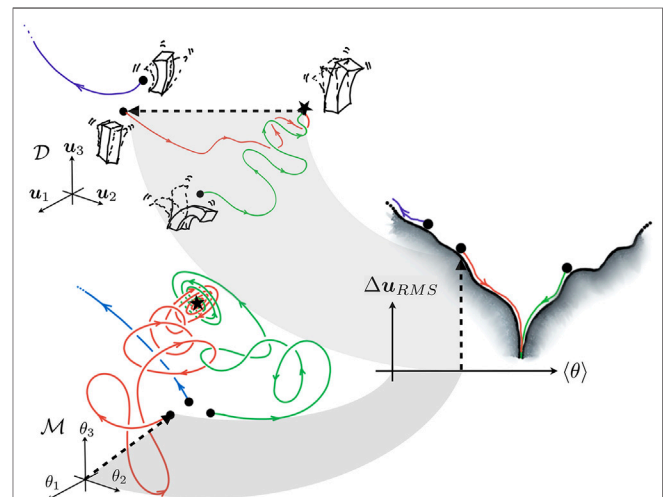


FIGURE 3 | A geometric view of the cost surface's lower dimensional representation. The trajectories in $\mathcal{D}$ and $\mathcal{M}$ (red, green, and blue) are meant to represent how difficult it is to visualize the paths in these high dimensional spaces. In MRE, the dimension of both is proportional to the number of voxels. To visualize the process of inference, we map our trajectories to a lower dimensional space made of the negative log posterior (root mean square of the displacement errors), and the mean property value. The RMS is proportional to the L_2 distance between the data displacements, $\mathbf{u}^D$, and model displacements, $\mathbf{u}^M$. The mean property value is proportional to the L_1 distance from the "no property" origin to the model point, θ . During single property inference the resulting lower dimensional representation is 1-dimensional, while for two property inference it is 2-dimensional. Note how the red and green trajectories eventually leads to the minimal RMS. The blue trajectory begins at a high enough error (near vanishing posterior), that our epistemic agent is incapable of finding the correct direction to move in $\mathcal{M}$. The dimensionality of $\mathcal{M}$ is so large that nearly every direction leads to an increase in RMS displacement error, and the lower dimensional representation interprets this as rolling up the cost surface, leading to divergence.

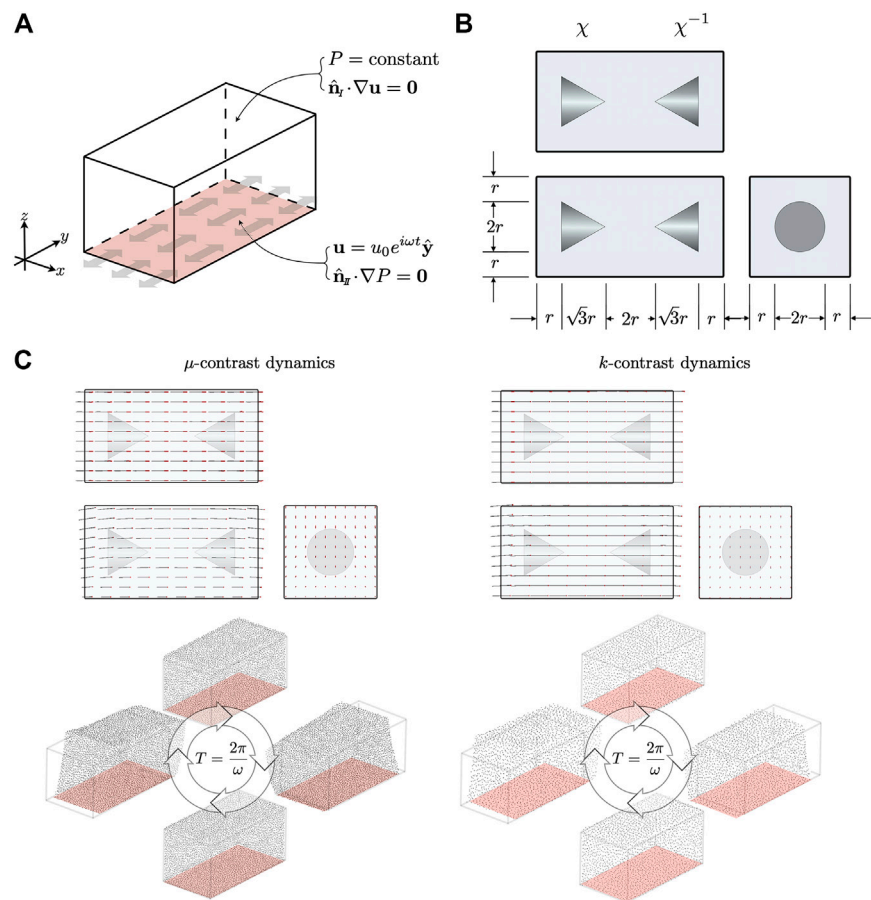


FIGURE 4 | (A) Boundary conditions on the top/sides(l) and bottom(ll) surfaces for the 10 Hz simulation. $\hat{n}$ are outward pointing normals. Pressure has Dirichlet BCs on l and Neumann BCs on ll . Displacement has the opposite. The bottom plate oscillates in the y direction at frequency ω . **(B)** Orthographic projection showing geometry of the conical inclusions. The left inclusion has positive property contrast, and the right one has a negative property contrast. **(C)** The dynamics of both scenarios - the left figure has inclusions with shear modulus contrast, while the right figure has inclusions with hydraulic permeability contrast. The MREf displacement field over one period is shown for both, the red dots representing in-phase displacements at the start of a cycle. Below these, the motion of the boundary nodes is displayed at four equally spaced times within the cycle of period T .

measured displacement fields to extract poroelastic properties. The former situation is referred to as the *forward problem*, MREf, while the latter the *inverse problem*, MREi.

The following tests examine MREi's ability to reconstruct spatial maps of both the hydraulic permeability, k , and the real shear modulus, μ . First we examined our code's baseline ability to infer distributions of a single property at a time. Next we considered the more complex requirement of inferring both material property distributions simultaneously. Simulated data were generated via MREf, followed by an exploration of the parameter space to see how initial conditions affect material property inference and find a range of starting conditions which converge to the known ground truth.

3.1 Single Property Inference

3.1.1 Forward Problem

Reconstructing the shear modulus or the hydraulic permeability one at a time was tested through configurations depicted in **Figure 4**(10 Hz) and **Figure 7**(1 Hz) - a long rectangular

block containing two conical inclusions. For both frequencies we tested two distinct scenarios. In the first scenario, inclusions were assigned shear modulus contrast. Relative to the background shear modulus μ_0 , the inclusion on the right has a shear modulus of $\mu = \chi\mu_0$, where $\chi > 1$, whereas the one on the left has a shear modulus of $\mu = \chi^{-1}\mu_0$. The shear modulus *contrast* of the right inclusion is positive $(\mu - \mu_0)/\mu_0 = \chi - 1 > 0$, while the one on the left is negative, $(\mu - \mu_0)/\mu_0 = -(\chi - 1)/\chi < 0$. In the second scenario the inclusions were given hydraulic permeability contrast. We ran simulations with χ ranging from 1.01 to 10, with similar results, and here, we report on the case $\chi = 2.0$, in which the inclusions have material property contrasts $-1/2$ and 1 - in the range reported for *in vivo* soft tissue MRE.

Referencing **Figure 4** and **Figure 7**, we chose $r = 4.25$ mm, making the block dimensions $1.7\text{cm} \times 3.2\text{cm} \times 1.7\text{cm}$. The displacements were supported on a tetrahedral mesh with 10,081 vertices and 55,593 elements. Although this is smaller than typical organs imaged with MRE in practice, results were similar to larger values of r , and the computational cost of running

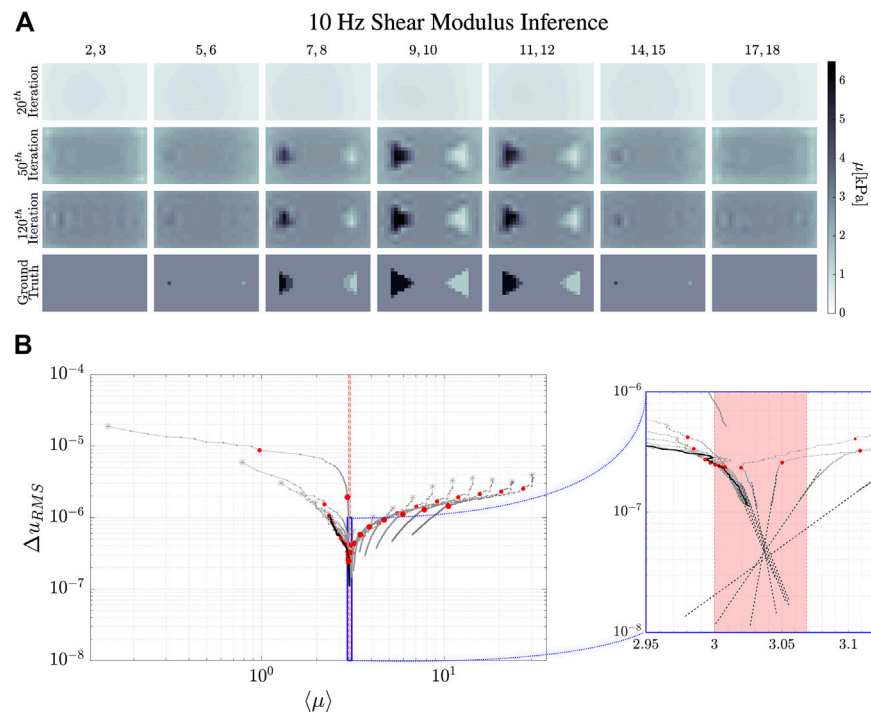


FIGURE 5 | Reconstruction evolution of the shear modulus in the 10 Hz simulation. **(A)** shows the reconstructed images of the shear modulus distribution inside the simulated phantom. The column images are averages of the numbered (xy)-slices (top row), each of which is 2 mm thick. The rows are labeled by the iteration number of the inference. **(B)** shows the lower dimensional trajectory of the shear modulus. As above, the shear modulus is measured in kilo-Pascals. The smaller diagram is a magnified region near the converged values. Initial estimates are noted by an asterisk and changes in descent strategy are indicated by small and large red markers, as explained in the text. The light red region represents the strip between the background value of the shear modulus and the ground truth mean value. The thicker trajectory is the one used to make the images in the above panel. Due to a rather long convergence time, best fit linear extrapolations of the trajectories are plotted to show that the convergence region is (nearly) the same for all converging runs.

a large number of simulations favored smaller geometries for detailed numerical investigations. Material properties were supported on a 0.5 mm cubic lattice mesh with 71,672 vertices and 66,429 elements. The conical inclusions each have a relative volume of $V_c/V = \pi(2\sqrt{3} - 3)/32 \approx 4.56\%$. The displacement mesh intersects the inclusions at 453 and 461 vertices for the left and right cones, respectively.

We report results the results for both frequencies, $f = \omega/2\pi = 1$ Hz and 10 Hz. For the shear modulus frequencies between 1 – 100 Hz were tested with similar results. For hydraulic permeability we evaluated a smaller range of frequencies, finding that the reconstruction worsened at frequencies above 10 Hz. At 10 Hz, see **Figure 4**, fixed displacement boundary conditions were imposed on the bottom face of the block to generate oscillations parallel to its long axis, $\mathbf{u} = u_0 e^{i\omega t} \hat{\mathbf{y}}$. At 1 Hz, see **Figure 7**, oscillations perpendicular to the bottom face were imposed, $\mathbf{u} = u_0 e^{i\omega t} \hat{\mathbf{z}}$. The displacement amplitude in both cases was chosen to be 0.5mm, justifying a linear treatment, $u_0/L \approx 0.03 \ll 1$.

Material property values used in MRE f are summarized in **Table 1**. Bulk density was chosen to be similar to brain tissue, while fluid density and dynamic viscosity were selected to fall in the range of cerebrospinal fluid [96–100]. The apparent density is

a coarse grained effective quantity coming from the interactions between the fluid and the pore space, and we chose a value based on prior studies, noting that a low sensitivity of results to ρ_a was demonstrated [34–36, 68]. Lamé constants have been well studied in MRE, our choices reflecting reasonable values [92]. Porosity was chosen based on mean values for white and grey matter using DTI, as was the background hydraulic permeability [101]. It should be noted that these last two properties are not well

TABLE 1 | The first six material properties were used in both MRE f and MRE i . The last two are background values used only in MRE f . Inclusions have multipliers relative to background values as discussed in the text. In MRE i , the last two properties, shear modulus and hydraulic permeability, are inferred not specified.

Material Property	Symbol	Value	Units	(Generalized)
Bulk density	ρ	1.0×10^3	kg/m ³	ML^{-3}
Fluid density	ρ_f	1.0×10^3	kg/m ³	ML^{-3}
Apparent density	ρ_a	1.5×10^2	kg/m ³	ML^{-3}
Dynamic viscosity	η	1.0×10^{-3}	Pa·s	$ML^{-1}T^{-1}$
Porosity	ϕ	4.0×10^{-1}	-	-
Lamé parameter	λ	1.0×10^4	Pa	$ML^{-1}T^{-2}$
Shear modulus	μ_0	3.0×10^3	Pa	$ML^{-1}T^{-2}$
Hydraulic permeability	k_0	1.01×10^2	D	L^2

measured, with the literature quoting values in a wide range [102–105].

3.1.2 Inverse Problem

Inference uses a coarser material property mesh in order to ensure enough constraints to solve the equations of motion. A side effect of the forward and inverse meshes not being identical is that there is no zero error solution. Here we have a 2.0 mm cubic lattice mesh, with 1700 vertices and 1296 volume elements.

We explore the question of how close to the true mean our initial values have to be in order to ensure convergence by examining ranges spanning ~ 2 orders of magnitude above and below the true mean. The background shear modulus is set to $\mu_0 = 3.0$ kPa, and the background hydraulic permeability is $k_0 = 101.3$ D. Thus, the ground truth mean values for $\chi = 2$ are:

$$\langle \mu \rangle_{GT} = \left(1 + \frac{V_c}{V} \frac{(\chi - 1)^2}{\chi} \right) \mu_0 \approx 3068 \text{ Pa}$$

$$\langle k \rangle_{GT} = \left(1 + \frac{V_c}{V} \frac{(\chi - 1)^2}{\chi} \right) k_0 \approx 103.6 \text{ D}$$

These expressions are, of course, only approximate for a discrete mesh - deviations on the order of 1% were observed for our constructed meshes. Since these values are so close to the background fields due to the tiny volume occupied by the inclusions, we considered an inversion successful if the measured mean property fell near the region between the background and mean property values.

We initialize inference with a homogeneous material property distribution of either $\langle \mu \rangle$ or $\langle k \rangle$. Twenty (20) values of shear modulus were chosen ranging an order of magnitude above and below the true mean, 150 – 30,000 Pa. Twenty (20) values of hydraulic permeability were chosen ranging nearly two orders of magnitude above and below the true mean, $10^0 - 10^4$ D.

The inversion used zones with 10% overlap. Zones were large enough that approximately 500 non-boundary nodes existed in each zone, with about 60 nodes in each zone overlapping with other zones. Gaussian smoothing regularization (spatial filtering) was applied, with the smoothing radius shrinking from 5 to 0.5 mm over the first 50 iterations and held constant for the remaining iterations. A conjugate gradient descent method was used for iterative inference on each zone. During the first 20 global iterations, each zone finds a single CG direction, and the distance moved is computed through a single iteration of a secant linesearch. The next 30 iterations use two of each, and the remaining use three.

Results of inference are summarized for the 10 Hz data in **Figure 5** for μ and **Figure 6** for k . For the 1 Hz data, the shear modulus results are shown in **Figure 8** and the hydraulic permeability in **Figure 9**.

3.2 Double Property Inference

3.2.1 Forward Problem

MREf simulation of combined shear modulus and hydraulic permeability contrast involved the configuration depicted in **Figure 10** - a square block containing eight inclusions. The

inclusions are spherically symmetric, and have material property multipliers of either 1, χ , or χ^{-1} , resulting in either null, positive, or negative material property contrasts, respectively. Corner inclusions had both properties contrasted, while the center inclusions have contrast in one. This setup tests all possible pairs of positive, negative, or null contrasts over all the inclusions. Multiple values of χ were tested ranging from 1.1 – 3.0, with similar results - we report the case $\chi = 2.0$, corresponding to inclusions with property contrasts of either 1 or $-1/2$.

Referring to **Figure 10**, We chose $r = 3.5$ mm, making the block dimensions $3.5 \text{ cm} \times 3.5 \text{ cm} \times 1.4 \text{ cm}$. MRPEf used a 1 mm tetrahedral displacement mesh (23,275 vertices and 132,084 volume elements) and a 1.0 mm cubic lattice material property mesh (23,104 vertices and 20,535 volume elements). Each inclusion took up $\pi/300 \approx 1\%$ of the total volume, containing ~ 240 vertices and ~ 215 elements - deviations from inclusion smoothness occur at ~ 0.35 steradians.

Boundary conditions were imposed to simulate the top face of the block being held fixed, while the bottom face was vibrated in the vertical direction, $\mathbf{u} = u_0 e^{i\omega t} \hat{z}$. The amplitude was chosen to be $u_0 = 0.5$ mm, ensuring that the size of vibrations relative to block geometry is small, $u_0/H = 1/28 \approx 0.04 \ll 1$, so that a linear treatment is justified. A frequency of $f = \omega/2\pi = 1$ Hz was applied. As in the case of single property inference, material properties were chosen to reflect known values associated with brain tissue. Our choices are summarized in **Table 1**.

3.2.2 Inverse Problem

MREi deployed a coarser material property mesh than MREf - a 2.5 mm cubic mesh with 1792 vertices and 1350 elements. Each inclusion sampled 12–13 material mesh nodes.

We examined the effects of initial conditions on reconstruction fidelity by applying 20 different homogeneous initial conditions around the mean values, with the shear modulus ranging between $9.13 \times 10^2 - 5.15 \times 10^3$ Pa, and hydraulic permeability ranging between $8.9 \times 10^{-2} - 2.7 \times 10^5$ D. Background shear modulus and hydraulic permeability were $\mu_0 = 3$ kPa and $k_0 = 101.2$ D. Ground truth mean values were

$$\langle \mu \rangle_{GT} = \left(1 + \frac{\pi}{100} \frac{(\chi - 1)^2}{\chi} \right) \mu_0 \approx 3,047 \text{ Pa}$$

$$\langle k \rangle_{GT} = \left(1 + \frac{\pi}{100} \frac{(\chi - 1)^2}{\chi} \right) k_0 \approx 102.9 \text{ D}$$

Once again, these were approximate values on the discrete mesh, but fell within $\sim 2\%$ of the observed values. Inference was considered successful if both mean property values fell within or near the region $[k_0, \langle k \rangle_{GT}] \times [\mu_0, \langle \mu \rangle_{GT}]$.

The first 10 global iterations used a single CG direction and a single secant line search for computing total distance traversed in parameter space while the next 30 applied two of each, and the final 250 invoked three of each. The first 50 iterations used Gaussian smoothing regularization (spatial filtering) on both properties, with the Gaussian standard deviation shrinking

linearly from 5 to 0.5 mm. If the change in the cost fell below 10^{-12} , inference was considered complete, and further iterations ceased.

Evolution of image reconstruction is summarized in **Figure 11** and the lower dimensional representation of the inference trajectory is in **Figure 12**.

4 DISCUSSION AND CONCLUSION

The dynamical evolution of our MREi code for single property inference and image reconstruction is displayed in **Figure 6** and **Figure 7** for $f = 10$ Hz data. The top panels of both display reconstructed images at four distinct iterations. By the 20th iteration, both hydraulic permeability and shear modulus are not very distinct, though the former is clearly partitioning the domain into positive and negative contrast regions. By the 50th iteration, both material properties show distinct localization that is similar to the actual inclusions. At this stage, spatial filtering has reached sub-voxel width; hence, fuzziness of the hydraulic permeability image reconstruction relative to the sharper shear modulus image reconstruction is solely due to the geometry of the information content surface.

The $f = 1.0$ Hz reconstructions are summarized in **Figure 8** and **Figure 9**. Hydraulic permeability starts to settle to the correct values by the 50th iteration, though nearly 100 more iterations are needed for the correct shape of the inclusions to appear in part. This is due to the coarseness of the MREi mesh. The $\langle k \rangle$ value enters the critical strip typically within the first 20 iterations. We do note that when the initial values of the hydraulic permeability fall two orders of magnitude below ground truth values, MREi begins to diverge. Similar difficulties were not found if initial values overestimated the true hydraulic permeability. The shear modulus did not show this behavior, with image reconstruction recovering inclusion geometry and relative distribution very well by the 50th iteration, although different initial conditions are seen to lead to different final absolute values - a peculiarity noted in prior work [106]. The lower panels of **Figures 5, 6, 8, 9** show the inference trajectories, with initial estimates noted by an asterisk and changes in descent strategy indicated by red markers and changes in descent strategy indicated by red markers.

Projecting onto the mean field error surface, hydraulic permeability approaches the correct mean field much earlier than shear modulus. Initial values do not affect the late stage reconstruction of the former when they are ~ 2 order of magnitude too small or up to ~ 3 orders of magnitude too high. The CG descent method does appear to have pathological behavior when initial values are more than ~ 2

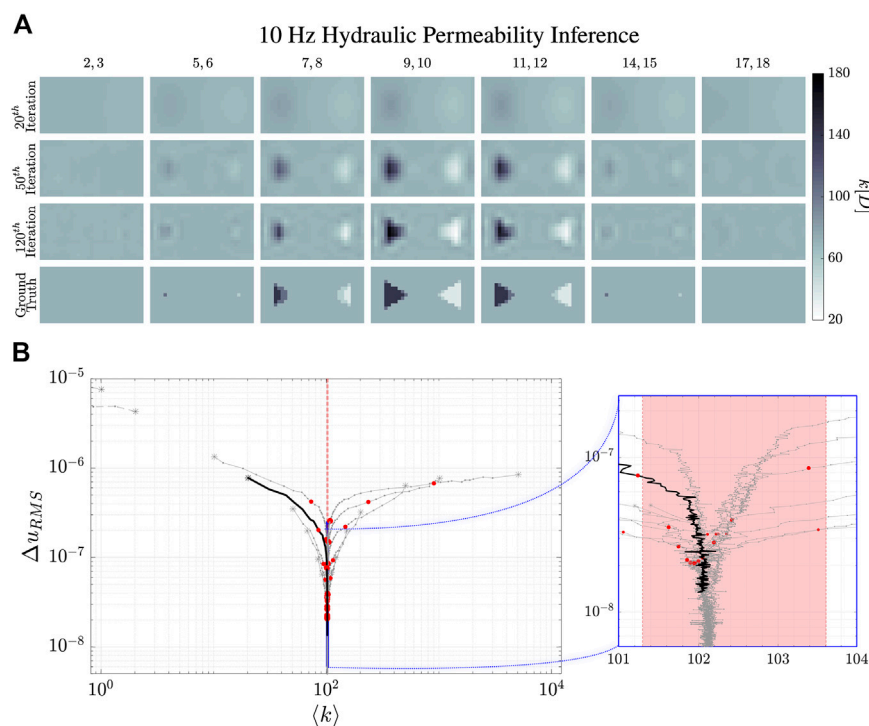


FIGURE 6 | Reconstruction evolution of the hydraulic permeability, k , at 10 Hz. **(A)** shows the reconstructed images of the hydraulic permeability distribution. The column images are the averages of the numbered (xy)-planes (top row). The rows are labeled by the iteration number of the inference. **(B)** shows the lower dimensional trajectory of the shear modulus. As above, the hydraulic permeability is measured in Darcy. The smaller diagram is a magnified region near the converged values. Initial estimates are noted by an asterisk and changes in descent strategy are indicated by small and large red markers, as explained in the text. The light red region represents the strip between the background value of the shear modulus and the ground truth mean value. The thicker trajectory is the one used to make the images in the above panel. One can see that the convergence of the permeability occurs well before the final change in descent strategy. It appears that proper inference is robust to initial conditions 2 orders of magnitude greater than the ground truth, while only 1 order of magnitude below.

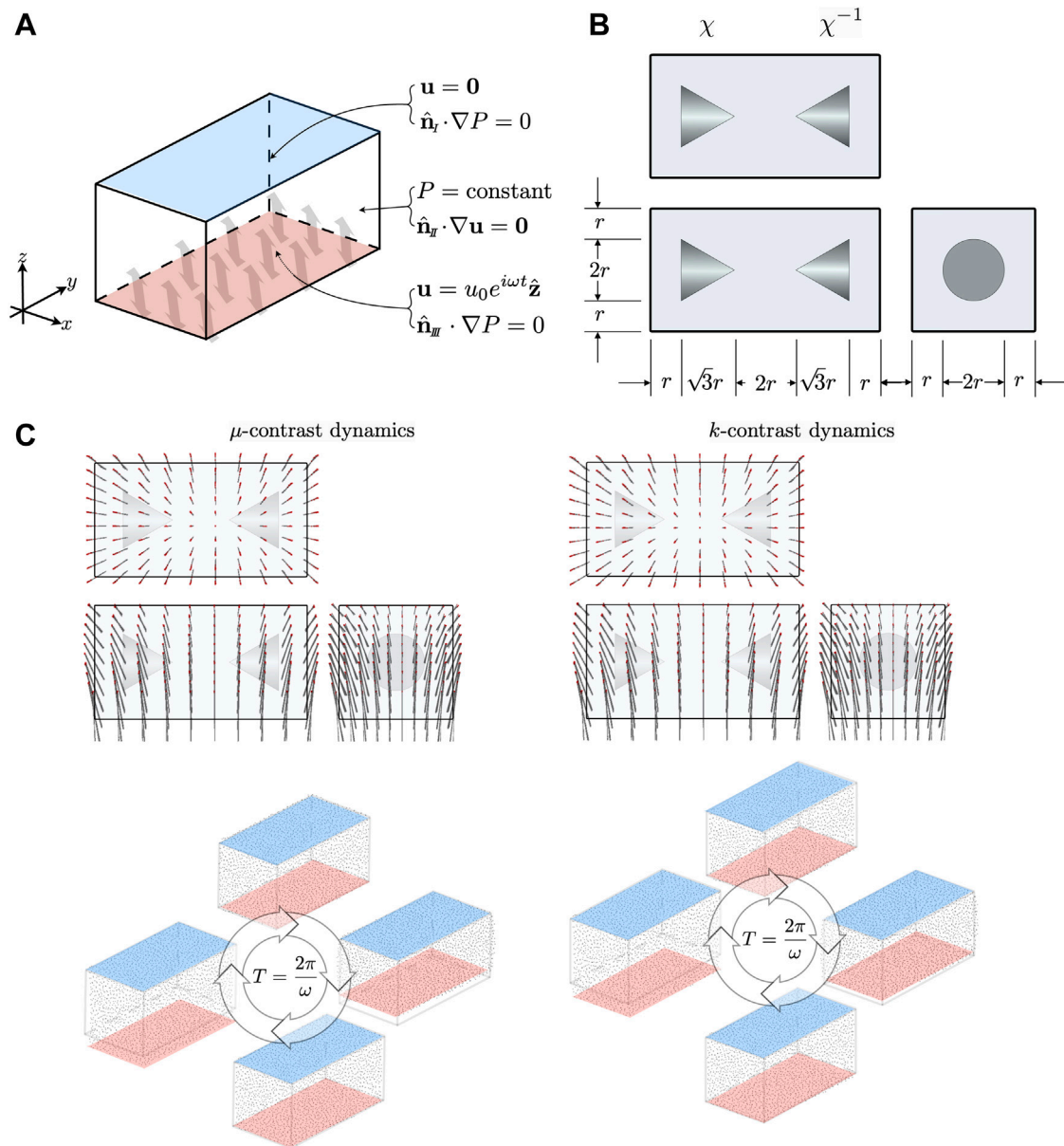


FIGURE 7 | (A) Boundary conditions on the top (I), side (II), and bottom (III) surfaces for the 1 Hz simulation. $\hat{n}$ are outward pointing normals. Pressure has Neumann BCs on I & III , and Dirichlet BCs on II . Displacement has the opposite. Top plate is held fixed, while the bottom oscillates in the z direction at frequency ω . **(B)** Orthographic projection showing geometry of the conical inclusions. The left inclusion has positive property contrast, and the right one has a negative property contrast. **(C)** The dynamics of both scenarios - the left figure has inclusions with shear modulus contrast, while the right figure has inclusions with hydraulic permeability contrast. MRE displacement field over one period is shown for both, the red dots representing in-phase displacements at the start of a cycle. Below these, the motion of the boundary nodes is displayed at four equally spaced times within the cycle of period T .

order of magnitude too small, resulting in an ascent of the error surface. A possible reason for this outcome is that low values of hydraulic permeability induce incompressible elastic behavior known to have stability issues in viscoelastic simulations [37]. The geometry of shear modulus reconstruction occurs with high fidelity within very few iterations, but the values converge much slower to the ground truth, an observation reported in prior work [106]. Projected trajectories of the error evolution in both

Figure 5 and **Figure 8**, however, converge at or near the critical strip for all initial conditions.

The results of the simultaneous property convergence appear in **Figure 11** and **Figure 12**. The former shows the fidelity of image reconstruction, while the latter inference trajectories as material properties are updated at each global iteration. Interestingly, the hydraulic permeability enters its critical strip well before the shear modulus. Mean value

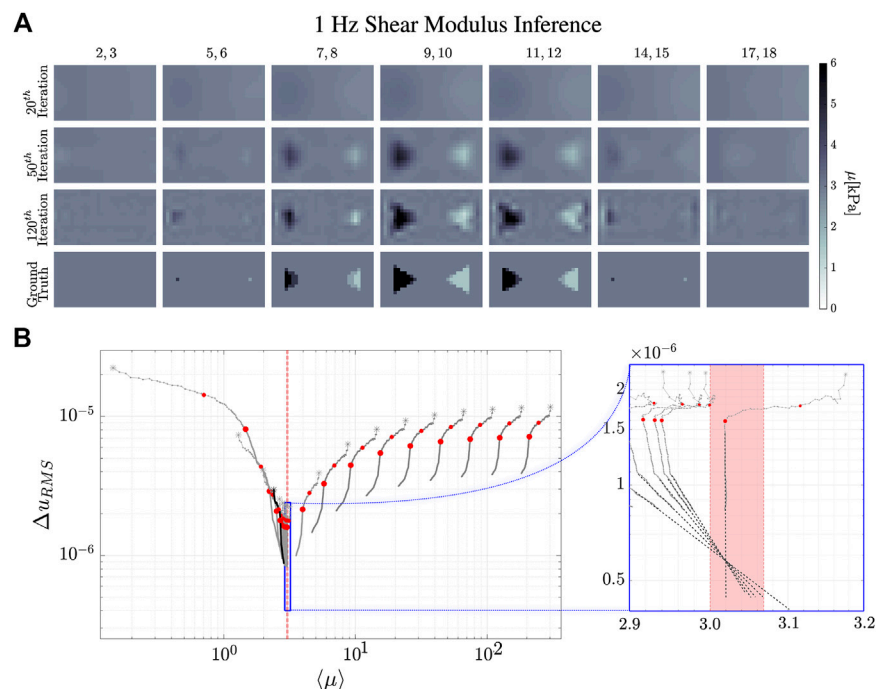


FIGURE 8 | Reconstruction evolution of the shear modulus, μ , at 1 Hz. **(A)** shows the reconstructed images of the shear modulus distribution inside the simulated phantom. The column images are the averages of the numbered (xy) -planes (top row). The rows are labeled by the iteration number of the inference. **(B)** shows the lower dimensional trajectory of the shear modulus. As above, the shear modulus is measured in kilo-Pascals. The smaller diagram is a magnified region near the converged values. Initial estimates are noted by an asterisk and changes in descent strategy are indicated by small and large red markers, as explained in the text. The light red region represents the strip between the background value of the shear modulus and the ground truth mean value. The thicker trajectory is the one used to make the images in the above panel. Once again we see that convergence of shear modulus is much slower than hydraulic permeability, yet images for a wide range of these simulated phantoms all show the correct structure of inclusions. Best fit lines are added to the zoomed in image to show that all the trajectories are approaching the same value.

convergence, however, is not a strong indicator of image fidelity. The shear modulus inclusion geometry is discernible by the 75th iteration, whereas nearly twice as many iterations are required before hydraulic permeability inclusion geometry begins to resemble ground truth. Furthermore, dependency between the two properties can be observed near the edges of the simulation - hydraulic permeability reconstruction has some vertical patterning that resembles the shear inclusions.

Interestingly, we find that the k -reconstruction trajectories always approach the critical strip from above. Inference tends to overestimate hydraulic permeability with several iterations, and then drives it down. As in the case of single property inference, k initial conditions have a lower limit of about ~ 1 order of magnitude below the ground truth in order for MRE_i to converge. Shear modulus is underestimated in almost all cases within about 50 iterations, and is then slowly driven upwards to the critical strip, not unlike the inference dynamics for the single property (μ) case.

Prior work [36, 107] has performed similar dual property reconstructions. These results, however, had MRE_i initial conditions very close to ground truth values, utilized the same property mesh for MRE_i as used for MRE_f, and required large amount of regularized smoothing. Our work shows that the first

two assumptions can be discarded, and the third relaxed significantly, while maintaining high fidelity for image reconstruction.

There seems to be some confusion in the literature between the terms hydraulic conductivity and hydraulic permeability, with many authors using the two interchangeably. We focused early in **Section 2** on making a clear distinction between the two in order to emphasize the specific contributions to fluid flow coming from the viscosity of the fluid and the geometry of the solid. Cerebrospinal fluid has a viscosity ranging between 0.7 – 1.0 mPa · s [99], blood between 3.0 – 67.7 mPa · s [108], ascitic fluid between 0.5 – 1.5 mPa · s [109] - the range covers two orders of magnitude so tissues with similar hydraulic permeabilities can have vastly different hydraulic conductivities. Hydraulic permeability measurements correlated with pathology can therefore be connected to changes in the *elastic matrix* of the tissue (via its effective continuum).

Our motivation for simulated values of porosity and hydraulic permeability rests on a general lack of knowledge by the scientific community of *in vivo* values of these parameters. *In vitro* experiments tend to find small values for both, which we hypothesize is due to pore collapse in sampled tissue. It is easy to imagine that once tissue is

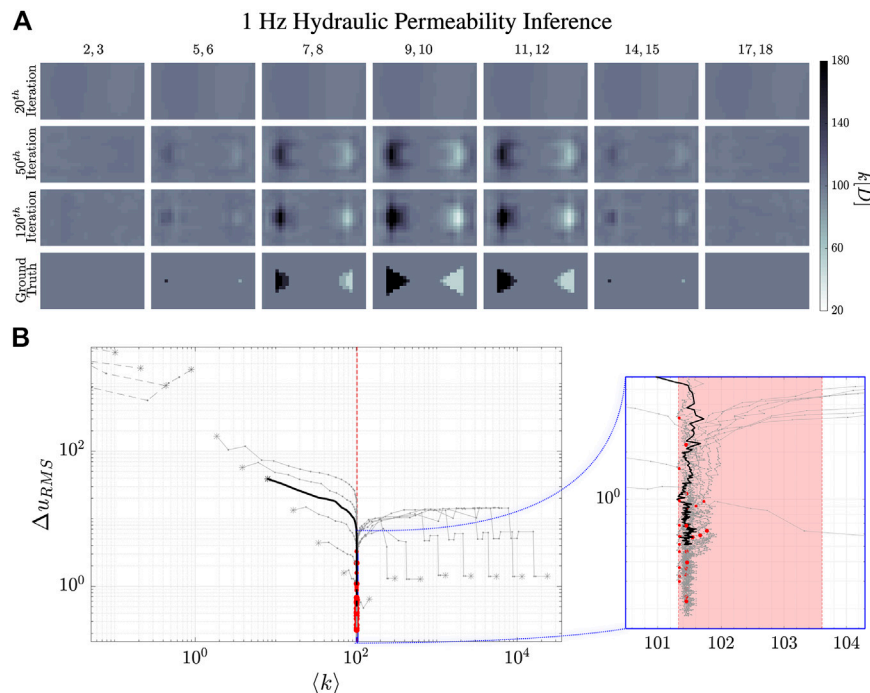


FIGURE 9 | Reconstruction evolution of the hydraulic permeability, k , at 1 Hz. **(A)** shows the reconstructed images of the hydraulic permeability distribution. The column images are the averages of the numbered (xy) -planes (top row). The rows are labeled by the iteration number of the inference. **(B)** shows the lower dimensional trajectory of the shear modulus. As above, the hydraulic permeability is measured in Darcy. The smaller diagram is a magnified region near the converged values. Initial estimates are noted by an asterisk and changes in descent strategy are indicated by small and large red markers, as explained in the text. The light red region represents the strip between the background value of the shear modulus and the ground truth mean value. The thicker trajectory is the one used to make the images in the above panel. Once again we witness that hydraulic permeability inference is robust to initial conditions over two orders of magnitude above the ground truth. With this simulation we see that the lower bound has gotten better as well, robust out to 2 orders of magnitude below the ground truth.

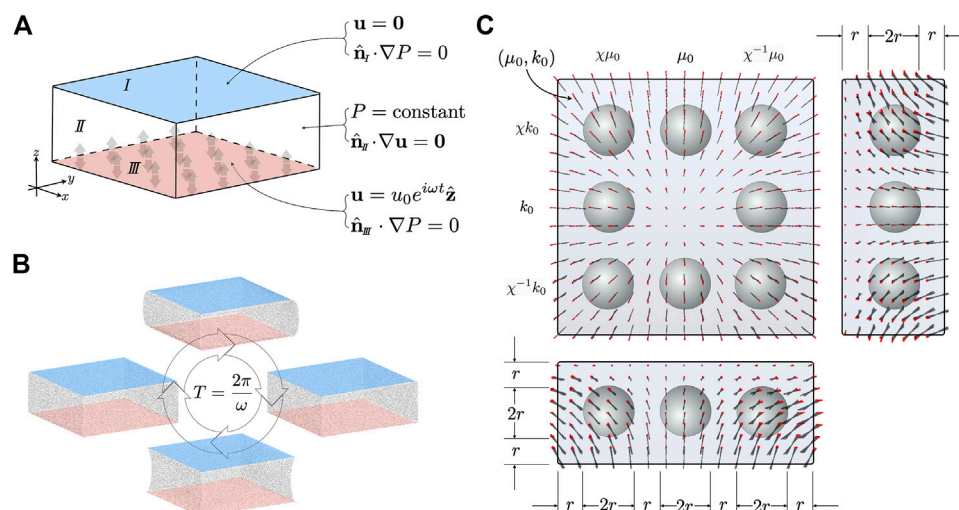


FIGURE 10 | **(A)** Boundary conditions on the top(I), side(II), and bottom(III) surfaces for the two property inference simulation at 1 Hz. $\hat{n}$ are outward pointing normals. Pressure has Neumann BCs on I & III, and Dirichlet BCs on II. Displacement has the opposite. Top plate is held fixed, while the bottom oscillates in the z direction at frequency ω . **(B)** The motion of the boundary nodes is displayed at four equally spaced times within the cycle of period T . One can see the Poisson effect during the apexes of the squeeze/stretch cycles. **(C)** Orthographic projection showing the geometry of the eight spherical inclusions. The left inclusions have positive shear modulus contrast, while the right ones have negative contrast. The top inclusions have positive hydraulic permeability contrast, while the bottom ones have negative. The MRE displacement field over one period is superimposed, the red dots representing in-phase displacements at the start of a cycle.

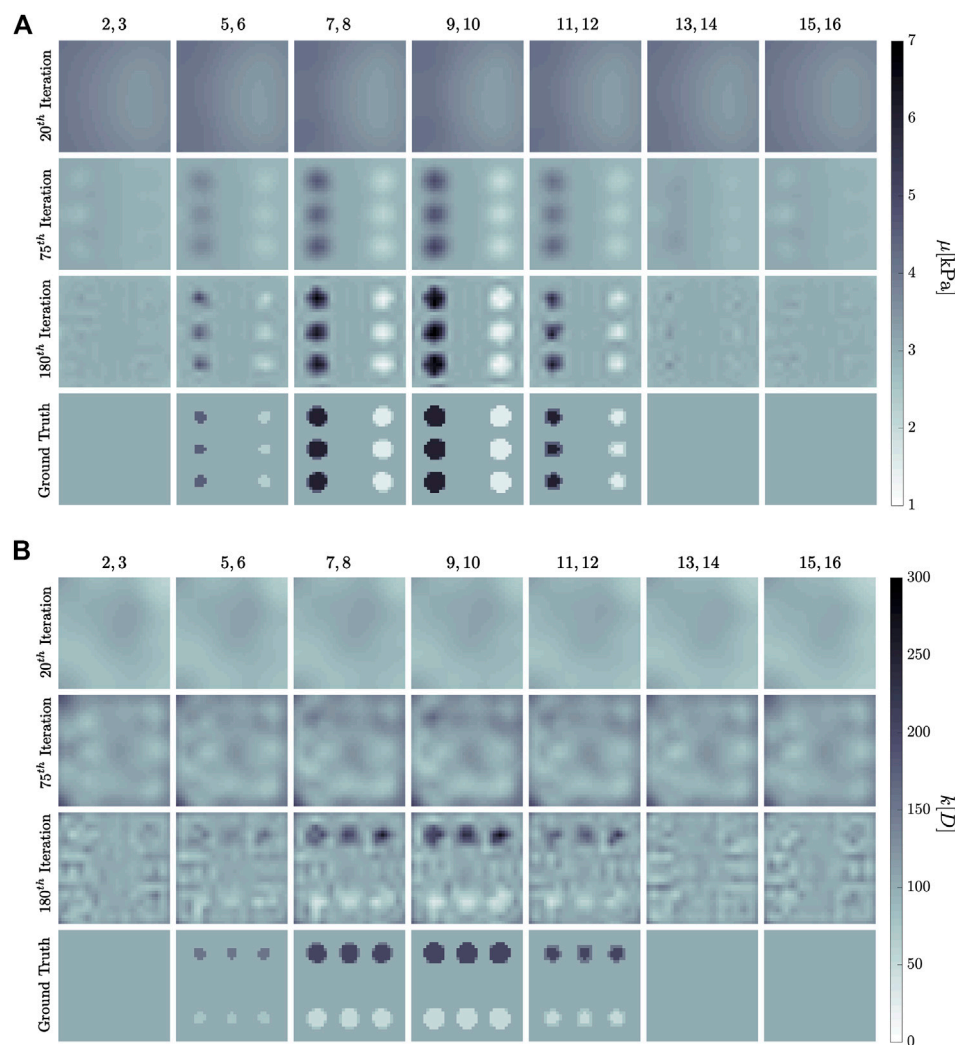


FIGURE 11 | Simultaneous inference evolution of both **(A)** shear modulus, μ , and **(B)** hydraulic permeability, k , in the 1 Hz experiment setup in **Figure 10**. The former is measured in kilo-Pascal, while the latter in Darcy. Columns are averages of the numbered (xy)-planes as in the other simulations. Rows are labeled by which iteration they are in MRE.

removed from the body, the pressure change and unjacketing of the sample results in fluid being expelled. The drop in pore pressure is such that the stresses in the elastic matrix are incapable of supporting the pore space under the influence of gravity, leading to collapse. Once pores are closed, surface to surface cellular interactions may prevent the process from being reversible. Even simple models like the Carman-Kozeny [110, 111] relationship between hydraulic permeability and porosity, $k \propto \phi^3$, would predict drastic changes in the former under reasonable changes in the latter. Furthermore, preliminary work shows that higher values of hydraulic permeability result in higher likelihood, giving us confidence that *in vitro* measurements of permeability are being underestimated by up to several orders of magnitude. A full analysis is forthcoming.

The shear modulus, on the other hand, is much better understood, with far less variance in the literature. In

viscoelastic models, white matter typically falls in the range of 1.5 – 2.5 kPa, depending on frequency, while gray matter is between 0.75 – 1.5 kPa [107, 112]. Viscoelastic estimates lump together resistance to deformation from both the solid and fluid constituents. The poroelastic shear modulus is the value for the drained solid, so will likely be somewhat lower than viscoelastic approximations, although a similar order of magnitude for most reasonably solid tissues. The frequency dependence of both is interesting, since it means that there is a memory effect in the tissue dynamics that has not been accounted for in the equations of motion. In order for this memory effect to be causal, in the sense that only past dynamics and not future dynamics affect the present state, certain constraints known as Kramers-Kronig relations must be satisfied by real and imaginary components of the moduli [113]. There is still some controversy over whether these conditions are satisfied by moduli measured *in vivo*. Finding

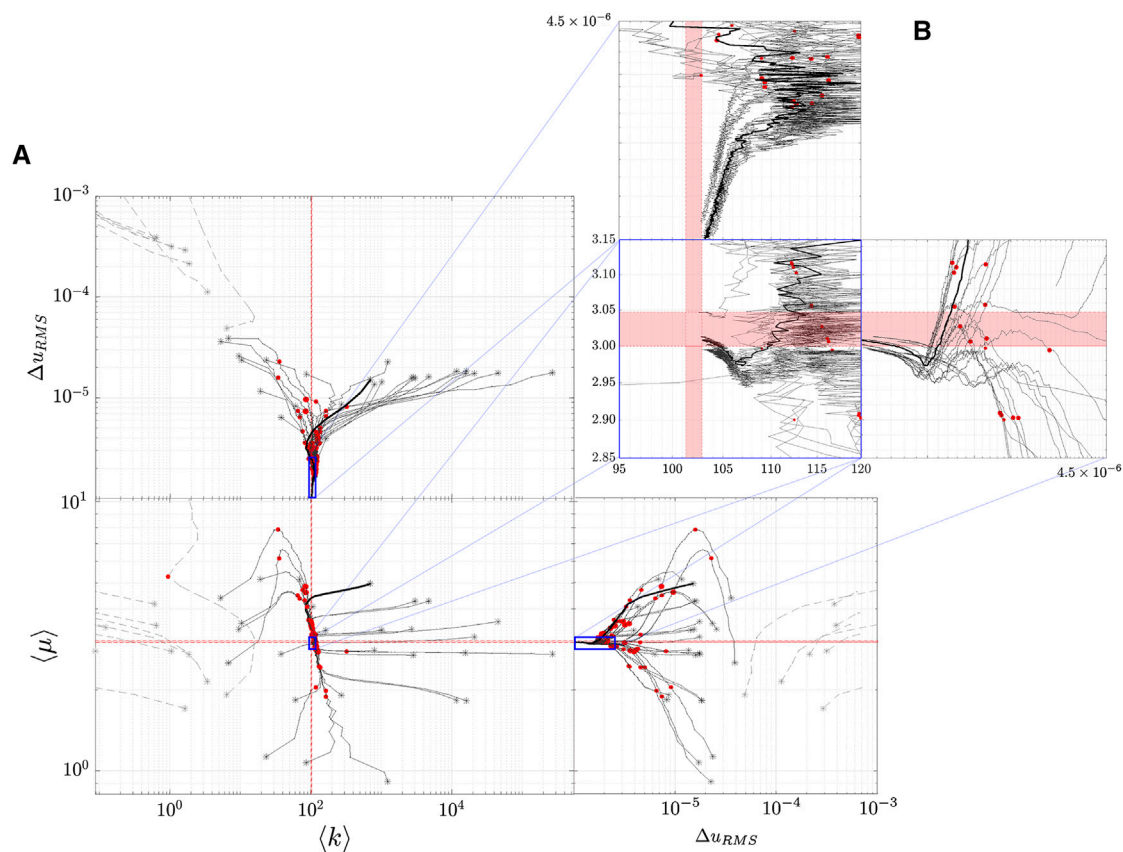


FIGURE 12 | Lower dimensional representation of the inference trajectories. Note that because two properties are being reconstructed, the lower dimensional representation is 2-dimensional. This plot conveys the trajectories through an orthographic projection. **(A)** shows a large region of $(\langle k \rangle, \langle \mu \rangle)$ -space, including starting points of the trajectories represented by asterisks. Right and top panels represent root mean square values for converging trajectories. Some diverging trajectories are also shown - they are dashed and lighter. The single thick trajectory is the one used for **Figure 11**. Small and large red markers represent points where the descent strategy changed. The light red horizontal and vertical regions are the critical strips laying between the background property values and the ground truth mean values. Note that, as before, scales are all logarithmic. **(B)** is zoomed into the blue squares located in the figures described above. The shaded red areas represent the region between the background property value, μ_0 or k_0 , and the mean value of the ground truth distributions, $\langle \mu \rangle_{GT}$ or $\langle k \rangle_{GT}$. Note that in these plots the scales have changed to be linear. Discussion of trends is in the text.

a frequency dependence of the poroelastic parameters would put similar constraints on the equations of motion.

The introduction of β_n in the poroelastic equations of motion creates a link between poroelastic and viscoelastic dynamics by means of the imaginary component of the parameter. Past numerical studies attempted to bridge the connection between the two types of behavior [107]. A full analytical treatment is in the works - understanding where the dynamics are most similar and where they are most different is a way of gaining insight into the microstructure of tissue. It also points towards better experimental design, since understanding the parameter spaces of the two can be harnessed to accentuate, and hence test for, behavior intrinsic to only one type of model. Forthcoming work will focus on understanding this connection.

One might wonder what happens when we attempt to infer more properties. Unfortunately, as the number of properties increases, the coarseness of the material meshes must also increase. This is simply due to matching displacement

constraints coming from MR measurements to the property degrees of freedom in the equations of motion. Theoretical considerations aside, our numerical suite has a much harder time converging with the addition of an extra property to infer - a significant contraction of the domain of convergence being observed. We believe that this is a numerical issue, not a theoretical one, and an active research direction within our group.

As we move forward with *in vitro* and *in vivo* testing, our *in silico* results give us a good picture at how ignorant we can be when guessing our background values. Hydraulic permeability reconstruction for noiseless data is robust to initial conditions ranging from an order of magnitude below, to 2+ orders of magnitude above the ground truth background. Meanwhile, shear modulus alone has not been observed to diverge, albeit its convergence rate can get quite slow. Adding noise to data introduces fluctuations that will affect reconstruction, as our group has shown in prior work [114]. We found our results to

be robust up to contrasts of an order of magnitude (it was small contrasts that were problematic) and believe that as long as signal to noise ratios are well above unity, noise should have only a minor effect on the domain of convergence.

4.1 Concluding Thoughts

Being able to image multiple poroelastic parameters will be a boon for preclinical diagnosis. Getting our computational suite to work *in vivo* will allow us to apply poroelasticity to the brain, where both cerebro-spinal fluid and blood play an active roll in homeostasis. The robustness under a wide range of initial conditions at low frequencies bodes well for using intrinsic actuation via the cardiac cycle. Tumors tend to have shear moduli between one and two orders of magnitude higher than surrounding tissue - these will be as visible to poroelastic parameter reconstruction as they are for viscoelasticity. The movement of fluid in the brain is critical in several venues. It is a mixture blood movement and interstitial fluid movement over the scale of a fraction of a voxel. We expect the dominant scale of fluid flow observed in MRE will be between that of perfusion which observes macroscopic bulk blood flow over a fraction of a meter and that of diffusion which observes microscopic fluid flow on a cellular scale. Observing fluid flow in this scale has potential to complement diffusion in characterizing edema and dementia. We postulate this scale of fluid flow will also illuminate tissue viability before diffusion becomes relevant allowing us to better identify and characterize reversible tissue damage. It might also provide additional information about functional changes secondary to brain activity.

It is an exciting time to watch as MRE enters its third decade of application. As interdisciplinary as its roots are, it is no surprise that with maturity the field is digging deeper into the fertile soil of its disciplinary constituents. Poroelasticity has been one of the crowning theoretical achievements of the geophysics community, and like every good idea, is ripe for cross-pollination. Perhaps it is no surprise that a model developed to describe rocks and soils is finding use in describing living tissue when in 1510 Leonardo da Vinci penned [115], into his *Codex Leicester*, that *The Earth is a living body. Its flesh is the soil, its bones are the strata of rock, its cartilage is the tufa, its blood is the underground streams, the*

reservoir of blood around its heart is the ocean, the systole and diastole of the blood in the arteries and veins appear on the Earth as the rising and sinking of the oceans.

4.2 Methods and Data Availability

Simulations were performed using an in-house FORTRAN MRE Computational Platform written and developed over the past decade by group members. Both forward and inverse problems were computed on the Discovery Cluster, a 3000+ core Linux cluster at Dartmouth College administered by Research Computing. The data that support the findings of this study are available from the corresponding author upon reasonable request.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available under reasonable request by the corresponding author, without undue reservation.

AUTHOR CONTRIBUTIONS

DS ran the simulations, did the data analysis, and wrote the manuscript with input and guidance from MM, SG, JW, and KP. MM and EH wrote the FEM code used for the simulations.

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Shear Wave Dispersion as a Potential Biomarker for Cervical Remodeling During Pregnancy: Evidence From a Non-Human Primate Model

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Shear wave dispersion (variation of phase velocity with frequency) occurs in tissues with layered and anisotropic microstructure and viscous components, such as the uterine cervix. This phenomenon, mostly overlooked in previous applications of cervical Shear Wave Elasticity Imaging (SWEI) for preterm birth risk assessment, is expected to change drastically during pregnancy due to cervical remodeling. Here we demonstrate the potential of SWEI-based descriptors of dispersion as potential biomarkers for cervical remodeling during pregnancy. First, we performed a simulation-based pre-selection of two SWEI-based dispersion descriptors: the ratio R of group velocities computed with particle-velocity and particle-displacement, and the slope S of the phase velocity vs. frequency. The pre-selection consisted of comparing the contrast-to-noise ratio (CNR) of dispersion descriptors in materials with different degrees of dispersion with respect to a low-dispersive medium. Shear waves induced in these media by SWEI were simulated with a finite-element model of Zener viscoelastic solids. The pre-selection also considered two denoising strategies to improve CNR: a low-pass filter with automatic frequency cutoff determination, and singular value decomposition of shear wave displacements. After pre-selection, the descriptor-denoising combination that produced the largest CNR was applied to SWEI cervix data from 18 pregnant Rhesus macaques acquired at weeks 10 (mid-pregnancy stage) and 23 (late pregnancy stage) of the 24.5-weeks full pregnancy. A maximum likelihood linear mixed-effects model (LME) was used to evaluate the dependence of the dispersion descriptor on pregnancy stage, maternal age, parity and other experimental factors. The pre-selection study showed that descriptor S combined with singular value decomposition produced a CNR 11.6 times larger than the other descriptor and denoising strategy combinations. In the Non-Human Primates (NHP) study, the LME model showed that descriptor S significantly decreased from mid to late pregnancy (-0.37 ± 0.07 m/s-kHz per week, $p < 0.00001$) with respect to the base value of 15.5 ± 1.9 m/s-kHz. This change was more significant than changes in other SWEI features such as the group velocity previously reported. Also, S varied significantly between the anterior and posterior portions of the cervix ($p = 0.02$) and with maternal age

($p = 0.008$). Given the potential of shear wave dispersion to track cervical remodeling, we will extend its application to ongoing longitudinal human studies.

Keywords: cervix, shear wave elasticity imaging, rhesus macaque, phase velocity, group velocity, singular value decomposition, pregnancy

1 INTRODUCTION

Preterm birth is the main cause of death in newborn children and its incidence rate is increasing [1, 2]. A factor contributing to this problem is the lack of objective methods to describe the structural and functional changes that gestational tissues go through during pregnancy and labor [3].

One of the gestational tissues that suffers the most dramatic transformation is the cervix. The cervix is a cylindrical-shaped structure at the lower uterus that, during pregnancy, serves as a protective barrier between the developing fetus and the exterior. As pregnancy advances, the cervix softens and, close to delivery, shortens, ripens, and dilates to allow for vaginal parturition. In both term and preterm births, this transformation is achieved through a dramatic biochemical and structural transformation of the cellular and extracellular components of the cervical stroma [4].

The extracellular matrix of the cervix is composed of layers of semi-aligned collagen fibers immersed in a viscous matrix of long-chain molecules such as proteo- and glycosaminoglycans [5]. As pregnancy progresses, mature crosslinks holding together collagen fibrils are replaced by immature crosslinks. As a result, collagen organization is gradually lost and the cervix softens [4]. During the ripening stage, the concentration of sulfated glycosaminoglycans increases, particularly that of hyaluronic acid (HA) [6]. Due to the hydrophilic nature of HA, cervical hydration augments, further separating collagen fibrils [7–14]. All these changes alter drastically the mechanical properties of the cervix, such as its stiffness and viscosity [15].

During the last decade, various groups have investigated the use of Shear Wave Elasticity Imaging (SWEI) to objectively quantify changes in cervical stiffness during pregnancy and to assess the risk of preterm birth [16–21]. SWEI is an ultrasound-based method that generates shear waves in the tissue of interest using an acoustic radiation force impulse (ARFI) produced with a clinical transducer. The ARFI-induced shear wave motion is tracked with pulse-echo imaging techniques, allowing analysis of the propagation of the shear wave. If the medium is assumed to be linear, purely elastic, isotropic, and homogeneous, a simple relationship between the shear wave group speed V_g and the tissue stiffness described by its shear modulus μ can be established: $\mu = \rho V_g^2$, where ρ is the medium mass density [22]. Various studies have demonstrated a significant reduction of V_g during pregnancy in *ex vivo* and *in vivo* studies in humans and animal models [17, 20, 23–25]. However, these studies are limited by the aforementioned assumptions behind the link between the group velocity and the shear modulus, which ignore the effects that the complex extracellular matrix has on shear wave propagation.

The layered collagenous microstructure of the cervix and the presence of the highly viscous ground substance cause shear wave dispersion, i.e., the phase speed (the speed at which different

frequency components of the shear wave move) varies with frequency. Shear wave dispersion has been demonstrated in other structurally complex tissues such as tendon and muscle [26, 27]. Using a multi-scale simulation strategy with different viscoelastic models Peralta et al. [28] demonstrated significant shear wave dispersion in the cervix. More recently, our group observed that the slope of the phase velocity vs. frequency in *ex vivo* cervix samples from a non-human primate (NHP) model was 5.5 m/s-kHz (interquartile range: 1.5–12.0 m/s-kHz) [20]. In the same study, we also demonstrated that shear wave dispersion could be indirectly assessed by comparing the group speeds determined with particle velocity and particle displacement. The time derivative relationship between the particle velocity and the particle displacement makes the former more sensitive to higher frequencies, thus resulting in higher group speed values than those obtained with the particle displacement [29]. Interestingly, no differences in dispersion were observed between normal cervix samples and those in which ripening was induced with the prostaglandin Misoprostol. This was attributed to the large variance of shear wave displacements, probably related to the larger stiffness of the rhesus macaque cervix compared to the human one [20, 30]. The quantification of descriptors of shear wave dispersion could provide quantitative imaging biomarkers that could be more sensitive than the group speed to changes in mechanical properties of the cervix.

Thus, in this study, we present evidence from a NHP model of the pregnant cervix that shear wave dispersion quantified with SWEI can be used as a quantitative imaging biomarker for cervical remodeling. We chose to work with the NHP model, in particular the Rhesus macaque, because of its similar maternal-fetal physiology to humans. Furthermore, because Rhesus macaques are non-obligated quadrupeds, the mechanical loading on the cervix is more similar to the human case [31–35]. Previous studies from our group have shown similar rates of shear wave reduction during pregnancy in humans and Rhesus macaques, thus showing the suitability of this model [21, 24]. To overcome the limitations of high displacement variance in the Rhesus macaque cervix, we performed a pre-selection of two possible descriptors of dispersion, the ratio of group speeds from particle velocity/particle displacement and the slope of the phase velocity vs. frequency, combined with two noise reduction strategies. After the pre-selection stage, the descriptor with larger changes due to dispersion was applied to SWEI data from the mid-pregnancy and late-pregnancy NHP cervix.

2 MATERIALS AND METHODS

2.1 SWEI-Based Descriptors of Dispersion

We investigated two possible SWEI-based descriptors of dispersion, one defined in the time domain, and another in

the Fourier domain. The time-domain descriptor is the ratio R of group speeds computed with particle velocity v_p over particle displacement u_p :

$$R = \frac{V_g(v_p)}{V_g(u_p)}, \quad (1)$$

where v_p can be determined from u_p by:

$$v_p = du_p/dt. \quad (2)$$

V_g is usually estimated by tracking the time of flight of the maximum particle displacement or particle velocity away from the ARFI excitation [36]. In an approximately purely elastic material, both estimates of V_g agree. However, this is not the case in viscoelastic media because the time-derivative in Eq. 2 acts as a high-pass filter in the frequency domain [29]. Thus, the more dispersive the medium is, the larger $V_g(v_p)$ is over $V_g(u_p)$ [20, 37]. In other words, for a purely elastic material, $R = 1$, and for a viscoelastic one, $R > 1$. This descriptor takes advantage of the various robust methods that have been proposed to quantify the group speed, such as the random sample consensus, or RANSAC, algorithm [38] or the Radon sum method [36, 37].

The Fourier-domain descriptor directly quantifies dispersion as the slope S of the phase velocity vs. frequency over the shear wave bandwidth [20]. To quantify S , we computed the particle velocity power spectrum $V(k, \omega)$, i.e., the square magnitude of the 2D-Fourier transform for the particle velocity $v_p(x, t)$, where k is the wave number and ω is the angular frequency [39]. To parameterize the frequency dependence of the phase speed $V_p(\omega)$ we used a linear model defined by an intercept $V_{p,0}$ and a slope $dV_p/d\omega$. To find the values of the parameters of the linear fit, a Radon-sum strategy proposed by [40] was used. Briefly, curved trajectories are defined on the velocity power spectrum as function of the linear fit parameters. The values of the parameters are selected from the trajectory along which the sum of the spectrum values is maximized. The sum was done over the -6 dB spatial and temporal frequency bandwidth. Finally, we used $S = dV_p/d\omega$ as the Fourier-domain descriptor of dispersion.

2.2 Pre-selection Stage

The pre-selection stage consisted of selecting the descriptor with the largest changes in dispersion in viscoelastic media over the estimation variance. To have control over the degree of dispersion, we used computational phantoms based on finite-element models of Zener viscoelastic solids implemented on LS-Dyna (Version 3.2 β, Livermore, Software Technology Corp., Livermore, CA) following the method described by [41]. Dispersion was controlled by varying the viscous component of the Zener model (one spring in parallel with a series of a spring and a dashpot). Shear waves were created through fields of axial stresses with spatial Gaussian symmetry that simulated acoustic radiation force impulse stimuli produced by clinical ultrasound transducers [29]. The width of the Gaussian profile was 0.1 mm, based on a previously reported computational optimization [42]. The stress fields were applied during 0.1 ms. The center of the

stress field was at a depth of 30 mm, corresponding the axial center of the simulated computational phantom [41].

The stress relaxation response of the Zener model has three parameters, the instantaneous shear modulus G_0 , the equilibrium shear modulus G_∞ , and the time constant b . The values of G_0 and G_∞ were fixed at 20 kPa and 2 kPa, respectively, and the value of b was varied from 3,000 s to 30,000 s, corresponding to changes in the viscous component from 0.6 to 6.0 Pa s. The temporal and spatial sampling rates of the simulated mechanical response (shear wave displacements) were 0.1 μs and 0.25 mm, respectively, for a total simulated time of 10 ms. For the shear wave frequencies that were simulated (peak temporal frequencies between 100 and 200 Hz as shown in Figure 1), there were at least 10 nodes per wavelength. To mimic the pulse repetitions frequencies used in commercial scanners such as the one used in the animal model study, the displacements were down sampled to 0.1 ms. The axial and lateral length was 60 mm and 20 mm respectively; additionally a 5-element thick perfect matching layer was added to all outward faces of the mesh to suppress reflections back into the analysis ROI. The analysis of shear wave propagation was done over a 5 mm axial extent centered at the ARF focus (30 mm deep), similarly to the analysis done in the animal model. Zero-mean Gaussian noise was added to particle displacements to analyze the compromise between changes of the descriptors due to dispersion and estimation variance [43]. The level of noise was controlled by a factor k that defined the standard deviation as a factor of the maximum displacement induced by the stress field in each simulation. The value of k was varied from 0 to 0.1.

The compromise between changes of each parameter and the estimation variance was quantified in terms of the contrast-to-noise ratio of each descriptor with respect to its value for the least dispersive medium. The CNR was mathematically defined as:

$$\text{CNR} = \frac{|P_{\eta_i} - P_{\eta_{\min}}|}{\sqrt{\sigma_{P_{\eta_i}}^2 + \sigma_{P_{\eta_{\min}}}^2}}, \quad (3)$$

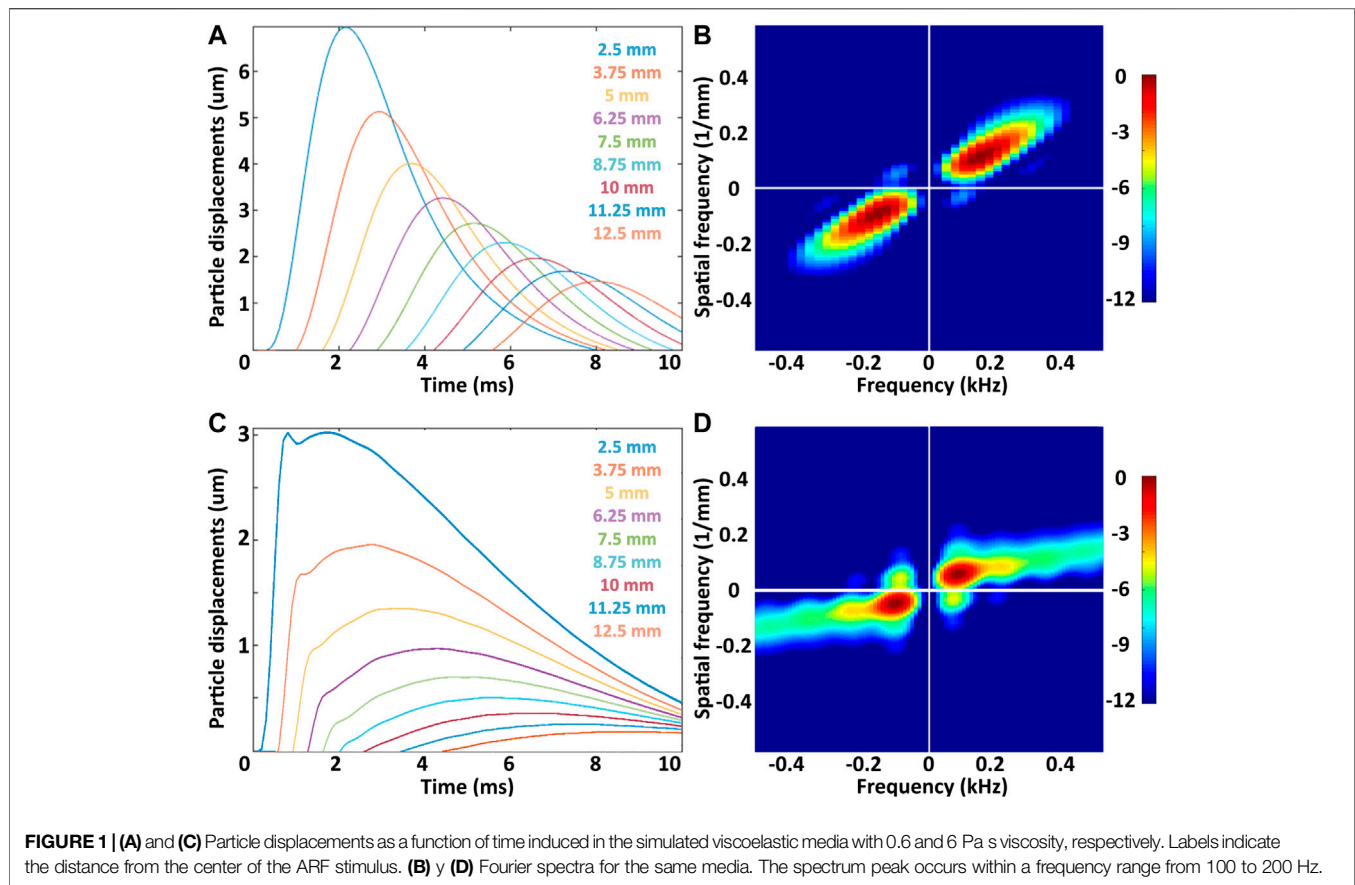
where P_{η_i} is the value of either S or R for the material with the i th value of viscosity η , and $P_{\eta_{\min}}$ is the value of the same parameter for the least viscous medium. $\sigma_{P_{\eta_i}}$ and $\sigma_{P_{\eta_{\min}}}$ are the standard deviations of these parameters. The value of the CNR for each parameter was computed for different combinations of viscosity and noise. Twenty independent realizations of the noise were added to the simulated shear wave fields to estimate the mean and standard deviation of the CNR.

2.3 Noise Reduction Strategies

Preliminary estimates of shear wave dispersion in the *ex vivo* NHP cervix [20] showed large variance possibly related to the high stiffness of cervical tissue compared to other soft tissues such as liver. To overcome this limitation and increase the sensitivity of dispersion descriptors, we implemented two noise reduction strategies.

2.3.1 Automated Low-Pass Filter

Because random noise dominates at high temporal frequencies [44], low-pass filters have been commonly used in previous



applications of shear wave elasticity imaging on cervical tissue [24]. We implemented this strategy and designed an automated criterion to select the cutoff frequency. This allowed us to automatically adapt the filter to the noise level in each simulated condition. To this end, we first obtained the maximum projection of the 2D spectrum of the particle velocity to obtain the velocity power spectrum vs. temporal frequency. Then, the cut-off frequency was defined by the intersection of two lines fitted to the velocity power spectrum vs. temporal frequency within a bandwidth that contains the shear wave signal (100–500 Hz) and a high-frequency bandwidth that contains noise (1–5 kHz). This is graphically represented in **Figure 2**.

2.3.2 Singular Value Decomposition Filter

The second strategy consisted of a filter based on the singular value decomposition (SVD) of shear wave displacements. This technique has been used to reduce clutter in other ultrasound techniques such as Doppler imaging and functional ultrasound [45]. The SVD filtering strategy is based on the fact that noise components of the displacement field have low spatial and frequency correlation compared to the components caused by shear wave propagation. Thus, unlike the low-pass filter strategy defined above, the SVD filter considers both temporal and spatial information. Also, in contrast to previous uses of the SVD, we aim at maintaining

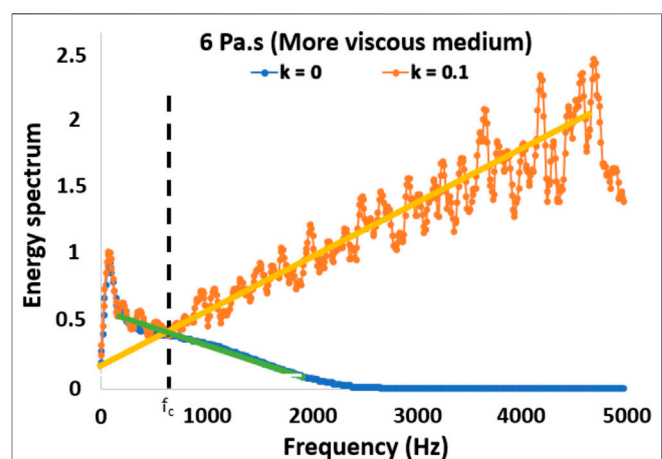


FIGURE 2 | Projected particle velocity spectrum of a viscoelastic solid with 6.0 Pa s viscosity and a $k = 0.1$ noise level (orange curve). The green and yellow lines are linear fits to the noisy spectrum at low (0.1 kHz–0.5 kHz) and high frequencies (1 kHz–5 kHz), respectively. The intersection of both fits allows determination of the cutoff frequency f_c . The blue curve shows the noise-free case to demonstrate the agreement of the fit over noisy data to the noiseless case.

highly correlated components of the displacement field. To apply this filter, the 2D displacements are sorted into a Casorati Matrix **S** with the spatial information in the

TABLE 1 | Characteristics of the macaques. For each macaque, tissue measurements were taken in the anterior and posterior region with respect to the cervical canal during gestation weeks 10 and 23.

Subject number	Age (yrs)	Weight (kg)	Previous births	Pregnancy length (weeks, days)
1	14.8	10.92	6	23,5
2	4.3	6.79	0	23,2
3	4.3	8.25	0	23,5
4	4.6	6.5	0	24,3
5	10.2	7.52	3	25,3
6	17	8.07	8	24,5
7	10.3	6.7	2	24,1
8	5.8	6.8	0	23,5
9	5.2	7.38	1	25,3
10	5.2	7.34	1	24,4
11	12.1	9.32	4	25,3
12	11.3	9.31	3	24,6
13	3.8	5.26	0	23,6
14	3.6	6.89	0	24,4
15	4.8	7.49	0	25,0
16	4.6	5.21	0	25,4
17	4.2	7.76	0	24,5
18	3.9	6.45	0	24,2

columns and the temporal information in the rows [45]. The Casorati matrix is decomposed into the product of three matrices, $\mathbf{S} = \mathbf{U}\mathbf{\Delta}\mathbf{V}^*$, where matrices $\mathbf{U}$ and $\mathbf{V}$ contain the eigenvectors of the covariance matrices $\mathbf{S}\mathbf{S}^*$ and $\mathbf{S}^*\mathbf{S}$, respectively, and $\mathbf{\Delta}$ is a diagonal matrix containing the singular values (square root of the eigenvalues of the covariance matrices) in decreasing order [46]. The first diagonal elements represent the contribution of the shear wave to the covariance (high contributions), while the last ones represent the contribution of random noise (low contributions). To filter noise, a displacement matrix $\mathbf{S}_i$ is reconstructed removing the eigenvalues smaller than the cutoff eigenvalue, corresponding to the seventh eigenvector. The cutoff index value was defined by minimizing the tradeoff between bias and variance in the estimation of dispersion based on simulations. This tradeoff was quantified in terms of the Contrast-to-Total-Error-Ratio (CTER), defined as the ratio of the contrast of parameter P with respect to its value in the least dispersive medium over the combined root mean squared error RMSE:

$$\text{CTER} = \frac{P_\eta - P_{\eta_{\min}}}{\sqrt{\text{RMSE}(\eta)^2 + \text{RMSE}(\eta_{\min})^2}}, \quad (4)$$

where RMSE was computed as:

$$\text{RMSE}(\eta) = \sqrt{\frac{\sum_{i=1}^N (P_{\eta,e} - P_{\eta_i})^2}{N}}, \quad (5)$$

where $P_{\eta,e}$ is the expected value of the parameter for each degree of viscosity.

The CNR of R and S for media with different dispersion (from different degrees of viscosity) and different noise levels were compared with and without each of the noise reduction strategies to identify the combination that maximized the CNR.

2.4 *In vivo* Application to Pregnant Rhesus Macaques

After identifying the combination of the dispersion descriptor and noise reduction strategy that maximized CNR, the combination was used to evaluate changes in the dispersion properties of the NHP cervix during pregnancy. To this end we used data from a longitudinal SWEI study on 18 pregnant Rhesus macaques acquired at weeks 10 and 23 of gestation, corresponding to mid and late pregnancy [21]. The study was performed at the Wisconsin National Primate Research Center and the protocol was approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. Characteristics of the subjects are described in **Table 1**.

SWEI data was acquired from the NHP cervix using a transrectal scanning approach on a longitudinal view of the cervix. Data was acquired with a prototype catheter transducer on a Siemens Acuson S2000 ultrasound scanner (Siemens Healthcare, Ultrasound Business Unit, Mountain View, CA, USA). Technical details about SWEI implementation can be found in [21]. Shear waves were induced in two propagation directions (from the uterine end to the vaginal end of the cervix, and vice versa) and were tracked over a $6 \times 5 \text{ mm}^2$ region of interest centered first on the posterior and then on the anterior portions of the cervix.

Shear-wave induced displacements were estimated from raw IQ data obtained from the system's Aixius Direct Ultrasound Research Interface [47] with Loupas' correlation method [48]. Displacement estimates were rejected based on values of the correlation coefficient (< 0.98) and filtered to eliminate low frequency biological motion using a second-order polynomial motion filter [40]. The first lateral millimeter of the displacement field was discarded due to ARF stimulus effects. *In vivo* data quality was assessed through three criteria (**Figure 3**):

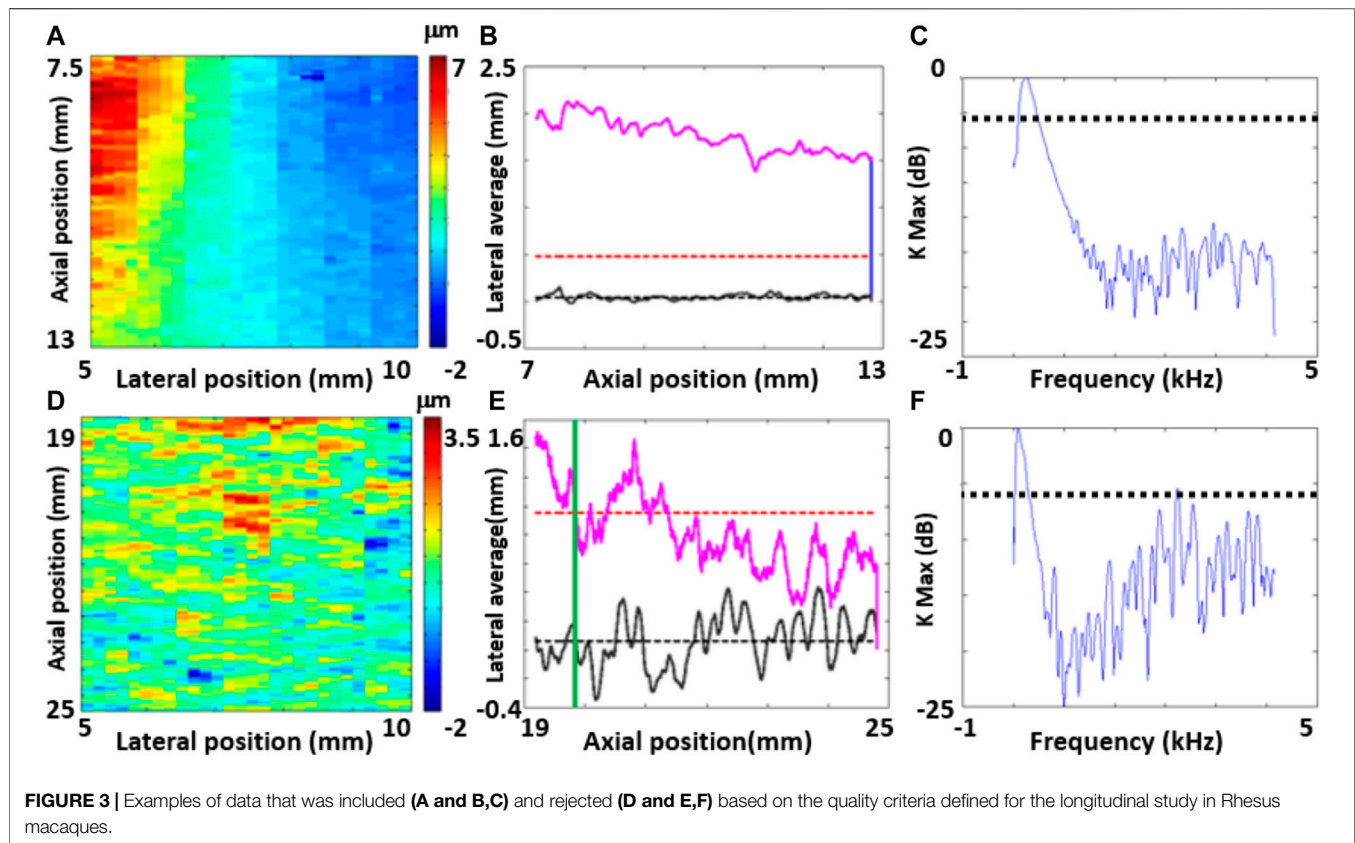


FIGURE 3 | Examples of data that was included (A and B,C) and rejected (D and E,F) based on the quality criteria defined for the longitudinal study in Rhesus macaques.

1. *Evidence of a planar wavefront:* Maps of the laterally-integrated particle displacement vs axial position and time must show no variation over the axial position (planar wavefront), and should gradually decrease over time mainly due to attenuation (Figure 3A). Data not showing these characteristics were rejected (Figure 3D).
2. *Statistically significant particle displacements.* Following a criterion proposed by [20], particle displacements were compared to those estimated from reference tracking frames acquired before the application of the ARF stimulus (Figure 3B). Data was rejected if time-averaged displacements (pink curve in Figures 3B,E) were not larger than a threshold (red dashed line) defined as three times the standard deviation of the reference particle displacements (black dashed line) over more than half of the axial span of the ROI. For the rest of the data, we only processed displacements that were continuously above the threshold (right side of vertical green line in Figure 3E).
3. *Data with high noise after filtering:* Finally, we rejected all the filtered data with noise levels at high temporal frequencies above -6 dB of the maximum signal (Figures 3C,F).

2.4.1 Statistical Analysis

We used a linear mixed effects model to evaluate the significance of the relationship between the optimized dispersion parameter, gestational age, and other experimental variables [49]. According

TABLE 2 | Variables of the mixed effects model applied to the optimized dispersion parameter.

Variable	Effect	Type of effect
e_1	Week of gestation	Continuous (weeks)
e_2	Mother's age	Continuous (years)
e_3	Cervix portion	Binary (anterior = 0, posterior = 1)
e_4	Weight	Continuous (kg)
e_5	Previous births	Categoric (integer)
e_6	Pregnancy length	Continuous (weeks)

to this model, the optimized parameter P can be written as a linear combination of each variable e_i and of their interactions $e_i * e_j$:

$$P = P_0 + \sum_i \left(C_i e_i + \sum_{j \neq i} C_{ij} e_i * e_j \right), \quad (6)$$

where P_0 is the intercept of the model, C_i is the contribution of each variable to the model, and C_{ij} is the contribution of the interaction of each pair of variables. Table 2 defines the variables e_i . We used a maximum likelihood strategy to fit the model using the Matlab's function *fitlme*. The model was applied in a sequential manner, discarding non-significant variables in each iteration. We report the final model that included significant variables only. A Shapiro-Wilk test was applied to the values of the optimized parameter grouped by gestation week and cervix portion in order to verify the assumption of normality.

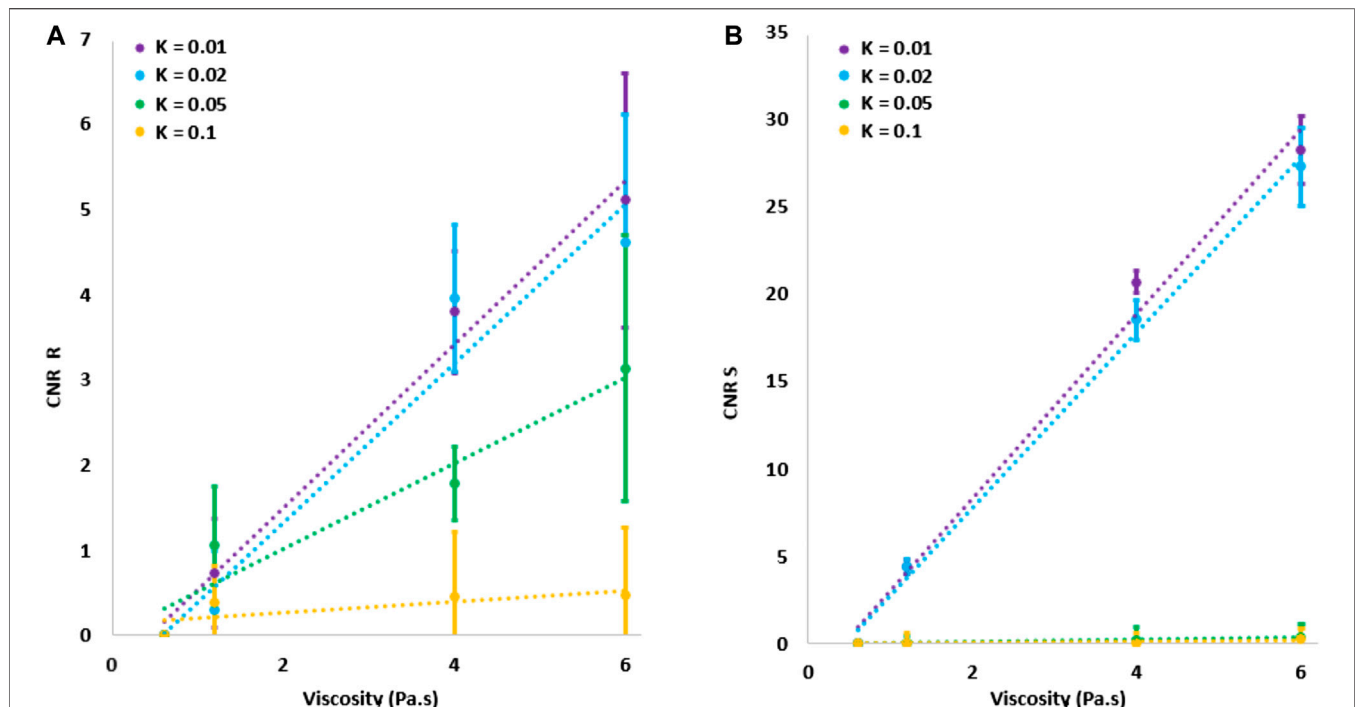


FIGURE 4 | Contrast-to-noise ratio for either R and S parameters in function of the viscosity for different levels of noise. Error bars represent standard deviations over twenty repetitions.

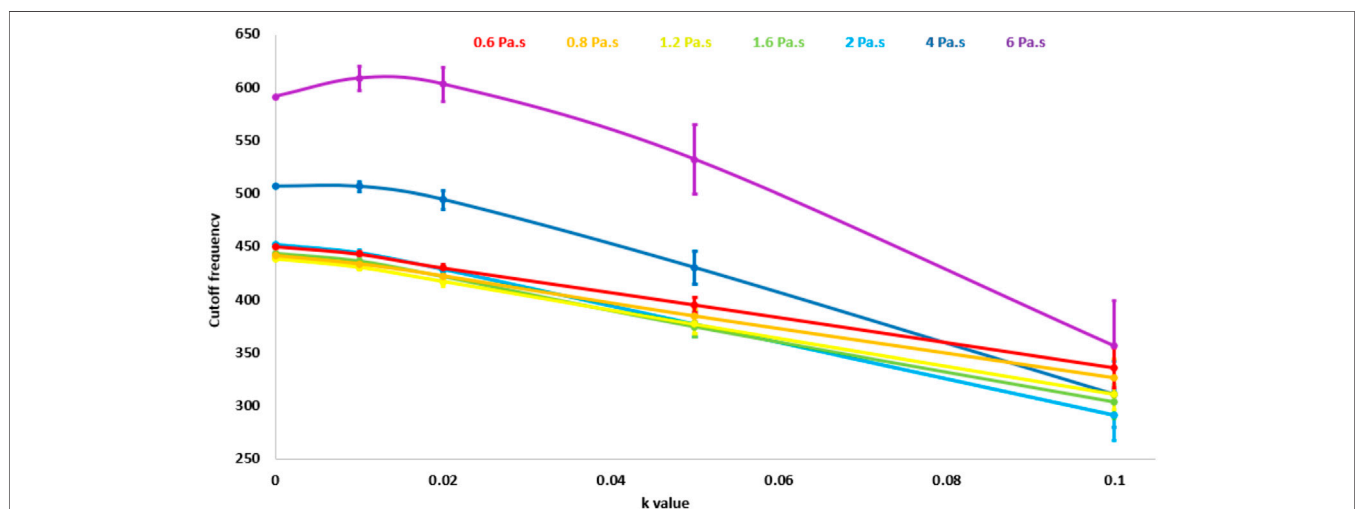


FIGURE 5 | Cutoff frequency values obtained through the automatic method as a function of the noise parameter k . Each curve corresponds to a different viscosity degree.

3 RESULTS

3.1 Simulations

The CNR of both dispersion descriptors R and S increased with viscosity. In addition, we computed the values of the dispersion descriptors R and S (see description below) for a purely elastic medium (no viscosity), obtaining $R = 0.998$ and $S = 0.01$ (close to

the values expected under no dispersion, i.e., $R = 1$ and $S = 0$). This demonstrates the absence of surface effects in shear wave propagation. Also, when no noise-reduction strategies were applied, R was found to be more robust to high levels of noise than parameter S . **Figures 4A,B** show the CNR of R and S , respectively, as a function of viscosity. Each curve corresponds to a different noise level. For low noise levels ($k \leq 0.02$), the CNR of S

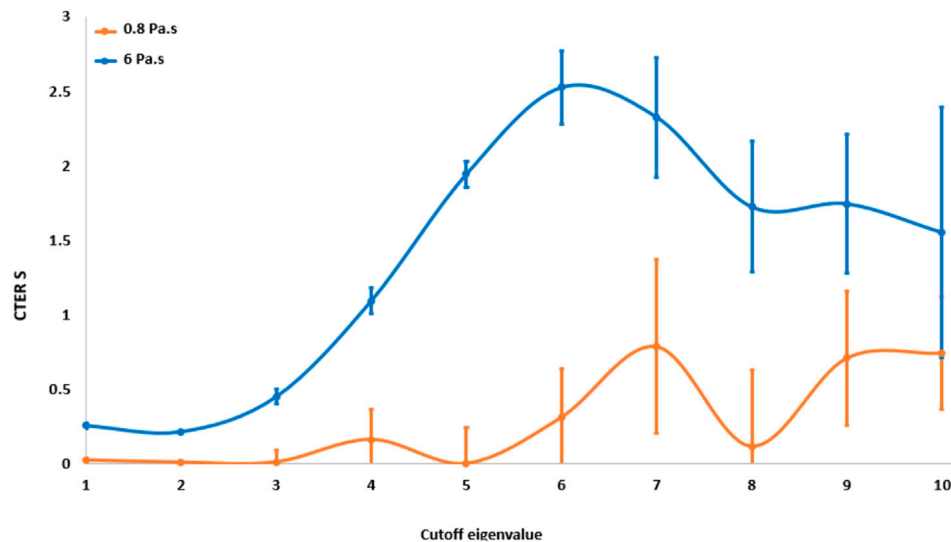


FIGURE 6 | CTER values of the S parameter as a function of the cutoff eigenvalue for the most viscous medium (6 Pa.s) and the less viscous (0.8 Pa.s).

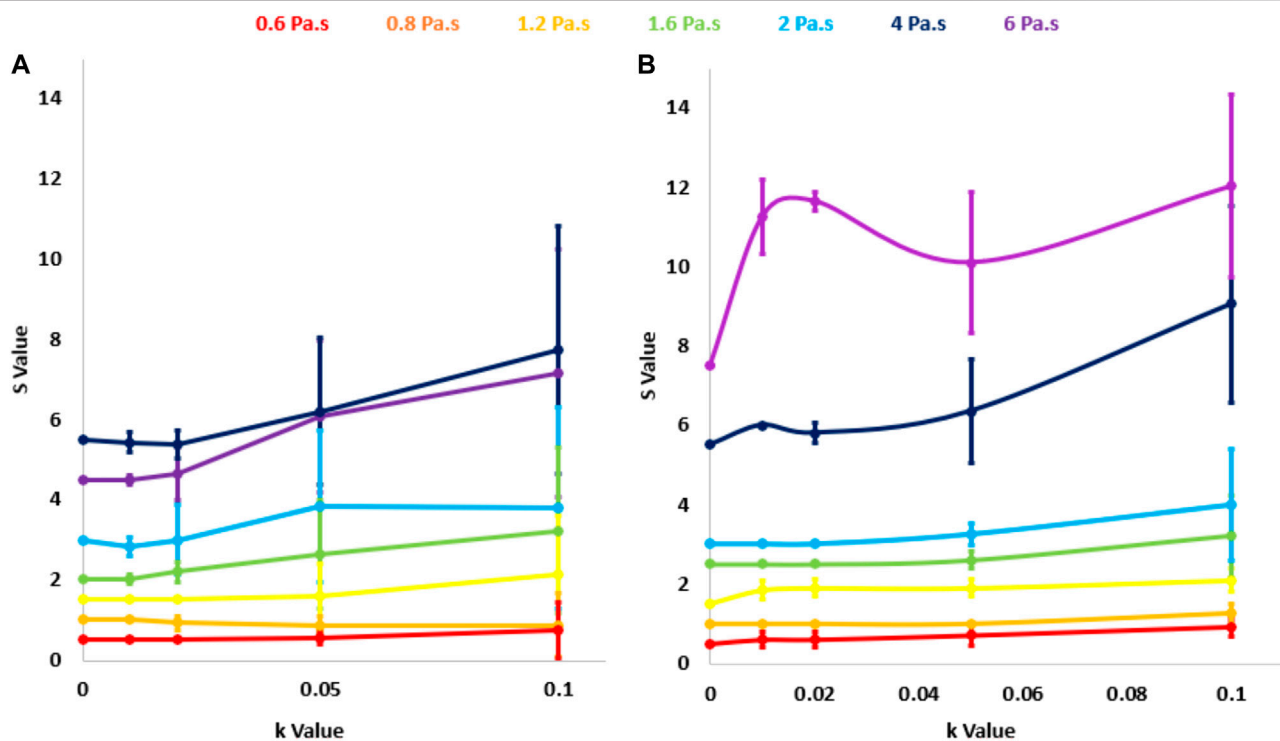


FIGURE 7 | S parameter calculated with the automated low-pass filter (A) and the singular value decomposition filter (B) as a function of the noise level k . Each curve corresponds to a different degree of viscosity η .

was higher than the CNR of R by a factor of six for the low viscosity material and a factor of 5.5 for the high viscosity material. However, beyond $k = 0.05$ the CNR of S was negligible compared to the CNR of R .

For the low-pass noise filtering method, the automatically-selected cut-off frequency increased as a function of viscosity and

decreased as a function of the noise level. This is shown in **Figure 5**, where the cutoff frequency is plotted as a function of the noise factor k . Each curve corresponds to a different degree of viscosity.

For the SVD noise filtering method, the optimum singular value cutoff was chosen based on the maximum value of the CTER. **Figure 6**

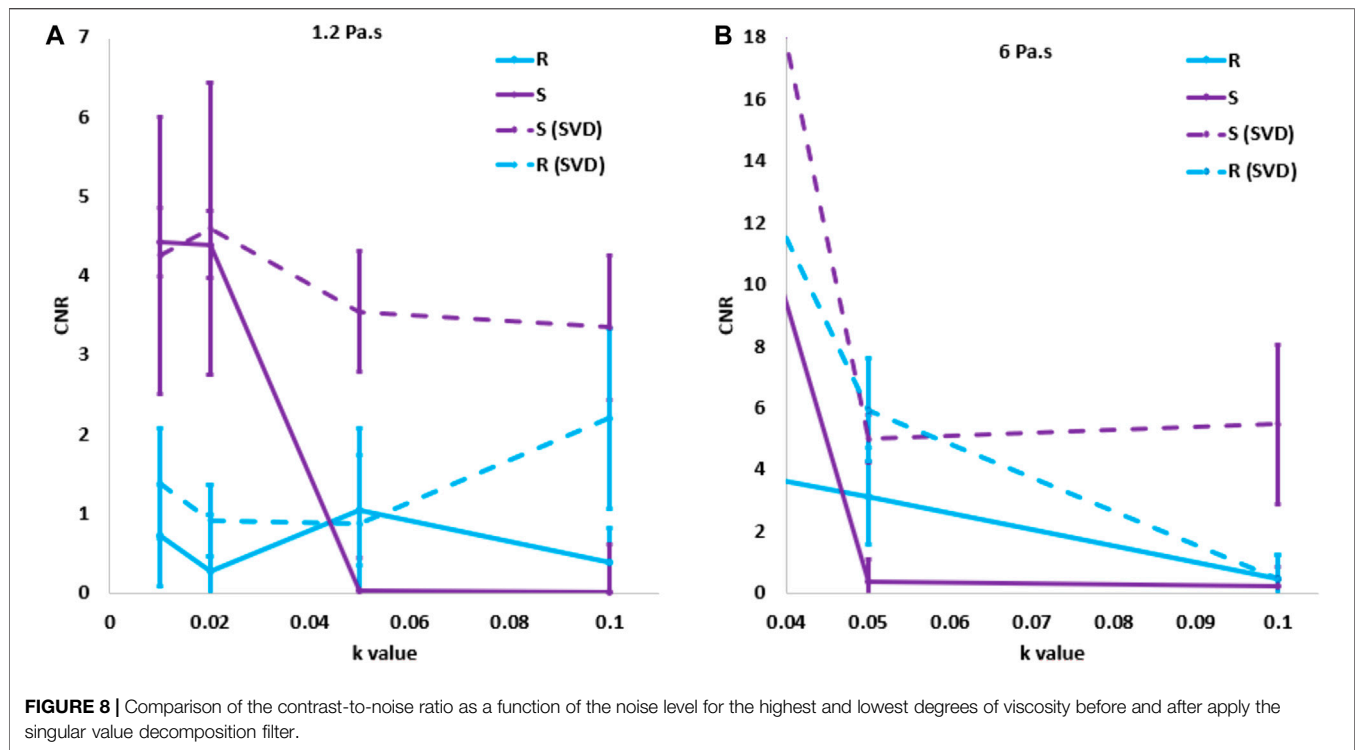


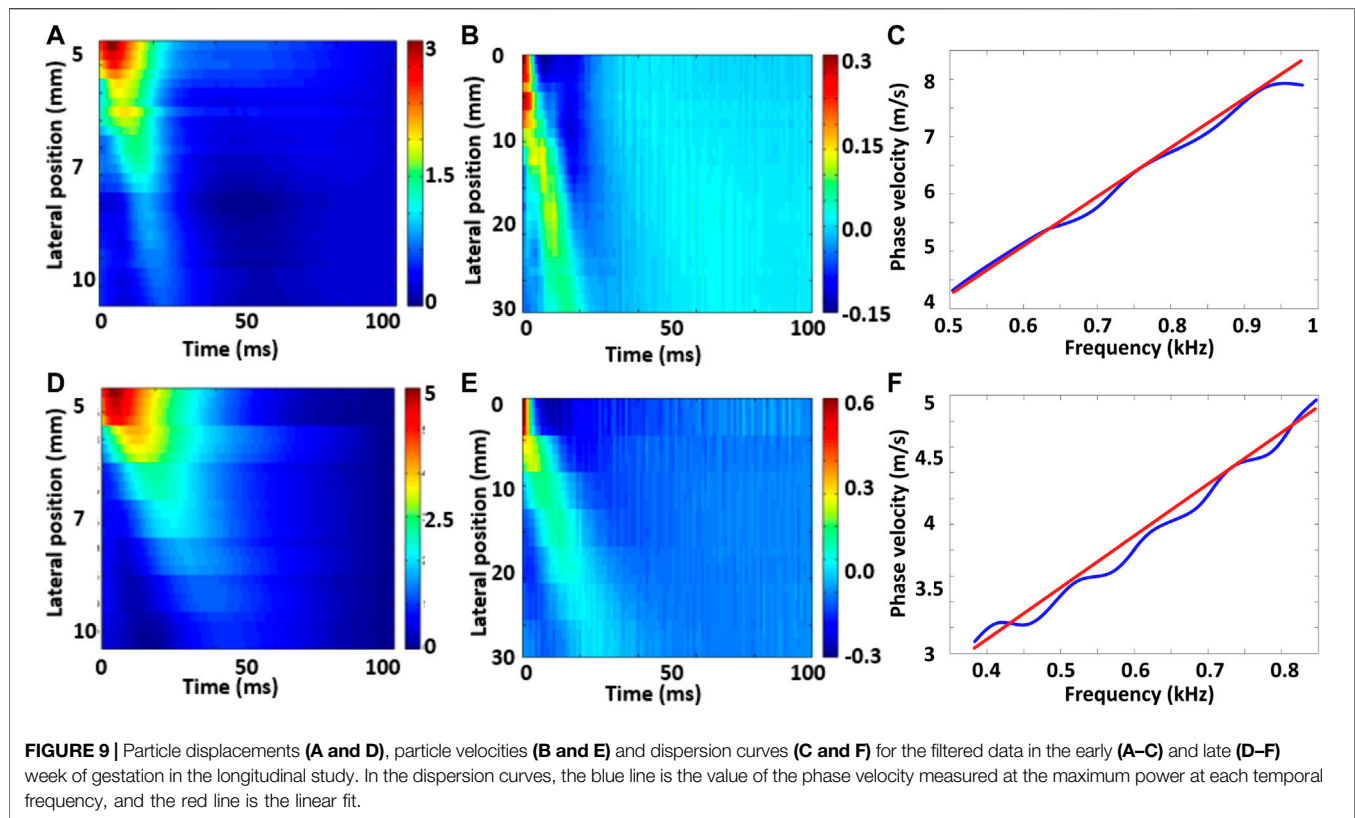
TABLE 3 | S values of each macaque calculated in the anterior and posterior region with respect to the cervical canal during gestation weeks 10 and 23. Cervical mean thickness is also showed for each macaque.

Subject number	S ₁₀ (m/s-kHz) anterior	S ₂₃ (m/s-kHz) anterior	S ₁₀ (m/s-kHz) posterior	S ₂₃ (m/s-kHz) posterior	Mean cervical thickness (cm)
1	—	—	—	—	1 ± 0.2
2	—	5.8 ± 1.8	5.8 ± 1.3	6.8 ± 1	1.1 ± 0.4
3	—	2.5 ± 1	—	2.8 ± 0.3	0.7 ± 0.3
4	13 ± 1.5	2.9 ± 2	6.5 ± ND	3.8 ± 0.3	1.0 ± 0.3
5	7.5 ± 1	1.5 ± 0.5	7.5 ± ND	3.5 ± 0.6	1.1 ± 0.3
6	—	—	5.8 ± 1.3	—	1.1 ± 0.2
7	8.5 ± ND	3 ± 1	8 ± 3	5 ± 1.4	1.1 ± 0.3
8	6.8 ± 2.3	5.01 ± 1.2	6.3 ± 2.8	4.8 ± 1	1.0 ± 0.2
9	—	—	—	—	1.1 ± 0.3
10	—	6.8 ± 2.3	6.5 ± ND	3.8 ± 0.8	1.0 ± 0.2
11	—	—	—	—	1.1 ± 0.2
12	—	—	—	5 ± 1.5	1.4 ± 0.3
13	—	5 ± 1	—	3.8 ± 0.3	1.0 ± 0.2
14	—	8.8 ± 0.8	—	6.8 ± 0.1	1.3 ± 0.2
15	—	—	—	4 ± 0.5	1.0 ± 0.4
16	7 ± ND	9.5 ± ND	0.5 ± ND	6 ± 1	1.4 ± 0.4
17	—	—	—	5.8 ± 0.3	0.9 ± 0.4
18	—	3.3 ± 0.3	—	4.1 ± 1	0.8 ± 0.2

shows the CTER as a function of the singular value cutoff index for the material with 0.8 Pa s viscosity and the material with 6 Pa s viscosity. The CTER had a maximum value around the index seven for both materials. Thus, we selected a value of seven for the cutoff index.

Figure 7 shows parameter *S* obtained after applying the low-pass filter (a) and the SVD filter (b), as a function of the noise parameter *k* and for various degrees of viscosity. With the low

pass filter, changes in *S* as viscosity increases were not significant as indicated by the overlapping error bars. Furthermore, the highest value of *S* does not occur with the higher values of viscosity, suggesting ambiguity in the values of *S* that result from the use of this filtering strategy. This did not occur with the SVD filter, for which *S* increased monotonically with viscosity and changes with viscosity did not overlap. Because of this, the low pass filter was rejected.



After applying the SVD filter, the S parameter showed the highest CNR for each degree of noise and viscosity. **Figure 8** shows the CNR of both R and S as a function of the noise factor without any filter and with the SVD filter. The combination of descriptor S and the SVD filtering for the highest level of noise resulted in 11.6 times higher CNR than R descriptor with the same filtering strategy. We applied the SVD filter to the estimation of descriptor S to data from the *in vivo* NHP study, zeroing singular values at and beyond index 7.

3.2 In vivo NHP Application

Table 3 summarizes the mean and standard deviations of the S parameter at weeks 10 and 23 for the anterior and posterior regions of the 18 macaques. Empty cells correspond to cases in which no measurements passed the quality criteria described above. No standard deviations (ND) are reported for those cases in which only one measurement passed the quality criteria. Mean cervical thickens for each animal is also reported in **Table 3**. **Figure 9** shows an example of the particle displacements (a,d), particle velocities (b,e) and dispersion curves (c and f) for data from different subjects at week 10 (top row) and week 23 (bottom row). In this example, the maximum values of the particle velocity propagate at a faster rate at early pregnancy than at late pregnancy, which indicates overall stiffer tissue in the former case. Dispersion curves show higher phase velocities vs. frequency at mid pregnancy than at late pregnancy.

In general, S decreased between weeks 10 and 23 in the anterior cervix portion (Wilcoxon $p = 0.03$). Shear wave

dispersion also decreased in the posterior portion, but the difference was not statistically significant (Wilcoxon $p = 0.051$). **Figure 10** shows box plots that group estimates of S for all the subjects that passed the quality criteria at weeks 10 and 23 for (a) the anterior and (b) the posterior portions of the cervix.

With exception of S values in the posterior side at week 23 ($p = 0.065$), all data complied with the assumption of normality. **Table 4** summarizes the results of the final LME model after sequentially rejecting non-significant effects. The most significant effect was that of the week of gestation ($p < 0.00001$), which resulted in a reduction of 0.37 ± 0.07 m/s-kHz per week from the initial value of 15.5 ± 1.9 m/s-KHz. Also, dispersion decreased with the mother's age ($p = 0.008$) and was higher in the anterior portion ($p = 0.0009$). Furthermore, significant correlations were found between the week of gestation and the cervical portion, and the maternal age and the cervical portion.

4 DISCUSSION

The goal of this study was to evaluate the changes in shear wave dispersion quantified through SWEI in an *in vivo* NHP model of the cervix between mid and late gestation. To this end, we performed a pre-selection of two SWEI-based descriptors of shear wave dispersion, R and S and noise reduction strategies using finite-element simulations of viscoelastic media. The most important findings of this study are:

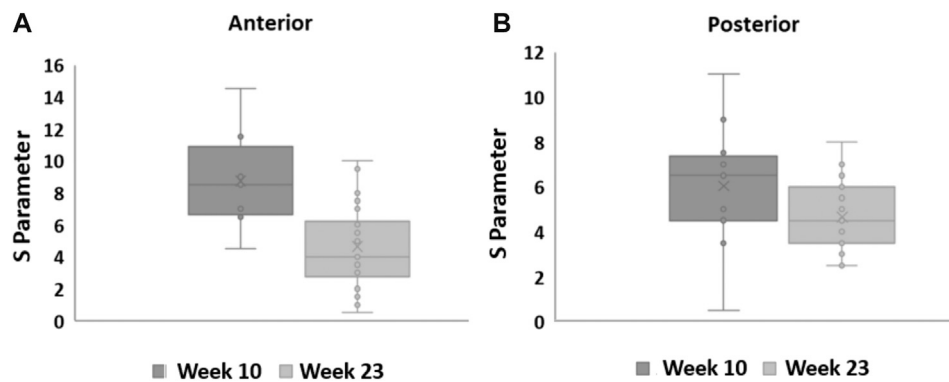


FIGURE 10 | Box plots for the S parameter estimated from *in vivo* NHP SWEI data at weeks 10 and 23 of pregnancy. The central line and cross indicate the median and mean, the top and bottom limits of the box represent the interquartile range, the whiskers indicate the range defined as 1.95 times the interquartile range above and below the 25 and 75 percentiles, and the circles indicate values for each subject.

TABLE 4 | Estimated coefficients, standard deviations, and *p* values for statistical significance of the linear mixed effects model for *S* in the *in vivo* NHP pregnant cervix.

Variable	Estimated	Std. dev.	<i>p</i> value
Intercept	15.51	1.91	< 0.00001
e_1 (Week of gestation)	−0.37	0.07	< 0.00001
e_2 (Mother's age)	−0.44	0.16	0.008
e_3 (Cervix portion)	−8.46	2.41	0.0009
$e_1 * e_3$	0.26	0.09	0.005
$e_2 * e_3$	0.45	0.19	0.02

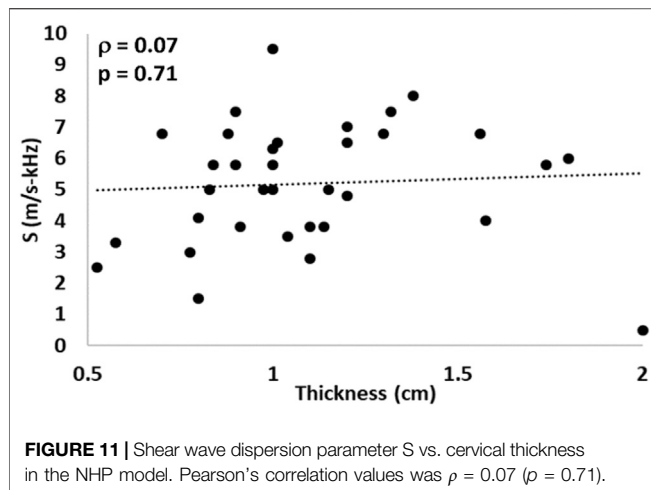
- The *S* parameter with the SVD noise reduction strategy showed the highest dispersion-based CNR compared to other combinations of parameter and noise reduction strategies.
- The combination of the *S* parameter and the SVD filter led to observe a significant reduction of shear wave dispersion in NHP cervix between mid and late pregnancy.
- As result of the application of the LME model, we found other significant effects that influence the value of the *S* parameter and included the mother's age and the cervix portion (anterior vs. posterior).

Based on a finite-element model, we observed that the frequency-domain parameter *S* in combination with the SVD filtering strategy produced the largest CNR among materials with different degrees of dispersion. The advantage of the SVD method over the automated low pass filter is based on its capacity to reduce both spatial and temporal contributions to noise. A disadvantage of this technique is the need to define an optimum cutoff value. A particularly complicated situation is how to treat frequency content due to bulk motion. In order to reduce the effects of such motion in the implementation of the optimized SVD strategy, we applied a second-order motion filter, as done by [40]. In the future, we will investigate the optimization of the selection of the singular value cutoff index to also reduce the contribution of bulk motion.

Other studies have applied SVD filtering in ultrasound studies like blood flow imaging [50]. However, the optimization of the cutoff value of SVD filtering for SWEI has not been widely studied. A particular case of SVD filtering in shear wave analysis can be found in geophysics applications. Chevrot and Girardin [51] used SVD filtering to reduce the noise in shear waves from seismic signals. In that study, a cutoff value was selected when a regular decrease of the singular spectrum was observed, which led to a cutoff index of 6. Here, we proposed a quantitative selection criterion considering the bias and variance in the estimation of the dispersion slope based on simulations with a large range of viscosity. The selected cutoff value of 7 was similar to that of [51].

In the animal study, we observed that the slope of the phase velocity vs. frequency decreased between mid and late pregnancy. Shear wave dispersion in the cervix can be attributed to the layered structure of collagen bundles, and to the presence of the viscous components of the extracellular matrix such as glycosaminoglycans. The collagen bundles are arranged in approximately three bands: an inner band with bundles aligned radially from the canal, a middle one with circumferential bundles, and an outer one with longitudinal bundles parallel to the canal [3]. Each band, with thickness of a few millimeters, can guide the propagation of the shear waves, as observed in tendon [26]. As pregnancy progresses, the layered collagen structure is lost and, therefore, shear wave dispersion caused by guided wave phenomena should decrease.

In addition to the bands of collagen, the thickness of the anterior and posterior portion of the cervix can lead to shear wave dispersion to the presence of Lamb waves. The average half-thickness (thickness of either the anterior or posterior portions) were 2.3 ± 0.3 and 1.1 ± 0.2 cm, respectively. We measured the half thickness instead of the full thickness because each half portion (anterior and posterior) is bounded by the cervical canal and the surrounding fascia. Because of this, we restricted the analysis of shear wave propagation to either the anterior or the posterior portions. To study the correlation between shear wave dispersion and the cervical thickness, we added a plot of the parameter *S* and cervical thickness (Figure 11). Even if the



wavelength is similar to the cervix half thickness, we did not find any correlation between the values of the S parameter and the cervical thickness and length (as indicated by the Pearson's correlation coefficient $\rho = 0.07$ ($p = 0.71$)). Also, the concentration of viscous components such as hyaluronan, decorin, and versican varies during pregnancy [52]. Thus, changes in shear wave dispersion during pregnancy result from competing phenomena. Taken together, our results suggest that the structural component of dispersion may have a larger influence on the observed changes between weeks 10 and 23 in the pregnant NHP cervix. To deepen our understanding of the influence of the different structural and molecular components of the cervix on dispersion measurements through SWEI, our laboratory is developing simulation tools based on the finite element methods.

After applying the criteria for reliability of dispersion measurements, eight of the 18 subjects showed reliable estimates in week 10, and 15 in week 23. The larger rejection of data in week 10 can be attributed to the fact that the NHP cervix is stiffer than the human cervix and other organs in which SWEI has been successfully applied, like the liver. The larger stiffness leads to noisier shear wave displacement values. Furthermore, the rejection of a large number of data is common in the initial steps of the development of quantitative imaging. For example, Castañeda-Martínez et al. [53] rejected data from 11 out of 16 subjects while implementing Ultrasound Backscatter Spectroscopy in the neonatal brain. Also, Wang et al. [54] rejected 50% of measurements when using shear wave elasticity imaging in the liver. Because the human cervix is softer than the NHP, we are confident that we will achieve more reliable dispersion estimates in the clinical application of the technique developed in this work.

Various works have reported the use of the shear wave group speed V_g as a surrogate for cervical softness in primates and humans [20, 23, 24, 30, 55–58]. For example [59], reported a decrease in the group speed of shear waves between ripened and unripened *ex vivo* human cervical samples, 39% for the anterior region and 25% for the posterior region with a p value < 0.05 . Also, in a study in patients with a term pregnancy, V_g decreased

39% between pre and post-ripening in the context of labor induction, with a p value < 0.001 [18]. Here we observed a reduction in dispersion of 53% with a p value much smaller than the reported changes for the group speed. This indicates that the shear wave dispersion can be more sensitive to cervical remodeling than the group speed. This could be attributed to the more direct relationship between shear wave dispersion and structural properties of the cervix, as well as the optimization of noise reduction strategies.

Using the linear mixed-effects model on the values of S , we found that age and cervix portion (anterior vs. posterior) had a significant influence on shear wave dispersion. Rosado-Mendez et al. [21] observed significant differences in the group speed between the anterior and posterior NHP cervix. Carlson et al. [60] reported similar differences in humans. These differences can be attributed to spatial variations in the microstructural properties of the cervix, a weight gradient created by the fetus, and/or the rotated orientation of the cervix with respect to propagation direction of the shear wave. Another factor could be a pre-compression of the posterior cervix due to the presence of the transducer. Also, these differences could be attributed to variations in the shear wave frequency content due the attenuation and focusing of the pushing beam. The significance of the mother's age could be attributed to the changes in the production and reception of glycosaminoglycans as the mother age increases [61]. Despite the influence of these factors, the most important effect on shear wave dispersion was the week of gestation, thus indicating the potential of this technique to track cervical remodeling during pregnancy.

This study had various limitations. The noise model that we used in the simulation part was Gaussian and modulated by the maximum shear wave displacement of each displacement field [38]. Although simplistic, this model allowed us to performed a comparative selection of the descriptor of dispersion that maximized the detection of different levels of dispersion. The second limitation is the large fraction of rejected data at week 10. This can be attributed to the stiffer state of the cervix at mid pregnancy compared to late pregnancy, which results in lower signal/noise of displacements estimates. This might be a limitation when performing future comparisons of the early vs. late pregnancy cervix. Since the human cervix is slightly softer than the NHP cervix, we expect that the rejection rate will not be as high in clinical applications of the proposed technique. Finally, the S parameter does not provide a quantitative assessment of the changes in the structural components that determine the dispersive response of the cervix. We are currently exploring the quantification of shear wave attenuation and the fit of rheological models to shear wave dispersion and attenuation to obtain a more detailed characterization of the cervix structural components. Although this strategy has been proposed by other groups, they used simplistic viscoelastic models such as Voigt's [28]. Other models such as the Prony series may provide a more accurate assessment of the structural components.

5 CONCLUSION

In this study we used shear wave dispersion as a surrogate of cervical remodeling during pregnancy in a non-human primate

model. We compared the sensitivity of two dispersion descriptors extracted from either a time-domain or a frequency-domain analysis of shear wave propagation in finite element simulations of viscoelastic media. We observed that the frequency-domain parameter S , defined as the phase velocity slope vs. frequency, in combination with noise-filtering based on singular value decomposition provided better differentiation of viscoelastic materials with different levels of dispersion than other combinations of parameters and filtering strategies. When applied to the NHP model, we observed a significant reduction of shear wave dispersion between mid and late pregnancy in the NHP model, likely due to the disorganization of cervical collagen during pregnancy. Our results suggest the potential of shear wave dispersion as a quantitative imaging biomarker of cervical remodeling. Future studies will apply this analysis to data from longitudinal human studies of normal and preterm pregnancy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison.

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AUTHOR CONTRIBUTIONS

AT contributed with data processing, analysis and discussion of results, and manuscript drafting. MP contributed with generation of simulations and discussion of results. HF contributed with data acquisition, analysis and discussion of results, and manuscript drafting. TH contributed to the discussion of results and manuscript drafting. IR-M contributed with data acquisition, supervising data processing, manuscript drafting, and discussion of results.

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Shear Wave Elastography Based on Noise Correlation and Time Reversal

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Shear wave elastography (SWE) relies on the generation and tracking of coherent shear waves to image the tissue's shear elasticity. Recent technological developments have allowed SWE to be implemented in commercial ultrasound and magnetic resonance imaging systems, quickly becoming a new imaging modality in medicine and biology. However, coherent shear wave tracking sets a limitation to SWE because it either requires ultrafast frame rates (of up to 20 kHz), or alternatively, a phase-lock synchronization between shear wave-source and imaging device. Moreover, there are many applications where coherent shear wave tracking is not possible because scattered waves from tissue's inhomogeneities, waves coming from muscular activity, heart beating or external vibrations interfere with the coherent shear wave. To overcome these limitations, several authors developed an alternative approach to extract the shear elasticity of tissues from a complex elastic wavefield. To control the wavefield, this approach relies on the analogy between time reversal and seismic noise cross-correlation. By cross-correlating the elastic field at different positions, which can be interpreted as a time reversal experiment performed in the computer, shear waves are virtually focused on any point of the imaging plane. Then, different independent methods can be used to image the shear elasticity, for example, tracking the coherent shear wave as it focuses, measuring the focus size or simply evaluating the amplitude at the focusing point. The main advantage of this approach is its compatibility with low imaging rates modalities, which has led to innovative developments and new challenges in the field of multi-modality elastography. The goal of this short review is to cover the major developments in wave-physics involving shear elasticity imaging using a complex elastic wavefield and its latest applications including slow imaging rate modalities and passive shear elasticity imaging based on physiological noise correlation.

Keywords: ultrasound, shear wave, elasticity imaging, near field effect, passive elastography

1 INTRODUCTION

The goal of Shear Wave Elastography (SWE) is to measure the tissue's shear elasticity μ (i.e. elasticity). To this end, SWE relies on shear wave propagation inside soft tissues, since under the assumption of a purely elastic isotropic medium, the shear wave speed c_s is directly linked to μ through the relation $\mu = \rho \cdot c_s^2$ (ρ being the tissue's density). The standard sequence in many SWE modalities is the following: first, shear waves are generated by carefully applying an external controlled shear wave source (e.g., mechanical actuator or ultrasound radiation force). Then, the induced displacements are imaged, usually by using ultrasound or a magnetic resonance imaging (MRI) system. In ultrasound based SWE ultrafast frame rates (i.e. between 1 to 20 kHz) are required

to track the shear wave propagation. Alternatively, for low frame rates imaging modalities, a phase-lock synchronization between acquisition and shear wave source is needed (e.g., in magnetic resonance elastography). Finally, the tissue's shear elasticity is deduced from the measured displacement field by estimating the shear wave speed, or alternatively, the shear wavelength (i.e., if the shear wave excitation frequency is known). Recent technological developments have allowed SWE to be implemented in commercial ultrasound and MRI systems, quickly becoming a new imaging modality in medicine and biology [1–3].

In SWE, the shear wave speed is usually estimated from the coherent or ballistic shear wave propagation. However, there are many applications where coherent shear wave tracking is not possible due to the interference of scattered waves coming from tissue boundaries and internal inhomogeneities. Moreover, additional waves generated by muscular activity, heart beating or other sources of vibrations may interfere with the coherent shear wave resulting in a complex elastic wavefield. Directional filtering has been proposed to solve this issue [4, 5]. However, other researchers focused in developing alternative approaches to measure μ (or equivalently c_s) from a complex elastic wavefield [6–18]. For example, in the TREMR technique (Table-Resonance Elastography with MR), the elastic field created by the vibrations of the patient table of a MRI system was used to image the elasticity of a tissue phantom and the brain [7]. Moreover, several authors have chosen to take advantage of the complex elastic wavefield naturally present in the human body due to pulsatility, heart beating and muscular activity to conduct an elastography experiment [8–11, 13, 14, 19–21]. Due to the absence of any external shear wave source this approach is usually termed as passive elastography.

Recently, inspired by seismic noise correlation [22, 23] and time reversal [24] a novel method [12, 25, 26] to extract the shear elasticity of tissue from a complex elastic field was developed. The first step is to record the complex wavefield generated by random internal or external sources. Then, by cross-correlating the elastic field at different positions, which can be interpreted as a time reversal experiment in the computer [27], shear waves are virtually focused on any point of the imaging plane. From the computed time reversal field there are different independent approaches to image the shear elasticity [12–15, 18], for example, tracking the coherent shear wave as it focus; measuring the focus size which is directly linked to the shear wavelength (λ_s) and hence to the shear wave velocity; or evaluating the vibration amplitude at the focusing point, since for a given frequency, the vibration amplitude is larger in a soft tissue than in a hard one. Because this method takes advantage of a complex elastic wavefield it has found important applications in passive elastography [13, 14].

The pioneering work in this field was done at the Laboratorio de Acústica Ultrasonora (LAU) in Montevideo, Uruguay in collaboration with Stefan Catheline, who was at the LAU for a 2 year mission. The work of Catheline et al. [25] presented the observation of a time reversal experiment using shear waves in a tissue-mimicking phantom for the first time. In [25] it was shown that, contrary to the scalar field case (e.g., in fluids), for an elastic field in the bulk of a soft tissue, the focus is no more isotropic.

Instead, it has an ellipse-like shape leading to a direction dependent Rayleigh criterion. Then, Benech et al. [12] established numerically that the focus width at -6 dB was approximately equal to one shear wavelength. This approach was termed the focus size (or width) method and was used to measure the elasticity of a tissue mimicking phantom. This method was shown to be independent of the source kind, shape, and time excitation function. This robustness regarding the shear wave source allowed envisioning its application to passive elastography. Alternatively, in [12], the phantom's elasticity was measured by tracking the phase of the coherent shear wave as it focused back to the source (i.e. the phase method). Both methods (phase and focus size), were also used by Brum et al. [26] to measure the elasticity of a tissue mimicking phantom and cheese using surface waves. Finally, the feasibility to conduct 2D elasticity imaging *in vivo* using passive elastography was demonstrated by Gallot et al. [13] in the human liver and belly muscle. To image the shear wave speed they used an ultrafast ultrasound scanner along with the phase and focus size methods. The main advantage of the focus size method is its compatibility with low imaging rates modalities. This was first shown in [12] and then used in [13] to conduct a shear wavelength tomography of a two-layered tissue-mimicking phantom. In Catheline et al. [14] it was further demonstrated that the loss of time and/or spatial coherence of the recorded wavefield is not an obstacle for a wavelength tomography, which led to new passive elastography experiments using optical coherence tomography (OCT) [21] and MRI [20]. Finally, in [15] an analytical expression that allowed converting the wavelength tomography into shear elasticity was derived. The compatibility with low imaging rates modalities has led to innovative developments and new challenges in the field of multi-modality elastography. Particularly, it has boost the concept of passive elastography to new applications involving ultrafast and ultraslow imaging modalities like ultrasound, optical methods (digital holography or OCT) and MRI.

In this context, the goal of this short review is to cover the major developments in wave-physics involving elasticity imaging using time reversal and noise correlation of shear waves together with its latest applications. These include time reversal physics and near field effects of shear waves in soft tissues [15, 28, 29], passive brain elasticity imaging using MRI [20], cornea elasticity imaging using OCT [21], thyroid [14] and breast [18] elasticity imaging using low frame rate ultrasound scanners, liver elasticity imaging [13, 30], passive muscle elasticity assessment [11, 19] and most recently cellular elasticity imaging [31].

2 TIME REVERSAL AND NOISE CORRELATION OF SHEAR WAVES

Time reversal was first proposed by Mathias Fink [24] and is based on the time-reversal invariance of the wave equation in a lossless medium. Time-reversal focusing is a two-step process. In a first step, the direct wave scene is recorded: the impulse response of a point source placed in $\vec{r}_0$ is measured by a set of receivers forming a closed cavity around $\vec{r}_0$. In a second step, the recorded wavefield is time reversed and sent into the medium through the

same position where it was received. As a consequence of the $t \rightarrow -t$ invariance of the wave equation in a lossless medium, the wave will travel the same original path but in opposite direction to finally focus at the original source position $\vec{r}_0$ and then diverges. Due to the impulsive character of the source, the time reversal field $\psi^{TR}(\vec{r}_0, \vec{r}, t)$ around $\vec{r}_0$ can be written as [32]:

$$\psi^{TR}(\vec{r}_0, \vec{r}, t) = G(\vec{r}_0, \vec{r}, -t) - G(\vec{r}_0, \vec{r}, t) \quad (1)$$

where $G(\vec{r}_0, \vec{r}, t)$ is the Green's function between $\vec{r}_0$ and $\vec{r}$. **Equation 1** clearly reflects the focusing process: the causal Green's function $G(\vec{r}_0, \vec{r}, t)$ corresponds to a diverging wave from $\vec{r}_0$ while the acausal Green's function $G(\vec{r}_0, \vec{r}, -t)$ corresponds to a converging wave toward $\vec{r}_0$. The maximum amplitude is found to be at $\vec{r}_0$ (focusing point) at time $t = 0$ (focusing time).

In practice, an ideal time-reversal cavity as described above is not necessary and can be replaced by a time-reversal mirror using reverberation and/or a multiple scattering medium [33]. For elastography applications one can take advantage of multiple sources, reverberation and diffusion to conduct a time reversal experiment. Specifically, the step where the time reversed field is sent back into the medium (i.e., the second step in a time reversal experiment) is replaced by a virtual time-reversal experiment based on spatial reciprocity and cross-correlation of the wavefield [12].

Usually the imaging systems used in elastography (e.g., ultrasound, OCT or MRI) allow recording at least one component of the elastic wavefield within a given region of interest (ROI). Let this component be $\psi(\vec{r}, t)$. The correlation field at a given position $\vec{r}_0$ is computed by cross-correlating the field $\psi(\vec{r}_0, t)$ with the field $\psi(\vec{r}, t)$ acquired at all other positions $\vec{r}$ within the ROI.

$$C(\vec{r}_0, \vec{r}, t) = \psi(\vec{r}_0, -t) \otimes \psi(\vec{r}, t) \quad (2)$$

If the field is diffuse, **Eq. 2** allows the retrieval of the Green's function [34]:

$$\frac{\partial}{\partial t} C(\vec{r}_0, \vec{r}, t) \propto G(\vec{r}_0, \vec{r}, -t) - G(\vec{r}_0, \vec{r}, t) \quad (3)$$

The analogy between time reversal and cross-correlation follows directly from comparing **Eqs. 1, 3**: the time derivative of the correlation field is proportional to the time-reversal field. The proportionality constant in **Eq. 3** will depend on the propagation equation, medium and source properties, propagation regime, etc. For instance, in the case of a viscoelastic medium with homogeneously distributed white noise sources Gouedard et al. derived **Eq. 3** with the factor $4a/\sigma^2$ on the left hand side, with a being the attenuation and σ the average source spectrum [35]. Moreover, if the displacement field has a finite bandwidth (as in most experiments), the correlation and its time derivative only differ in a constant phase change [36] and the correlation field may be directly interpreted as the time reversal field. Formally, the relation between cross-correlation and time-reversal can be expressed by a representation theorem of the

correlation type [37]. Consider a lossless elastic medium with volume V and bounding surface S . Let $G_{mn}(\vec{r}_0, \vec{r})$ represent the Green's function between a point harmonic source at $\vec{r}_0$ acting in direction m and observed at $\vec{r}$ along direction n . The representation theorem in the frequency domain relates the field at two arbitrary points $\vec{r}_0$ and $\vec{r}$ within V with the traction and displacement at the surface S :

$$\begin{aligned} - \int_S \left[G_{mj}(\vec{r}_0, \vec{R}) T_{jn}(\vec{R}, \vec{r}) - G_{nj}^*(\vec{r}, \vec{R}) T_{jm}(\vec{R}, \vec{r}_0) \right] dS \\ = G_{mn}(\vec{r}_0, \vec{r}) - G_{mn}^*(\vec{r}_0, \vec{r}) \end{aligned} \quad (4)$$

where $T_{jm}(\vec{R}, \vec{r}_0)$ is the traction along direction j at a point $\vec{R}$ on the surface S created by an harmonic point source at $\vec{r}_0$ along direction m . The right-hand side of **Eq. 4** is the superposition of the field at $\vec{r}$ due to a point source at $\vec{r}_0$ and of its time-reversed version. Thus, the point $\vec{r}_0$ can be interpreted as a virtual source embedded in the medium. The surface S of **Eq. 4** can be interpreted as the mirror position in a time reversal experiment, or the position of noise sources for cross-correlation. In a fully diffuse field, the left-hand side of **Eq. 4** is the spatial average of the cross-correlation field over all sources [38]. The scalar version of the representation theorem is also the basis of time reversal acoustic introduced by Cassereau and Fink [32].

3 INVERSION METHODS IN CROSS-CORRELATION BASED ELASTOGRAPHY

Equations 1–4 are the point of departure of many inversion methods developed during these past years to image μ from the correlation field interpreted as a time-reversal experiment. **Figure 1** illustrates the main steps involved in an cross-correlation based elastography experiment: from the wavefield acquisition (**Figure 1A**) to the final shear elasticity image (**Figure 1D**). The estimation of μ by cross-correlation requires the presence of a diffuse field. In practice, the way that elastography methods have found to create such field is the use of multiple uncorrelated sources in time and space. The complexity of the field comes from the interference of the direct and reflected waves created by the different type of sources: external (active methods), internal (passive elastography) or both.

Cross-correlation methods allow to reconstruct a refocusing wavefield from an apparently random and disorganized wavefield. **Figure 1B** shows two snapshots of the cross-correlation field $C(\vec{r}_0, \vec{r}, t)$. At $t = -6$ ms the shear wave front (indicated by a black dashed line) converges toward the focusing point $\vec{r}_0 = (50, 40)$ mm. Long time acquisitions ensured that each point within the ROI receives waves from all directions.

From the correlation field there are different independent inversion methods to image μ or equivalently c_s (**Figure 1C**), e.g. tracking the coherent shear wave as it focus (phase method), measuring λ_s from the focus size or evaluating the vibration amplitude at the focusing point. In this section a brief overview of these and other inversion methods will be given (specific details can be found in [14, 15, 28, 29]). A summary of these methods

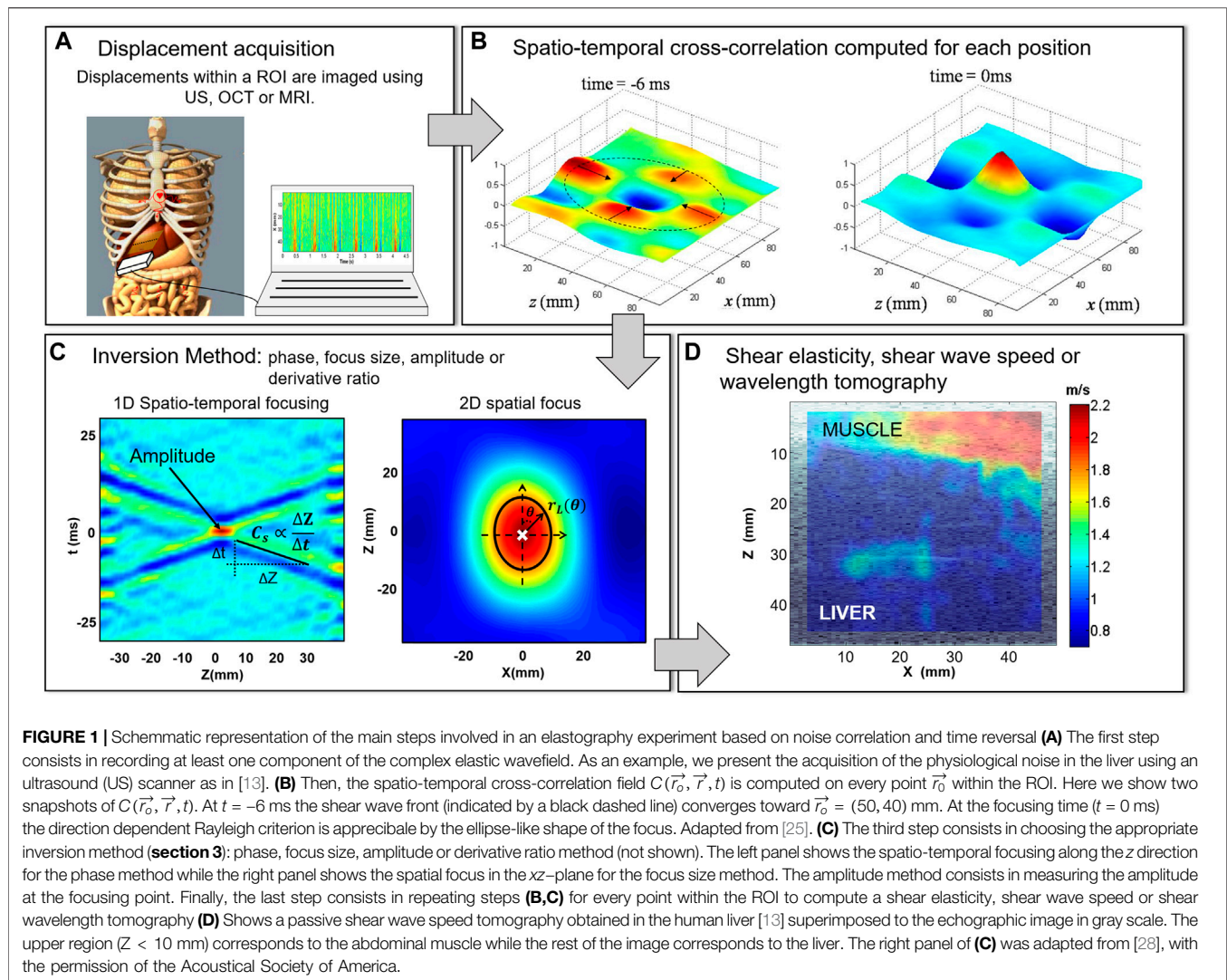


TABLE 1 | Summary of the methods and applications based on noise correlation elastography.

Method	Applications	Imaging modality	Compatibility with ultraslow imaging rates	References
Focus size	Liver, cells, cheese	Ultrasound, ultrafast microscopy, surface sensors	Yes, but not quantitative	[13, 26, 31]
Phase	Muscle, cheese	Surface sensors, ultrasound	No	[11, 19, 26]
Amplitude	Breast	Ultrasound	Yes	[18]
Derivative ratio	Cornea, brain, thyroid, liver	Ultrasound, OCT, MRI	Yes, but not quantitative	[14, 20, 21, 30]

along with its applications, advantages and references is given in **Table 1**.

3.1 Phase Method

The phase method consists in measuring the phase velocity V_ϕ of the shear wave as it focus back to the source. The left panel of **Figure 1C** shows the evolution of the correlation field along z direction, with focusing point at $\vec{r}_0 = (0, 0)$ mm. The cross-like

shape of this figure indicates a field coming from positive and negative z direction that converges at the focusing point and then diverges. As in a classical SWE experiment, the slope of each branch of the cross is linked to V_ϕ and hence to the shear wave speed c_s . Directional filters can be applied to separate each direction of propagation [4]. Then, by changing the focusing point $\vec{r}_0$ all over the ROI a shear elasticity image is constructed. This procedure was followed by Gallot et al. [13] to image the

shear elasticity of a two layered phantom. The main advantage of this method is that in the far field the local phase velocity equals the shear wave velocity ($V_\phi \cong c_s$). However, in the near-field ($\sim \lambda_s/2$) a correction to convert the phase velocity into the shear wave speed is needed. The correction factor between V_ϕ and c_s depends on the observation direction as [28]:

$$V_\phi = \frac{3c_s}{4} (1 + \gamma_m^2) \quad (5)$$

where γ_m^2 is the direction cosine between the recorded component of the wavefield $\psi(\vec{r}, t)$ and the direction of observation. Thus, near-field corrections are needed to compute the final elasticity image. For example, for ultrasound systems the recorded component of the wavefield usually coincides with the direction of the ultrasound beam (defined as z in **Figure 1**). Therefore, $\gamma_m^2 = \cos(\theta)$ and $V_\phi = 3c_s/2$ in **Figure 1C**. Finally, we note that for this method an ultrafast frame rate is needed in order to follow the time evolution of the cross-correlation field.

3.2 Focus Size Method

An alternative inversion method that has shown to be compatible with low imaging rates modalities is the focus size method. In a time reversal experiment with shear waves, the size of the focus is limited by diffraction to dimensions comparable to the shear wavelength. As a first approximation, one can consider that the size of the focus Δ , given by the width at half maximum, is related to λ_s via the Rayleigh criterion for a scalar wavefield: $\Delta = \lambda_s/2$. Given the central frequency f of the field, the shear wave speed can be retrieved via $c_s = 2\Delta f$. This procedure was used by Gallot et al. [13] in a passive elastography experiment. However, as described in the early works of Catheline and Bence et al. [12, 25], the focus width is direction dependent. As it focuses, the converging shear wave gives birth to near-field effects, even if it was recorded in the far-field [25]. These near-field effects (observable in **Figure 1B**) imply a direction dependent focus size, which is related to the dipole characteristics of the simple source for shear waves in elastodynamics [28]. Therefore, a more detailed analysis was needed to establish a quantitative relation between the focus size and λ_s .

In [28], by substituting the free space Green's function for elastodynamics [39] on the right hand side of **Eq. 4**, the following expression relating the focus size $r_L(\theta)$ with λ_s was derived:

$$r_L(\theta) = \frac{\lambda_s}{2\pi} \sqrt{\frac{10(1-L)}{2 - \cos^2(\theta)}} = \frac{c_s}{2\pi f} \sqrt{\frac{10(1-L)}{2 - \cos^2(\theta)}} \quad (6)$$

where $r_L(\theta)$ denotes the distance from the focusing point to the contour level at which the correlation field takes a fraction $L < 1$ of its maximum value (i.e. $L = 1$ corresponds to the focusing point) and θ corresponds to the polar angle. The right panel of **Figure 1C** shows the 2D focus in a tissue mimicking phantom with $c_s = 1.85$ m/s. The full black line corresponds to the contour level at -3 dB ($L = 0.7$), the white cross indicates the focusing point and the distance $r_L(\theta)$ is represented by a black arrow.

Equation 6 can be used to retrieve λ_s or c_s if the frequency f is known. However its main drawback is that it was derived for single frequency f . The signals in the experiments are composed

of multiple frequencies within a given bandwidth. Brum et al. [15] modified the above expression to include broadband signals by defining an effective frequency. This effective frequency corresponds to the frequency root mean square of the bandwidth using the power spectrum of the correlation field as weighting function. Finally, in [15] the validity of such expressions was demonstrated numerically and experimentally on a tissue-mimicking phantom consisting of two different elastic layers demonstrating the potential of the technique to quantitative shear elasticity imaging.

One advantage of the focus size method is that the time and spatial coherence are decoupled in **Eq. 6**. Thus, it is still possible to retrieve λ_s even if the field is under sampled in the time domain, using a frame rate well below the Nyquist-Shannon sampling limit (i.e. no frequency information). This fact was shown for the first time in [12]. In this work, using 1D ultrasonic elastography, the focus size of a wavefield at 100 Hz central frequency was measured twice in an agar-gelatin phantom. The first time using ultrafast electronics (1 kHz sampling frequency) and the second one the field was under-sampled at 40 Hz rate. The focus sizes were shown to be equal for both experiments. The potential to image the elastic properties using low frame rates was later taken up in other works. In the work of Brum et al. [15], an experiment referred to as “ultraslow” (in contrast with ultrafast) was able to image the elasticity of a bilayer phantom using 10 Hz sampling frequency. In addition, the spatial resolution of the method was discussed. The conclusion is that the contour level L should be chosen as high as possible to improve the spatial resolution. Nevertheless, a trade-off exists because the presence of noise in the autocorrelation peak could make the contour level curve meaningless.

3.3 Amplitude Method

The focus size method discussed above makes use of the right-hand-side of **Eq. 4**. However, if the temporal frequency is unknown, as in the case of ultraslow experiments, it is not possible to convert the focus size into shear wave speed. To solve this issue, in the work of Rabin et al. [18] they chose to work out the left-hand-side of **Eq. 4**. As a result a quantitative expression relating the vibration amplitude at the focus (i.e., autocorrelation value) with the shear elasticity was derived. To this end, the traction $T_{jm}(\vec{R}, \vec{r}_0)$ was expressed in terms of the far-field approximation of the elastodynamic Green's function assuming that the observation points are many wavelength away from the surface. By making use of the Betty-Rayleigh identity [39] for mediums without volume sources and under the hypothesis of spatio-temporal uncorrelated sources, the shear elasticity is given by:

$$\mu(\vec{r}_0) = \left[\frac{NT^2 A_t}{6\pi\mu_0^{1/2} C(\vec{r}_0)} \right]^{2/3} \quad (7)$$

where $C(\vec{r}_0)$ is the autocorrelation value at the focusing point $\vec{r}_0$, N is the number of traction sources, A_t is the contact surface of each source and μ_0 is the shear elasticity at the surface of the tissue. For example, in [18], they used the mean shear elasticity of breast reported in the literature as a value for μ_0 .

3.4 Derivative Ratio Method

This method was first proposed by Catheline et al. [14] and was further developed in [29] by taking into account the near field effects in the vicinity of the focusing point. This method relies on the proportionality between Eqs. 1, 2 in the presence of a diffuse field and is valid for any field obeying a wave equation. If $\psi(\vec{r}, t)$ satisfies the wave equation, then, its temporal derivative $V(\vec{r}, t) = \partial\psi(\vec{r}, t)/\partial t$ and its spatial derivative $\xi(\vec{r}, t) = \partial\psi(\vec{r}, t)/\partial z$ will satisfy the same wave equation, consequently, they are time-reversal invariants. Let ξ^{TR} and V^{TR} designate the time reversal field associated to the spatial and temporal derivative, respectively. As an example, it suffices $\psi(\vec{r}, t)$ to be one component of the elastic displacement field. Then, its spatial and temporal derivatives correspond to the strain and the particle velocity field, respectively.

In an ideal isotropic diffuse field, plane wave decomposition at a given frequency allows to use the following approximations for the spatial and temporal derivatives: $\xi \approx ik\psi$ and $V \approx -i\omega\psi$. Thus, their time reversal fields can be written as: $\xi_0^{TR} \approx -k^2\psi_0^{TR}$ and $V_0^{TR} \approx -\omega^2\psi_0^{TR}$, with $\psi_0^{TR} = \psi^{TR}(\vec{r}_0, \vec{r}_0, t=0)$. Consequently, the ratio between the time reversal fields associated to the spatial and temporal derivatives allows to retrieve the local wave speed as:

$$c_s(\vec{r}_0) = \frac{\omega}{k} \propto \sqrt{\frac{V_0^{TR}}{\xi_0^{TR}}} \quad (8)$$

Or equivalently, for the case of ultraslow experiments the local shear wavelength can be estimated as:

$$\lambda_s(\vec{r}_0) \propto \sqrt{\frac{\psi_0^{TR}}{\xi_0^{TR}}} \quad (9)$$

In [14] the proportionality constants for Eqs. 8, 9 were assumed to be 1 and 2π , respectively, and it was demonstrated through simulations and experiments that the loss of time and spatial coherence of the recorded wavefield was not an obstacle for a tomographic reconstruction. Later, in the work of Zemzemi et al. [29] a proportionality constant of $\sqrt{1/5[2 - \cos^2(\theta)]}$ was found for Eq. 8 by introducing the near field effects described in [28].

3.5 Discussion

In this section the main advantages and drawbacks of the inversion methods presented above will be discussed. All of them rely on the presence of a diffuse field. Consequently, there is no need to control or to know the position of the shear wave source(s) nor the direction of its applied force. Moreover, reflected waves do not pose a problem as in a standard SWE experiment. This approach is advantageous in tissues where is difficult to isolate a coherent shear wave propagation from its reflections, for example, in tissues with complex boundary shape or containing several inhomogeneities. Here the more complex the wavefield the better, since waves arriving from all directions are required to improve the Green's function retrieval from the cross-correlation. In addition, the specific shape of the shear wave sources is not relevant either.

Paradoxically, the need of a diffuse field is also the main drawback of these methods. The hypothesis of an homogeneous repartition of sources (i.e. isotropic shear wave distribution within the ROI) is needed for the exact Green's function retrieval through cross-correlation [35]. However, this is difficult to achieve in practice. First, wave attenuation may set a limit to the diffusion process. To minimize the effects of attenuation many authors used multiple sources in their experiments [18, 29]. Second, in an internal organ, waves usually come from a given region inside/outside this organ. This may be due to the limited access when using external sources or because physiological noise comes from a preferred region within the body. For example, in the work of Gallot et al. [13] it was noted that the physiological noise recorded in the liver was highly directive coming mainly from the heart region. As result only one branch of the cross-correlation evolution used in the phase method (left panel of Figure 1C) was observed. This directivity of the wavefield can lead to biases in the reconstructed shear wave speed tomography. Nevertheless, some strategies are envisaged to overcome this drawback. One of them is to use a passive inverse filter [40] which allows an optimal spatial redistribution of the incoming energy. Another strategy is to enhance the isotropic distribution shear waves by using multiple sources. This was done in Rabin et al. [18] for the breast and in Ormachea et al. [17] in the liver by including multiple active vibration sources in the patient's clinical bed. The use of multiple sources may also be advantageous to increase and control the frequency content of the diffuse field. The knowledge of the shear wave excitation frequency allows the shear wavelength tomography conducted with ultraslow imaging modalities to be converted into shear elasticity.

The main advantage of the phase method is a direct estimation of V_ϕ . However, for a non diffuse wavefield, where the focusing field may exhibit a preferred direction of propagation, only the projection of the wave vector along the direction of observation is measured. As a result, V_ϕ will be overestimated. Finally, we note that this method is not compatible with low rate imaging modalities since it requires ultrafast frame rates to track the time evolution of the correlation field.

The focus size method relies in the relation between the focus size and the shear wavelength given in Eq. 6. Again, this relation is valid in the presence of a diffuse field where the Green's function can be retrieved from cross-correlation. However, since the method uses an average value in all directions, the lack of diffusivity is not as critical as in the phase method. In addition, the size is measured around the focusing point where the signal to noise ratio is highest. Thus, the method is robust in the presence of noise. Finally, the spatial coherence is shown to be independent of the time acquisition rate. As a consequence, this method is compatible with low frame rate imaging modalities like standard ultrasound, MRI or OCT. However, at ultraslow frame rates, only the shear wavelength can be estimated because the frequency information is lost.

The amplitude method is based on the relationship between the peak of autocorrelation function and the local shear elasticity as expressed in Eq. 7. This relationship overcomes the need of knowing the frequency of the field to quantitatively measure the shear elasticity. Thus, it is fully compatible with low frame rate imaging modalities. Nevertheless, Eq. 7 was derived from the free space Green's function. Therefore, its validity is limited in the

presence of inclusions where scattering must be considered. Thus, the spatial resolution of this method as well as its quantitative value near internal boundaries or inhomogeneities needs further revision.

Strictly, the derivative ratio method does not require the presence of a diffuse field since the governing equations of this method are valid for any field obeying the wave equation. This is a great advantage compared to the other methods. However, the lack of diffusivity makes the result dependent on the specific orientation between the direction of observation and the preferred direction of the incoming energy. This method is compatible with low frame rate imaging modalities where the shear wavelength is imaged.

Viscosity is an inherent property to any biological tissue that may influence elastography methods based on noise correlation and time reversal. Although viscosity measurements were early integrated into magnetic resonance elastography [41], how to incorporate and quantify viscosity is still a matter of debate among the ultrasound-based SWE community [42]. Wave attenuation is known to mitigate the focusing quality in time reversal and cross-correlation based methods [43]. However, since spatial reciprocity remains valid even in the presence of attenuation, the best signal-to-noise ratio will be found on the focusing point at the refocusing time, i.e. time reversal acts as a spatio-temporal matched filter [44]. Consequently, in a dissipative medium the refocusing is likely to emerge from the reverberant field allowing the focus size estimation. In the work of Benech et al. [12] the influence of viscosity in the phase and focus size methods was evaluated experimentally in agarose phantoms with different Q-factors. In [12] it was found that for high Q-factors (where several reflections take place) the field can be considered as being diffuse and the mechanical properties may be accurately retrieved through both methods. Contrary, for low Q-factors the focus size method provides a more robust estimation. Moreover, as demonstrated by the numerous works and applications cited along this review, cross-correlation elastography experiments were not hindered by viscosity. Nevertheless, future works should aim to include viscosity while solving the inverse problem through the phase, focus size, amplitude or derivative ratio method.

4 APPLICATIONS OF NOISE CORRELATION AND TIME REVERSAL ELASTOGRAPHY

The compatibility with low imaging rates modalities has led to innovative developments and new challenges in the field of multi-modality elastography. Particularly, the use of diffuse and complex wavefields has boost the concept of passive elastography to new applications involving ultrafast and ultraslow imaging modalities like ultrasound, optical methods (e.g., digital holography or OCT) and MRI.

The work of Sabra et al. [11] was the first proof of concept in the field of passive elastography based on noise correlation. In [11] they used sixteen miniature skin-mounted accelerometer placed along the vastus lateralis to measure muscular noise in the 40–55 Hz frequency band. The shear wave phase velocity dispersion was estimated from the correlation field by using a Morlet wavelet transform and by computing the slope of the shear wave arrival time for increasing sensor separation distances. The muscle's shear

elasticity and viscosity were retrieved by fitting a Voigt model to the phase velocity dispersion curve. They found that elasticity and viscosity increased with muscle load. In the work of Brum et al. [26] they also used surface sensors to record the reverberated elastic field generated by a shaker applied at the medium's surface. In this work they used the phase and the focus size methods to measure the elasticity of a hard and a soft gelatin-based phantoms and cheese. Experiments performed in cheese allowed envision applications in the food industry, for example, evaluating the cheese ripening time.

Later, the work of Gallot et al. [13] set a milestone regarding passive elasticity imaging inside the human body. To this end they used an ultrafast ultrasound scanner to record the natural displacements in the human liver and belly muscle. To conduct the shear elasticity imaging they used the focus size method which has shown to be more robust for low signal to noise ratio. To retrieve the shear wave speed, the focus size at -6 dB was assumed to be half a shear wavelength as in the case of fluids. Nevertheless, a good agreement was obtained between the shear elasticity image and its corresponding echographic image with shear velocity values close to the ones reported by other studies. This idea was taken up in the work of Catheline et al. [14] where they used the derivative ratio method and its compatibility with low frame rate imaging modalities to conduct the first experimental *in vivo* demonstration of passive shear wave speed imaging using an ultrasound scanner working at a conventional frame rate. The experiment was done on the thyroid of a healthy volunteer and the ultrasonic probe was hand held during the acquisition of 800 frames at 25 Hz. Breathing and moving was prohibited during the 32 s long acquisition.

The compatibility with low rate imaging modalities boosted several innovative applications involving standard commercial ultrasound scanners, OCT and MRI systems for elasticity imaging. In the work of Zorgani et al. the derivative ratio method was used to realize a passive shear wavelength tomography in the brain using MRI [20]. The experimental validation of the sequence and method was first conducted in a calibrated tissue mimicking phantom. Then, the proof of concept was demonstrated *in vivo* in the brain of two healthy volunteers. Compared to other magnetic elastography techniques, this approach does not need any synchronization with the shear wave source. Later, in the work of Nguyen et al. a low frame rate spectral-domain OCT system was used to demonstrate the feasibility of a passive shear wavelength tomography on the eye of an anesthetized rat [21]. The results were cross-validated with an active elastography experiment at ultrafast frame rate. But it was not until the work of Rabin et al. [18] that quantitative elasticity imaging using ultraslow imaging systems was achieved. In their work they used the amplitude method to image *in vivo* the shear elasticity of healthy and tumorous breast. The B-mode images from a conventional ultrasound scanner (frame rate 30–50 Hz) were used to measure the diffuse displacement field. Since in the breast the signal to noise ratio of the physiological noise as well as the sensitivity of the algorithm used to measure the displacements are low, in this work they used an array of mechanical shakers to create the diffuse field.

Recently as in [18], many authors decided to use one or multiple shakers to generate a controlled diffuse field. In this way the signal to noise ratio inside the region of interest can be increased and the frequency content of the field can be controlled [16, 30, 31, 45]. For example, in Reverberant Shear Wave Elastography (R-SWE) a

narrowband diffuse field is generated with several mechanical actuators, then, the shear wavelength is derived from the spatial autocorrelation function [16, 17, 46, 47]. Regarding the application of diffuse field interpreted within the frame of noise correlation and time reversal in the work Grasland et al. [31] a 15 kHz vibrating pipette was used to create a complex reverberated elastic field inside a cell. This novel approach was termed “cell quake elastography.” The displacement field inside the cell was imaged at 200 kHz using a microscope together with a high speed camera and the shear wavelength was estimated from the curvature of the focusing point. Elasticity images allowed identifying different cell zones (e.g. zona pellucida, cytoplasm and nucleus) in isolated and multiple cells configurations. Moreover, in [31] it was shown that elasticity decreases when the cell cytoskeleton was disrupted with cytochalasin B. This technique allow shear wave elastography at microscopic scale, opening a new research field in mechanobiology cellular properties. Moreover, in the work of Barrere et al. [30] the feasibility of monitoring high-intensity focused ultrasound (HIFU) treatments in the liver using the derivative ratio method was investigated. To this end, bovine livers were heated up to 80°C using a planar HIFU transducer and the displacements generated by a mechanical shaker were imaged with a high-frame-rate ultrasound imaging device. The formation of ablated tissue was monitored and evidenced by a tissue stiffening like in [48]. More recently, in the work of Marmin et al. [49] digital holography was used to capture a diffuse shear wave field induced by a piezoelectric actuator with a sensitivity of up to 10 nm. A shear wavelength tomography was conducted in agarose phantoms and *ex vivo* pork liver. Digital holography allow envisioning contact-less passive elasticity imaging with high sensitivity and spatial resolution [29]. Finally, it is important to mention that the wave-physics and algorithms developed in the frame of noise correlation and time reversal elastography are being applied in other fields of research. For example, Hillers et al. [50] analyzed seismic data with the focus size method to image the San Jacinto fault zone.

5 CONCLUSIONS AND FUTURE DIRECTIONS

Throughout this short review we covered the major developments in wave-physics involving elasticity imaging using cross-correlation of a complex elastic wavefield along with its latest applications. The main advantage of this approach is its

compatibility with low imaging rate modalities which has boosted the concept of passive elasticity imaging. Compared to standard SWE with an active shear wave source, a passive approach is a smart solution when shear wave generation is difficult (e.g. in well protected organs as the brain) or even dangerous, as in the case of the cornea. The various works cited along this review demonstrate the significant potential of cross-correlation elastography to become a new imaging tool in the field of multi-modality elastography. Nevertheless, it is important that future works seek to incorporate the mechanical properties inherent to tissue such as viscosity and anisotropy into the inversion methods. Moreover, cross-correlation elastography will clearly benefit from three dimensional and multiple component measurements of elastic wavefields, now possible with MRI or new 3D ultrasound imaging technologies. Over the past thirty years, different technological advances allowed SWE to be incorporated in commercial ultrasound and MRI systems, becoming a new imaging modality in clinics. Cross-correlation elastography is a very recent approach that has demonstrated its feasibility in different medical applications, however its clinical significance still needs to be demonstrated. Therefore, future works should attempt to translate this approach into clinics by proving its reproducibility, repeatability, diagnostic value and its ability to improve *in vivo* results. If succeeded, we believe that cross-correlation elastography will certainly become a novel multi-modality imaging tool in clinics.

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JB conceived the manuscript. JB and NB wrote the manuscript. JB, NB, TG, and CN revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Elastography Validity Criteria Definition Using Numerical Simulations and MR Acquisitions on a Low-Cost Structured Phantom

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MR Elastography is a novel technique enabling the quantification of mechanical properties in tissue with MRI. It relies on a three-step process that includes the generation of a mechanical vibration, motion capture using dedicated MR sequences, and data processing involving inversion algorithms. If not properly tuned to the targeted application, each of those steps may impact the final outcome, potentially causing diagnostic errors and thus eventually treatment mismanagement. Different approaches exist that account for acquisition or reconstruction errors, but simple tools and metrics for quality control shared by both developers and end-users are still missing. In this context, our goal is to provide an easily deployable workflow that uses generic validity criteria to assess the performance of a given MRE protocol, leveraging numerical simulations with an accessible experimental setup. Numerical simulations are used to help both determining sets of relevant acquisition parameters and assessing the data processing's robustness. Simple validity criteria were defined, and the overall pipeline was tested in a custom-built, structured phantom made of silicone-based material. The latter have the advantage of being inexpensive, easy to handle, facilitate the fabrication of complex structures which geometry resembles the anatomical structures of interest, and are longitudinally stable. In this work, we successfully tested and evaluated the overall performances of our entire MR Elastography pipeline using easy-to-implement and accessible tools that could ultimately translate in MRE standardized and cost-effective procedures.

Keywords: MR elastography, validation, simulations, silicone phantom, complex shear modulus

INTRODUCTION

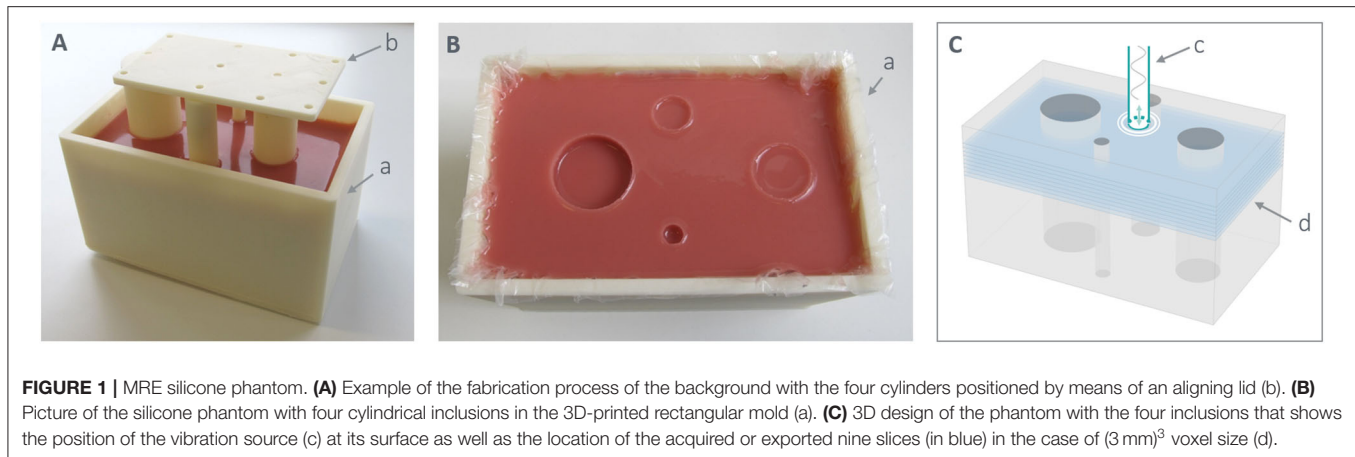
Magnetic Resonance Elastography (MRE) non-invasively assesses and quantifies mechanical properties of tissue *in vivo* [1, 2]. Various MRE techniques have been developed, involving the combination of interdependent processes (mechanical vibration generation, motion-sensitized MR sequences, image processing algorithms) all of which may impact the qualitative and quantitative outcomes. As a result, MRE, despite being available for decades, has not entered clinical routine

application yet; a consensus on precision and accuracy of this technique still needs to be defined. Indeed, one of the major drawbacks of MRE is the lack of a gold-standard that could enable to compare elastograms with each other. Rheometry may provide a reference measurement of bulk mechanical properties of various materials [3–7], but the precision of common rheometers or dynamic mechanical analysis (DMA) systems depend on multiple factors, like the type of instrument used or the sample's size and experimental environment [6, 8, 9]. Furthermore, performing reliable rheometry over a range of frequencies comparable with those employed in MRE may not be possible with common rheometers and specific high-frequency measurement systems may be required [8, 9]. In addition, biomechanical properties of *ex vivo* tissue specimens that lack perfusion are very different than those of perfused *in vivo* body parts, making a direct comparison between classical rheometers and MRE difficult. In this context, biases and errors intrinsic to the technique should be assessed and controlled to avoid the generation (and further the interpretation) of unreliable elastograms. Since MRE relies on the encoding of the tissue displacement into the phase of the complex MR signal, metrics that take into account phase errors are good candidates to measure raw data quality. Phase-to-noise ratio (PNR) can be used to evaluate the impact of noise on the reconstructed displacement. Given that the phase standard deviation in radians is equal to the inverse of the magnitude SNR [10], PNR is proportional to both the SNR and the encoded phase shift. PNR can be computed in numerous ways, either by including the specific details of a given experiment [11] or independently of them, but at the expense of extra acquisitions [12]. Displacement-to-noise ratio (DNR) has been similarly used as a metric, either by exploiting the knowledge of the encoding sequence details and SNR [13], or by estimating a motion SNR from the displacement noise [14, 15]. The octahedral shear strain (OSS) and the derived OSS-SNR have been introduced as indicators of reconstruction accuracy showing better performance than the motion SNR [15]. However, OSS is obtained from spatial derivatives of the motion field, which makes it dependent on the processing [16]. The ratio of local shear wavelength λ to pixel size a has also been introduced as an indicator of reconstruction accuracy [17, 18], and values were reported for different methods [19, 20]. From these studies, the range of optimal λ/a appears to be broad (~ 5 – 20), although best performances are generally achieved in the range 6.7 – 10 , especially at low SNR.

Numerical simulations can be used as a validation tool by generating displacement fields (forward problem) for given boundary conditions, visco-elasticity distributions, and vibration sources. The displacement fields are then fed into the MRE reconstruction pipeline, which solves the inverse problem and retrieves the mechanical properties for comparison with ground-truth values. Simulations are generally based on analytical formulations [5, 14, 17, 21] or finite element methods (FEM), either with custom-developed tools [22–24] or dedicated commercial softwares [20, 24–30]. Simulations are very useful to assess the error linked to reconstruction algorithms, however they do not suffice to reflect all potential limitations of an

MRE experiment (transducer, B_0 and B_1 inhomogeneities, SNR, motion sensitivity, susceptibility issues, and heterogeneity of the material at very small scales). Validation is thus typically obtained from self-made or commercial phantoms mimicking biological tissues, which shape and properties depend on a targeted application. A thorough overview of materials used for ultrasound elastography is given in [31], and here we will focus on those commonly used in MRE. Water-based phantoms are largely spread and include natural or synthetic polymers. Natural polymers comprise gelatin-based phantoms, easy to make, that are characterized by a linearly elastic behavior, low viscosity and frequency dependency [32]. However, they are sensitive to temperature changes [33] and also to the fabrication process [27, 34, 35]. Agar can be used alone [1, 2, 5, 20, 36, 37] or added to gelatin [38] with a multi-step heating and cooling procedure at precise temperature steps. Over a period of about 1 year post fabrication, such phantoms have shown a general decrease of storage modulus following an initial increase phase (1–5 months). Synthetic polymers like polyvinyl alcohol cryogels (PVA) are obtained by performing repeated cycles of freezing and thawing of boiled and stirred PVA solutions. Their mechanical properties depend both on the number of cycles and the PVA concentration [14, 39–42]. This fabrication process enables phantoms with anisotropic mechanical properties [21], yet inhomogeneous temperature changes within the entire volume cause inhomogeneities leading to uneven inclusion-background interfaces [39, 43]. Commercially available phantoms (CIRS Inc., USA), made from patented Zerdine hydrogel, are more expensive than water-based phantoms [44]. They contain well-defined inclusions but are essentially non-viscous and the measured values often differ from those declared by the manufacturer [19, 20, 45–48]. Polyvinyl chloride (PVC, also known as plastisol) phantoms are fabricated by heating-up a mixture of PVC and a softening agent to high temperatures (160 – 177°C) while constantly stirring before pouring into a heat-resistant mold [8, 49]. Arunachalam and colleagues have assumed that a 90-day period was sufficient to reach stable mechanical properties [9]. They also reported weak MR signal and very low loss moduli of the material, causing increased reflections. Finally, silicone has been used for Digital Image Elasto-Tomography (DIET) [50, 51], and for mechanical imaging using pressure sensors [52], but little so far for MRE [53, 54]. Compared to the other materials described above, silicone simplifies considerably the phantom fabrication process. Typically, silicones are created by mixing two components, and the final stiffness can be controlled by the addition of a softener or hardener. Besides, many silicones are room temperature vulcanizing (RTV), which eases the fabrication process and enables the realization of homogenous structures with smooth interfaces.

Our objective is to establish simple and easily transferable validity criteria, setting the ground for basic procedures in MRE quality control and efficient deployment of the technique in the clinics. In the presented work, we assess the limitations of MRE for a given protocol, comparing reconstruction performance from both fully simulated and acquired datasets in a low-cost silicone-based phantom.



MATERIALS AND METHODS

Sample Preparation and Phantom Fabrication

Different samples were obtained by mixing the two components of a silicone rubber (Eurosil 4 A+B, Schouten SynTec, the Netherlands) together with an oil softener (Eurosil Softener, Schouten SynTec, the Netherlands). An empirical density of 1,000 kg/m³ yielded accurate volume estimation of the final material. The silicone components were stirred manually (1–5 min depending on total volume) in a plastic container until a homogeneous mixture was obtained. Different stiffness properties were obtained by fixing the weight ratio between the silicone bi-components (A and B) and varying the softener composition, expressed as 1A : 1B : Sx. For rheological measurements, silicone mixtures were poured into small cylindrical plastic containers (diameter 25 mm) to create seven samples of about 6 mm height, with softener compositions of S1.0, S1.5, S1.7, S1.9, S2.1, S2.3, and S2.5. All samples were made on the same day.

An MRE phantom was made that consisted of four cylindrical inclusions of various diameter in a homogeneous, rectangular background (**Figure 1**). The silicone mixture for the background (1A : 1B : S1.9) was first poured into a 3D-printed mold (Fortus 450 m, Stratasys, USA) with 4 removable 3D printed cylinders (**Figure 1A**). As the 3D print may produce rough surfaces, which promotes the formation of micro air bubbles while pouring uncured silicone, a plastic sheet was placed to cover the inner surface of the mold (**Figure 1B**). Furthermore, an aerosol release agent (Pol-Ease 2500, Schouten SynTec, Netherlands) was applied on the surface of the cylinders to facilitate the extraction of the phantom from the mold. We allowed the silicone to fully cure for 1 day before extracting the cylindrical inserts, and filled the remaining volume with a softer mixture 1A : 1B : S2.1 to create the inclusions. The final size of the phantom was 182 × 108 × 90 mm³ with inclusions of diameter 10, 20, 30, and 40 mm referred to as I1, I2, I3, I4 according to their size. All samples were stored away from direct light at room temperature, in a closed plastic container together with a two-way humidity control bag (“Humidipak,” D’Addario, USA) to ensure 45–50% humidity.

Silicone Stability

Stability over time of the prepared seven cylindrical silicone samples was investigated using a rotational rheometer (MCR series, 302 and 702, Anton Paar, Austria) to extract their complex shear modulus $G^* = G' + iG''$ (G' : storage modulus, G'' : loss modulus). For each measurement, the upper rotating plate (flat model PP25) which diameter is identical to the samples, was positioned with an automatic set point of 5-N normal force to enable a sufficient compression and rotation. A sweep of the shear strain amplitude in the range 0.01–10% was performed at 10 Hz to verify the linear behavior of each material. Subsequently, a shear strain amplitude of 1% was used for G^* measurement with a linear frequency sweep from 100 to 1 Hz. For each sample, two consecutive measurements of the frequency sweep were acquired. The same measurements were repeated at several time points, respectively at 1, 2, 4, 7, 15, and 22 weeks *post* fabrication.

An example of the frequency sweep measurement obtained with the silicone samples is given in **Supplementary Figure 1**. An increased standard deviation for the two consecutive measurements can be observed for frequencies >20 Hz (**Supplementary Figure 1A**). Similarly, larger G'' than G' are observed above 35–40 Hz, even though the material kept its solid structure. Therefore, we assumed that the measurements corresponding to 10 Hz provided reliable information and we used those values to evaluate the sample stability over time.

Simulations

Forward Problem

Numerical simulations were performed using COMSOL Multiphysics 5.4 (COMSOL AB, Sweden). The geometry of the simulated phantom was defined as a rectangular solid with dimensions identical to the MRE silicone phantom described above, including the same four cylindrical inclusions (**Figure 1C**). The vibration area was defined as a circular region of 16 mm diameter on the front face of the phantom, at half distance between I3 and I4, similarly to the physical vibration setup used later.

For the simulation’s physics definition, the Solid Mechanics interface from the Structural Mechanics module was used. The front face of the phantom was set as a “Free” boundary, while

all the other faces were assigned to a “Fixed Constraint” node to reproduce the condition of the containing mold. A “Prescribed Displacement” node was assigned to the vibration area with an imposed sinusoidal displacement perpendicular to the surface with a 200- μm amplitude and frequency f_{vib} of 57 Hz. For both types of regions (background and inclusions) the material properties were defined using a “Linear Elastic Material” domain node with a “Viscoelasticity” domain, without any particular contact definition between the two materials.

In order to define $G^* = G' + iG''$ of the different regions, we employed an isotropic Standard Linear Solid model (see **Supplementary Material**). The simulation consisted in a “Time Dependent” study over a time interval of 12 vibration periods $T = 1/f_{vib}$, sufficient for the waves to propagate in the whole volume, with a time step equal to $T/20$, for a total simulation time of 41 min (Intel Xeon CPU E5-2680 v4 @ 2.4 GHz, 14 cores, RAM 128 GB). The exported simulated data consisted in the displacement field components u , v , w (in X , Y , and Z direction, respectively) at time points $\tau = i^*T/N$ (with N the number of wave phases sampled during MRE acquisitions, five in this case) of the last vibration period. A spatial grid of 9 contiguous axial slices was defined for three different isotropic voxel sizes of 2.5, 3.0, and 3.5 mm³, respectively. The central slice (i.e. slice 5) was set at 22 mm from the surface (**Figure 1C**).

Synthetic MR Data

Displacement field components were converted into synthetic complex MRE data via a custom MATLAB (R2020a, The MathWorks Inc., USA) script. Phase-encoding was artificially achieved by multiplying the displacement data by the fractional encoding factor (Equation 1 from [12]) of the MRE encoding scheme, with a motion-encoding-gradient (MEG) of 54 mT/m:

$$\begin{aligned}\varphi &= \gamma u \int_0^{TR} MEG(t) \sin(2\pi f_{vib}t) dt \\ &= \frac{\gamma A_{MEG} u}{f_{MEG}} \frac{\sin(\pi q)}{\pi(1-q^2)} \quad (1) \\ \text{with } q &= \frac{f_{vib}}{f_{MEG}} \\ \text{when } MEG(t) &= A_{MEG} \sin(2\pi f_{MEG}t)\end{aligned}$$

with φ the vibration-encoded phase, γ the gyromagnetic ratio, u and f_{vib} the amplitude and frequency of the vibration at a given coordinate in space, A_{MEG} and f_{MEG} the amplitude and frequency of the sinusoidal motion encoding gradient $MEG(t)$. Magnitude of the synthetic data was arbitrarily set to one everywhere. Since a reference dataset without MEG is required for our reconstruction, a separate dataset was generated with identical geometry, magnitude set to 1 and phase to 0 everywhere (no additional noise was considered).

MR Imaging

MRE was performed on a clinical 3 T Magnetom Prisma scanner (Siemens Healthineers, Germany) using a fractional encoding gradient-echo sequence [12] and a 20-channel head coil. To constrain the bulk motion, the phantom was kept inside its plastic

TABLE 1 | Motion encoding parameters used for MRE acquisitions on the phantom for different voxel sizes.

Isotropic voxel size (mm)	F_{vib} (Hz)	F_{MEG} (Hz)	A_{MEG} (mT/m)
2.5	57.00	105.26	54
3.0	57.00	104.39	54
3.5	57.00	104.39	54

F_{vib} , vibration frequency; F_{MEG} , frequency of motion-encoding-gradients (MEGs); A_{MEG} , amplitude of MEGs.

mold. The accessible phantom face was oriented parallel to the axial plane of the scanner. A custom vibration system was used, similar to the ones described in [42, 55]. The latter consists of a loudspeaker (12NW100-8, B&C Speakers, Italy) and a waveguide (flexible, 16-mm diameter plastic tubing, Tecnotubi Picena, Italy) which circular opening was positioned in contact at the surface of the phantom. A vibration frequency of 57 Hz was used that corresponded to one of the resonant frequencies of the waveguide (calibrated separately). The vibration was produced via a wave generator (33500B Trueform, Keysight, USA) coupled to an audio amplifier (XLS 1502, Crown, USA). The MRE sequence encoded five time points of the vibration phases with a four-point scheme, acquiring three motion-encoded data series (each for one of the three spatial MEG directions) and a reference series (without motion encoding). Three experiments were performed at different spatial resolutions with the following acquisition parameters: MEG amplitude 54 mT/m, TE/TR 12.3/180 ms, acquisition matrix 96×54 , 9 contiguous axial slices, isotropic voxel size of 2.5, 3.0, and 3.5 mm³, MEG frequency of 105.26, 104.39, and 104.39 Hz, respectively, as summarized in **Table 1**. The central slice was positioned at about 1/4 of the phantom's height, i.e. at around 22 mm from the surface. The scans were scheduled with time points identical to the rheometric measurements run in the silicone samples, plus 1 day.

In each MRE session, relaxation times T_1 and T_2 of the phantom were also quantified (see **Supplementary Material**).

Data Processing and Analysis

MRE Reconstruction

Both the synthetic and experimental raw MRE data were processed using a custom software performing a 3D curl-based direct inversion [12]. All the elastograms were then exported for further processing using MATLAB scripts. In this paper we focus only on G' , the MRE output information most commonly used by numerous research groups.

A mask was drawn manually around the phantom boundaries before unwrapping the phase data. Since the reconstruction software requires to calculate multiple spatial derivatives, the generated elastograms are by default cropped by two voxels in 3D with respect to the initially defined volume of interest (VOI). In addition, we chose to discard two more pixels for each slice in order to take into account possible calculation error propagation that often translates in G' underestimation at boundaries. Elastograms of the five central slices were used for the analysis of synthetic data. Alternatively, elastograms of the

three central slices were used with experimental data as more discrepancy was observed on surrounding slices.

Regions of Interest (ROIs)

For synthetic data, maps of the G parameter were exported at the same coordinates as the displacement field data, in order to obtain a map of the inclusion positions on the same spatial interpolation grid. This way, ROIs were automatically available for the location of the inclusions inside the background. For the experimental data, ROIs were defined as circles centered in the inclusions, segmented from T_2 maps. The respective masks were co-registered with the MRE datasets. To avoid partial volume effects due to the larger voxel sizes employed in MRE, the mask for the background was eroded by two voxels surrounding the inclusions. Mean, median, and standard deviation values of G' were calculated within the different ROIs. All the segmentation steps were based on data quality considerations detailed below.

MRE Validity Criteria

In order to evaluate the quality and reliability of raw MRE data, simple metrics were chosen, easily transposable to any MRE method. In particular, SNR was calculated voxel-wise for each set of raw data and converted into phase and displacement amplitude uncertainty maps using Equation 1. The mean SNR and standard deviation of the reference dataset (acquired without motion encoding gradients) were used to discard voxels with low SNR and therefore large phase error on final MRE maps. This threshold was empirically set to 2 standard deviations below the mean SNR: voxels on the elastograms were discarded when at least one dataset of the three encoded directions did not have a sufficient SNR. Additionally, due to derivative calculations on the reconstructed data, we discarded two more voxels on each side of the voxels excluded by the SNR threshold by dilating the SNR mask in all three directions.

To calculate the ratio of shear wavelength λ to voxel size a for the three resolutions, λ was estimated via Equation 2 [56]:

$$\lambda = \frac{1}{f_{\text{vib}}} \sqrt{\frac{G'}{\rho}} \quad (2)$$

where f_{vib} is the vibration frequency of 57 Hz, ρ the density of the material (assumed to be 1,000 kg/m³), and G' is the estimated storage modulus. For G' values, we considered the median values obtained with the 3-mm resolution within the background (silicone softener composition S1.9) and within the largest inclusion I4 (silicone softener composition S2.1).

MRE Validation

Reconstruction algorithms can have an impact both on the calculated stiffness and the apparent objects geometry. To validate the MRE pipeline, the contrast-to-noise ratio CNR_i between the i -th inclusion and the background [19, 20, 57, 58] was calculated using Equation 3:

$$CNR_i = \frac{2(m_i - m_B)^2}{\sigma_i^2 + \sigma_B^2} \quad (3)$$

where m_i , m_B are the mean values of G' within the i -th inclusion and the background, and σ_i , σ_B are the corresponding standard

deviations of G' . Here, the median was used instead of the mean to allow for non-Gaussian distributions.

Root-mean-square-error RMSE was calculated for the voxels in the different regions according to Equation 4:

$$RMSE = \sqrt{\frac{1}{N} \sum_j^N \frac{(G'_j - G'_{0,j})^2}{G'_{0,j}{}^2}} \quad (4)$$

where $G'_{0,j}$ is the storage modulus defined in the simulation and G'_j is the reconstructed value in a voxel j . For MRE data, $G'_{0,j}$ in the background was taken as the median value at 3 mm resolution, whereas $G'_{0,j}$ values in the inclusions were estimated as $G'_{0,j}$ in the background divided by the ratio $G'_{S1.9}/G'_{S2.1}$ measured via rheometry.

Statistical Analyses

For each considered resolution, the probability distribution was tested using the Kolmogorov-Smirnov method. Differences between G' values in the inclusions and those in the background were assessed with the Kruskal-Wallis test ($\alpha = 0.05$), followed by a Mann-Whitney test for the two-by-two comparisons between the four inclusions and the background. The significance level was adjusted for multiple comparisons using the Bonferroni correction, and a p -value of 0.0125 was considered as significant.

RESULTS

Silicone Stability

Figure 2 summarizes the evolution over time of G' measured at 10 Hz for all samples, showing an initial variation of the material properties leading to good stability after 2–4 weeks post fabrication. The increase in G' never exceeds 1.1 kPa for all samples, except in the case of softener composition S1.0 where the variation almost equals 2 kPa. In addition, the silicone samples exhibit, as expected, a clear distinction in terms of G^* values, with higher values in the case of lower softener proportion (**Figure 2**).

Simulations

Analysis of the reconstructed elastograms for the simulated phantom is shown in **Figure 3**. As described above, the reconstruction does not provide any value within a 2-voxel rim around the object. The inclusions are circled in red and the sections (A-A and B-B) show G' values across the five central slices (**Figure 3A**).

Despite having identical compositions, large inclusions, compared to small ones, are better discriminated from the background even though they all showed significant differences ($p < 0.0125$) at all resolutions (see boxplots in **Figure 3B**). This trend is confirmed by the CNR values, greater in the inclusions I4 and I3 than in I2 and I1. The reconstruction software yields more accurate values in the background and in I4 and I3 than in I2 and I1, close to the ground-truth defined in the simulation (**Figure 4**). In fact, the error is similar in the background and in the inclusions I4 and I3, while it increases more than 2-fold for the smaller inclusions I2 and I1. In general, **Figures 3, 4** indicate that

a smaller voxel size corresponds to a greater standard deviation of G' within each region and to a larger error in the background.

The wavelength-to-voxel size ratios λ/a are calculated for each spatial resolution and summarized in **Table 2**. This ratio decreases with increasing voxel size, with all values included between 10 and 15.7.

Phantom MRI and MRE

The employed phantom fabrication technique produced cylindrical shapes with sharp contours and overall proper adherence between the different silicone regions at the inclusions

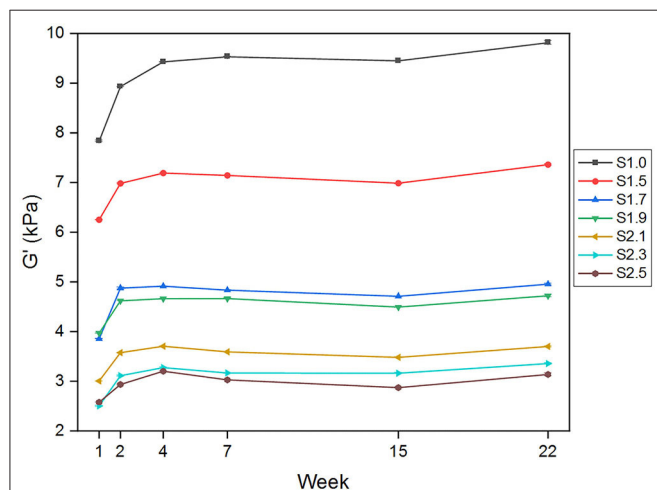


FIGURE 2 | Rheometry measurements at different timepoints (weeks post fabrication) of the complex shear modulus component G' at 10 Hz angular frequency for all the silicone samples prepared with various softener compositions.

boundaries (cf. **Figure 1**). The values of both T_1 and T_2 of the two silicone compositions differ by <10 ms, with minor variations over time post fabrication (**Supplementary Figure 2**). The inclusions shape and position were identified on T_2 maps, which presented less B_1 inhomogeneity at the center of the phantom than T_1 maps (**Supplementary Figure 3**), and were thus used for ROI segmentation.

The SNR and the error values both on the MR phase and the corresponding displacement amplitude are reported in **Table 3**. These errors were rather small and did not contribute in the overall data thresholding. An example of the reconstructed G' maps is represented in **Figure 5** for the central slice at week 4 *post* fabrication. The number of voxels near the edges of the phantom with underestimated values of G' is between 2 and 5 voxels greater than what is observed in synthetic data. In general, the voxels discarded by our SNR threshold are located near the boundaries of the phantom and at its center, in correspondence with partial volume effects and the location of the vibration source.

Figure 6 presents the reconstructed G' values in the segmented regions of the phantom at week 4 *post* fabrication.

TABLE 2 | Simulated values of G' in the inclusions and background with corresponding shear wavelength λ and the calculated ratio of wavelength to voxel size λ/a for the considered values of a .

Voxel size a (mm)	Inclusions			Background		
	G' (kPa)	λ (mm)	λ/a	G' (kPa)	λ (mm)	λ/a
2.5	4.0	35.1	14.0	5.0	39.2	15.7
3.0	4.0	35.1	11.7	5.0	39.2	13.1
3.5	4.0	35.1	10.0	5.0	39.2	11.2

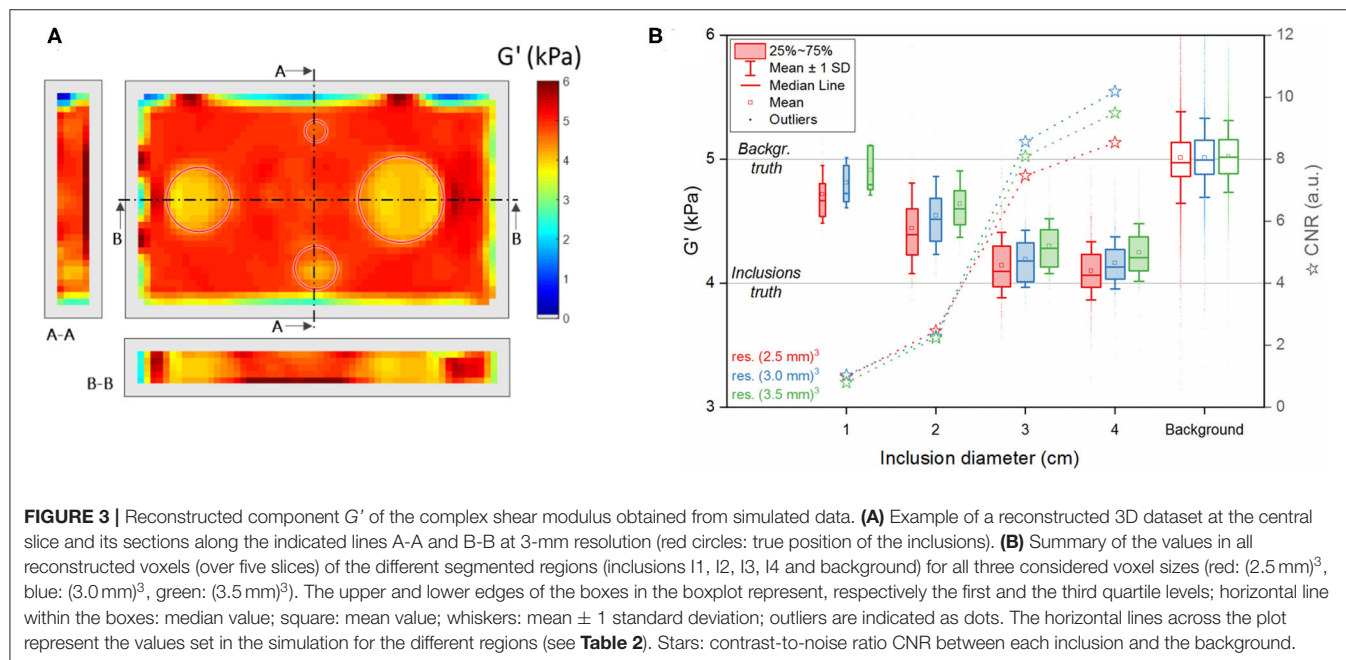


FIGURE 3 | Reconstructed component G' of the complex shear modulus obtained from simulated data. **(A)** Example of a reconstructed 3D dataset at the central slice and its sections along the indicated lines A-A and B-B at 3-mm resolution (red circles: true position of the inclusions). **(B)** Summary of the values in all reconstructed voxels (over five slices) of the different segmented regions (inclusions I1, I2, I3, I4 and background) for all three considered voxel sizes (red: (2.5 mm)³, blue: (3.0 mm)³, green: (3.5 mm)³). The upper and lower edges of the boxes in the boxplot represent, respectively the first and the third quartile levels; horizontal line within the boxes: median value; square: mean value; whiskers: mean $\pm$ 1 standard deviation; outliers are indicated as dots. The horizontal lines across the plot represent the values set in the simulation for the different regions (see **Table 2**). Stars: contrast-to-noise ratio CNR between each inclusion and the background.

As observed with synthetic data, the standard deviation within a given region decreases with increasing voxel size, and the values within the inclusions stand out of the background more clearly while inclusion size increases. This effect led to a higher CNR for the largest inclusions although CNR values in the phantom were smaller than those obtained from simulated data. As in synthetic data, G' in all the inclusions and for all the resolutions was significantly different from the background's ($p < 0.0125$). As expected, the background silicone with a lower softener content

of S1.9 has greater storage modulus values than the inclusions made with a softer composition of S2.1. The mean values in the background are however lower than their median, because of the skewed G' distribution and the contribution of underestimated voxels near the phantom's edges.

The RMSE values for G' measured via MRE in the different regions for each considered resolution are illustrated in **Figure 7**. The error is smallest for the inclusion I4, and largest for I1, with all values greater than observed for synthetic data. As for simulations, we observed an increased error in the background

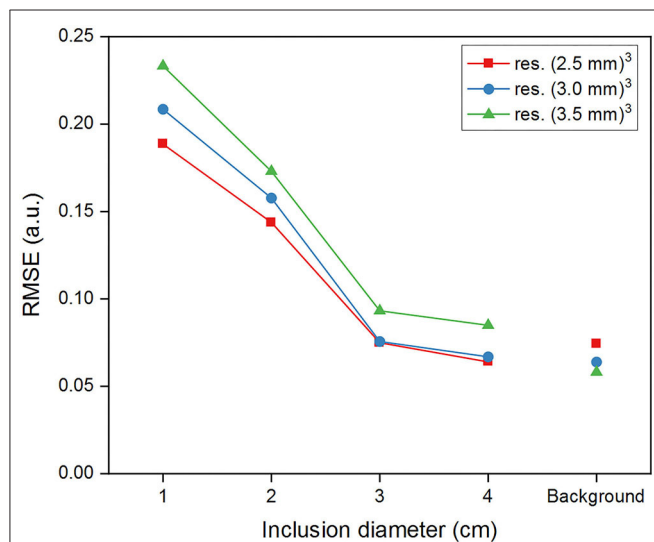


FIGURE 4 | Root-mean-square-error (RMSE) of G' reconstructed within the inclusions and in the background for the simulated data and for the different spatial resolutions.

TABLE 3 | Calculated SNR and corresponding phase and amplitude errors within the phantom for the MRE datasets at different resolutions and measurement timepoints post fabrication.

	Isotropic voxel size (mm)	SNR (a.u.)	Phase error (rad)	Amplitude error (μm)
Week 1	2.5	89.9 ± 8.0	0.011 ± 0.003	0.18 ± 0.05
	3.0	117.8 ± 10.5	0.009 ± 0.002	0.14 ± 0.03
	3.5	141.1 ± 14.3	0.007 ± 0.002	0.12 ± 0.03
Week 2	2.5	92.7 ± 9.0	0.011 ± 0.004	0.18 ± 0.07
	3.0	117.1 ± 10.6	0.009 ± 0.002	0.14 ± 0.03
	3.5	142.4 ± 14.3	0.007 ± 0.002	0.12 ± 0.03
Week 4	2.5	88.1 ± 8.1	0.012 ± 0.005	0.19 ± 0.08
	3.0	120.0 ± 11.5	0.009 ± 0.004	0.14 ± 0.06
	3.5	140.8 ± 13.8	0.007 ± 0.002	0.12 ± 0.04
Week 22	2.5	88.6 ± 8.1	0.012 ± 0.003	0.18 ± 0.05
	3.0	116.6 ± 10.4	0.009 ± 0.002	0.14 ± 0.03
	3.5	121.1 ± 10.8	0.008 ± 0.002	0.13 ± 0.02

All values are expressed as mean $\pm$ standard deviation.

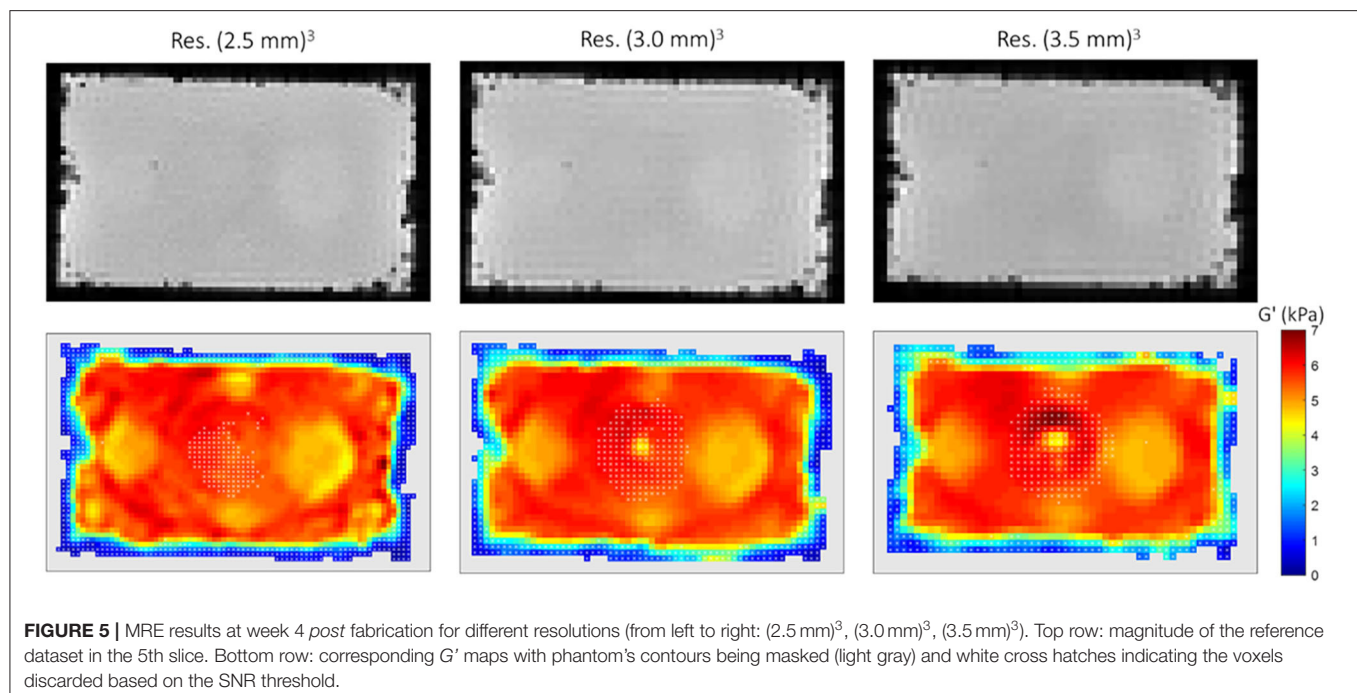
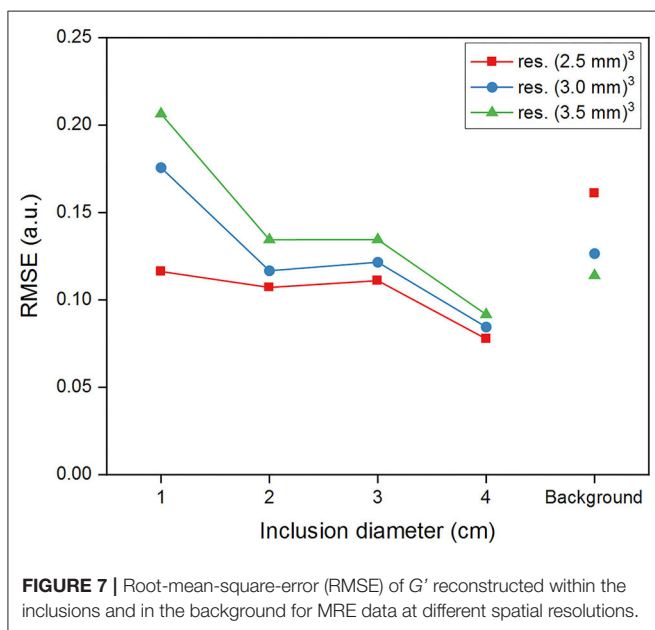
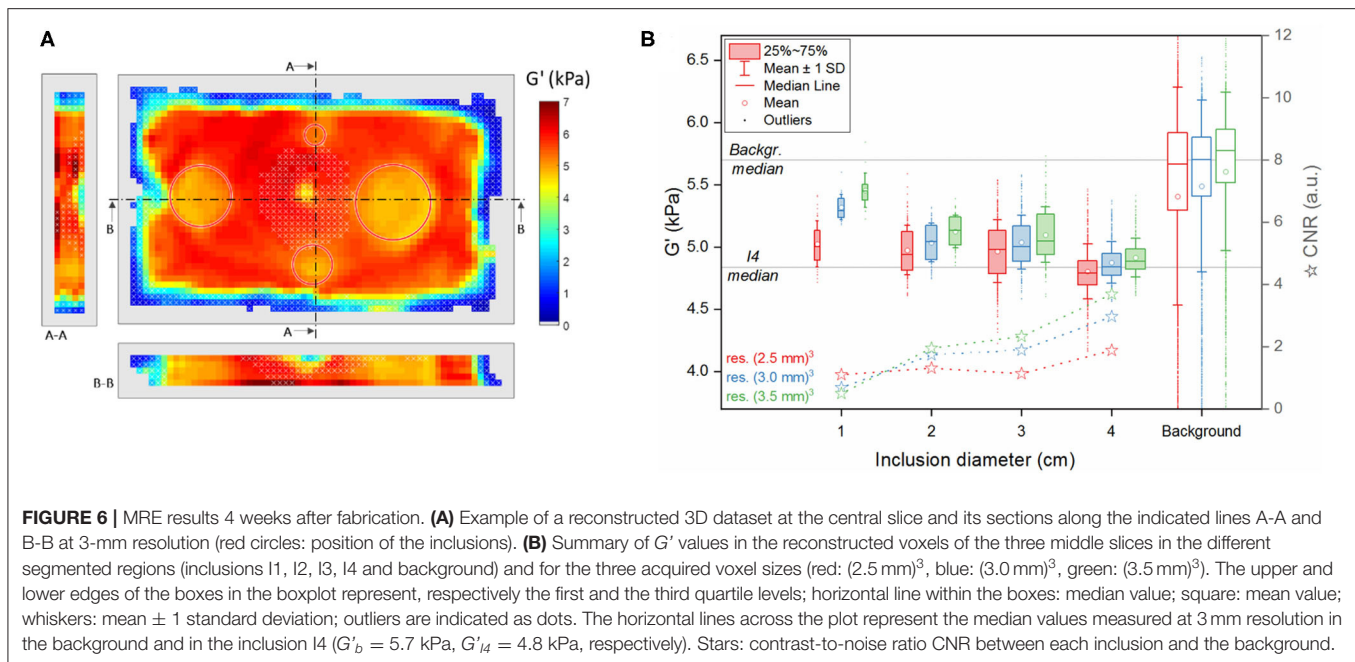
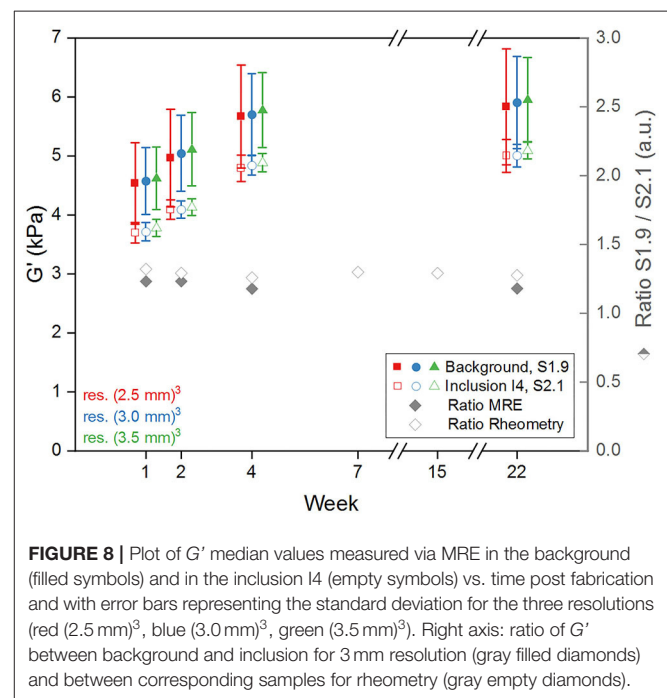


FIGURE 5 | MRE results at week 4 post fabrication for different resolutions (from left to right: $(2.5 \text{ mm})^3$, $(3.0 \text{ mm})^3$, $(3.5 \text{ mm})^3$). Top row: magnitude of the reference dataset in the 5th slice. Bottom row: corresponding G' maps with phantom's contours being masked (light gray) and white cross hatches indicating the voxels discarded based on the SNR threshold.



for the finest resolution, as opposed to the inclusions where the errors increased with increasing voxel size.

The evolution of G' values over time (**Figure 8**) shows a higher initial variation than in the case of the rheometry samples, with stability reached at around 4-weeks post fabrication. Furthermore, ratios of G' between the two silicone compositions S1.9 and S2.1 measured via MRE are similar to those measured via rotational rheometry. The wavelength-to-voxel size ratios λ/a estimated from MRE data are summarized in **Table 4**. This ratio increases over time for all the regions following the initial stiffening of the silicone material, with λ/a values ranging from 9.7 and 17.1.

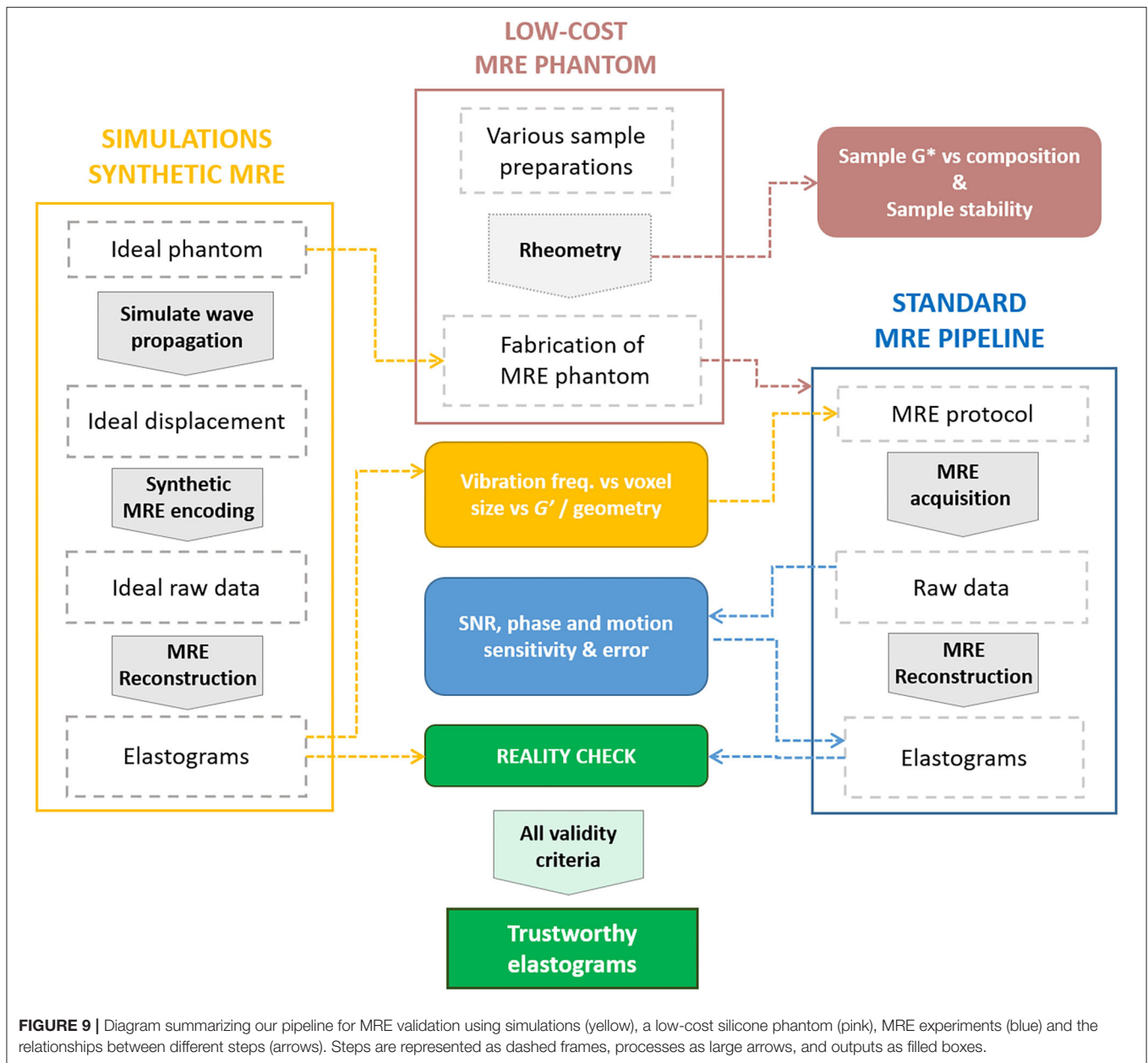


DISCUSSION

Silicone Based Phantoms for MRE

The proposed silicone-based preparation shows great promise as a low-cost, easy-to-reproduce, and durable option for quality control and calibration in MRE experiments.

Rheometry monitoring of the complex shear modulus showed good long-term stability after a 2- to 4-week delay, across all prepared samples. During this initial phase, an overall initial



stiffening of maximum 1.6 kPa was observed. Remarkably, despite the exceeding of the maximum recommended softener composition from the manufacturer, our results showed that G' could be changed and remained stable over time. From this, we obtained a range of realistic stiffnesses covering G' values from 2 to 10 kPa, similar to that observed in biological tissues.

The fabricated MRE silicone phantom exhibited similar evolution of G' over time, while maintaining its G' ratios (background/inclusions) (Figure 8). Yet, the overall variations in G' measured with MRE (~ 1.3 kPa) were generally larger than G' obtained with rheometry (~ 0.7 kPa). This may be caused by variations in the aging process due to their size differences. Furthermore, such differences may also be expected

since rheometry is not a gold standard and operates in a different frequency regime compared to MRE. Nevertheless, our results indicate that rheometry is a good candidate to provide an estimation of the expected values measured via MRE and consists in a good basis for fine-tuning future phantom preparations.

Here, we used two silicone compositions and a simple geometry for the purpose of the study. With appropriate modifications to our fabrication procedure, it should be possible to create more complex, multilayer geometries or anatomically-shaped features in an easy way by exploiting the good shape conservation and interface contact between silicone mixtures once cured. A potential difficulty in the fabrication process may arise from the formation of micro air bubbles. Gradually pouring

TABLE 4 | Measured G' in the largest inclusion (I4, softer composition S2.1) and background (BG, softer composition S1.9) at 3-mm resolution for different time elapsed since fabrication and corresponding estimated ratio of wavelength to voxel size λ/a for the considered voxel sizes a .

	Median G' (kPa)		λ/a (a.u.)					
			$a = 2.5$ mm		$a = 3.0$ mm		$a = 3.5$ mm	
	BG	I4	BG	I4	BG	I4	BG	I4
Week 1	4.6	3.7	15.0	13.5	12.5	11.3	10.7	9.7
Week 2	5.0	4.1	15.8	14.2	13.1	11.8	11.3	10.1
Week 4	5.7	4.8	16.8	15.4	14.0	12.9	12.0	11.0
Week 22	5.9	5.0	17.1	15.7	14.2	13.1	12.2	11.2

the silicone over a plastic sheet covering the internal faces of the mold helped mitigating this problem, yet some susceptibility artifacts due to air bubbles at the edges of the inclusions (see **Figure 5** in the proximity of I3) remained. To solve this issue, one can envision either using materials with smooth and solid surfaces for all the molding parts, such as PMMA or silicone molds with anti-adherent coating [59], scanners with lower magnetic field strengths, or a vacuum chamber for degassing during the fabrication process. Viscosity is another important aspect of the silicone material, and in our case it was greater than what is reported for other commonly used MRE phantoms. As biological tissue, our samples exhibit a dispersive behavior (**Supplementary Figures 1A,B**). The subsequent wave attenuation has the advantage to prevent successive reflections at boundaries and hence the presence of standing waves especially in small objects but may prevent from obtaining sufficient wave propagation inside large objects.

Finally, our results showed T_1 and T_2 values in the order of 1,030 ms and 240 ms, respectively, for both compartments of the MRE phantom (differences never exceeded 7.2 ms for both T_1 and T_2), and with little variation over time (overall <0.9 and 2.9% in relative terms). Further studies may consider tuning the silicone relaxation times by the addition of paramagnetic substances compatible with the silicone mixture during fabrication. This would help further the sequence optimization for a faster transfer to *in vivo* application, in particular when other markers than stiffness are required for segmentation for example [59].

Simulations

We showed that synthetic MRE data produced via numerical simulations can be used with our reconstruction pipeline. The obtained elastograms displayed distinct (and expected) differences in G' values between the defined inclusions and the background. By varying voxel size, we could investigate the resolvability of the latter inclusions as a function of their size, as well as the accuracy of the reconstruction algorithm. It was possible to accurately retrieve G' values within large homogeneous regions while observing some limitations for inclusions smaller than 3-cm diameter. For larger voxel size and/or for a smaller inclusion diameter, our results showed a decreased standard deviation of G' as well as an overestimation

of G' within the inclusions. Both findings may be partly explained by the local homogeneity assumption made in the inversion algorithm, and by the calculation of the derivatives within a local neighborhood. With large voxel sizes or small inclusions, more voxels are affected by the discontinuity in the distribution of the mechanical properties at the inclusion boundaries. The higher dispersion, especially visible in the background region when using small voxel sizes, can be explained by a sub-optimal λ/a ratio. The theoretical λ/a for the 2.5-mm resolution belongs to the higher end of the suggested optimal interval of 5–20 found in the literature. Furthermore, for this same resolution (data not shown), G' maps exhibit inhomogeneities that match the wave propagation pattern and ultimately lead to larger RMSE in the background. Considering that our simulations do not add any noise and ensure that no standing waves occur, one can conclude that such effects are exclusively due to the reconstruction pipeline and that large λ/a ratios should be avoided. All together, these results suggest that numerical simulations can provide a reliable prediction of reconstructed results for different experimental conditions in a simple, yet heterogeneous object. Possible challenges in designing accurate simulations of more complex geometries lie in the difficulties of realistic modeling, in particular of the boundary conditions and wave propagation at the various interfaces. Although simulations of a body organ may require a detailed knowledge of mechanical properties and their distribution at a fine spatial scale, their feasibility may be increased from the availability of structural datasets acquired via different imaging techniques (MRI, CT) in combination with appropriate, simplified physical assumptions. Finally, we believe that simulations could help improving the overall MRE performances by tuning either the MRE parameters, or by addressing specific aspects of the reconstruction pipeline. In this study we chose one single frequency and three spatial resolutions, but simulations can easily be performed using various frequencies and a large span of spatial resolutions.

MRE Experiments

Overall, experimental results appear very similar to the reconstructed simulation data (**Figures 3, 5, 6**). Some differences can be seen on the edges of the phantom exhibiting uneven contours and susceptibility artifacts at the air-silicone interface. Like the simulations, MRE demonstrated good accuracy for retrieving G' across large, homogeneous regions (background, I4, and I3), and limitations for inclusions smaller than 3 cm diameter. Those limitations are tied to the specific MRE protocol chosen in this study. We chose a vibration frequency of 57 Hz in the range commonly used in human studies for various diseases, both focal and diffuse (e.g. in the brain, breast, liver, muscle, prostate, heart), along with acquisition times that ensure patient comfort. Yet, if required, mapping smaller objects with confidence could be achieved by improving the inversion algorithm, in particular by assuming anisotropy and medium heterogeneity. Further development in the experimental protocol could also help in that direction and provide more accurate MRE results, typically by adapting the mechanical vibration settings along with the acquisition parameters or using a retrospective data resampling [60]. We also noticed biases introduced by the

reconstruction process, such as the underestimation of G' values near the boundaries of the phantom (Figure 5) or the greater error in the background at the finest resolution (Figure 7). That said, confidence in the estimation of errors from the MRE side must be balanced with the fact that RMSE calculation requires an actual ground truth, here estimated from the simulation data.

Validity Criteria for MRE

The λ/a ratio was always in the range suggested by the literature, gradually increasing with time from the initial stiffening phase in our phantom. Interestingly, we note (Table 4) that a relatively small change in voxel size (e.g. 3 mm vs. 2.5 mm) affects the ratio λ/a more than an increase in G' of almost 1.2 kPa, as could be measured from week 1 to week 4. Therefore, if decimation or interpolation of voxels are always possible after the acquisition, an appropriate native resolution remains essential for relevant reconstruction outcomes. More concretely, it shows that the quest for finer resolutions does reach a limit where it starts to impede MRE performance rather than improving it.

In our study, we used a fixed vibration frequency along with a variable resolution conversely to most studies that investigated the λ/a ratio by employing a wide range of vibration frequencies and a fixed pixel size. The range of chosen resolutions may not be sufficient to define the optimal λ/a ratio but is sufficient to show how much it impacts reconstruction, without being influenced by wave penetration or motion efficiency. Although this approach may lead to smoothing effects from filtering kernels in the processing of the raw MRE data, it is more relevant for mono-frequency MRE approaches and in organs where wave attenuation may vary significantly in a small range of frequencies.

Considering SNR, the computed values over time were high (Table 3) and translated into rather small phase and amplitude errors, defeating their relevance to discard unreliable voxels. In a more general case, however, phase uncertainty may be used to eliminate regions where the encoding of the vibration produces a net phase lower than the corresponding error. This may occur because of low SNR, either due to short T_2^* in general, a strong vibration, highly attenuated wave amplitude or insufficient encoding efficiency. It is particularly important to be able to distinguish the two latter conditions since a lack of waves may be due to physical properties or transducer settings, whereas the encoding efficiency may be improved acting on the sequence parameters. The motion encoding efficiency, however, is not always available to the end-user, and providing this information in terms of encoded radians per displacement at the scanner user interface would allow useful data quality considerations and an estimation of PNR. Using SNR only as a thresholding metric, on the other hand, was successfully applied. In our study, it appeared particularly useful in regions where large motion amplitudes led to strong losses of MR signal, i.e. in voxels near the transducer location. Such a metric is simple and has the advantage of not requiring knowledge of the sequence details. We strongly recommend to systematically calculate the SNR and to use it as a basic quality control check to prevent any potential diagnostic errors that will in turn lead to inadequate patient treatment.

CONCLUSIONS

The proposed work presents an effective and adaptable solution for testing, assessing, or comparing MRE methods performances, which is summarized in Figure 9. Synthetic MRE was successfully obtained from simulated displacement fields. We demonstrated the fabrication of a simple, low-cost, structured MRE phantom with controlled mechanical properties close to biological tissue that are stable over time, replicating the ground-truth phantom modeled in our simulations. Furthermore, the material is quite dispersive and hence suitable for studying multi-frequency MRE. Such a phantom can be easily adapted to various applications and deployed worldwide to study the performance of a given MRE experiment and reconstruction pipeline. Our simulations successfully matched experimental MRE results on the phantom. Similar results, trends, and limitations regarding the accuracy of both G' values and the inclusion shape were observed. As a consequence, simulations can be used to predict or estimate performance in MRE experiments *in silico* and showcase the possible intrinsic method limitations. This step could be cost and time effective for parameters fine tuning in anticipation of the optimization phases at the scanner. Finally, we showed how the interpretation of the reconstructed maps can be improved by discarding unreliable voxels. In particular, besides phase uncertainty based criteria which may be useful in certain cases and the importance of λ/a ratio, an SNR-based discrimination should be applied in general for direct thresholding to discard unreliable elastogram regions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MY, MS, JW, and NS: study design. MY: simulations. MY, MA, and NS: experiments. MY, MS, RS, and NS: data analysis. All authors contributed to writing and proofreading the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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On the Comparative Suitability of Strain Relaxation and Stress Relaxation Compression for Ultrasound Poroelastic Tissue Characterization

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Poroelastic tissue strain imaging measures the time-varying and spatially varying deformation of a soft-tissue matrix during compression as the tissue fluid flows out of the compartmental boundaries. With the help of ultrasound, it has been carried out by observing the evolution of the images of the ultrasound echo strain over time, which shows that, in a stress-relaxation experiment (constantly applied global axial strain), a front of negative dilatation (volumetric strain) propagates slowly from the boundaries of a sample toward the center of the compressed region. The fitting of equations that predict this behavior to experimental data has earlier allowed quantitative imaging of the product of aggregate modulus and permeability of a tissue phantom, $H_A k$, and its Poisson's ratio, ν . An ability to image and measure such novel tissue characteristics is likely to benefit biomedical research and have a wide range of clinical applications, including the assessment of lymphoedema, the diagnosis of cancer, the prediction of anticancer drug effectiveness, and monitoring of the tissue response to various treatments. This method is problematic, however, for application *in vivo* because the calculation of the volumetric strain requires the lateral and elevational strains, which are not easily measured accurately with conventional ultrasound strain imaging. This paper investigates for the first time whether the ultrasound observation in a strain-relaxation experiment (constantly applied uniaxial stress) could be used to observe the same mechanical behavior and provide the same information about the properties of a poroelastic sample as in a stress-relaxation experiment. The analytical theory was used to demonstrate that the propagation of dilatation shown in stress relaxation should also be observable in strain relaxation and that it should be detectable using axial strain, which is relatively easily measured *in vivo*. Finite element modeling (FEM) was employed to simulate all strain components within a homogeneous poroelastic material first during strain relaxation and then during stress relaxation, again demonstrating their equivalence for the observation of the propagation of a dilatation. The validity of using the strain relaxation conditions

as an alternative to stress relaxation for measuring a poroelastic material's response was further confirmed by a fitting of the analytical models to the results of FEM. This allowed for an inversion of the time-varying volumetric strain, to recover the images of $H_A k$ and ν , for either loading configuration. The strain-relaxation configuration offers not only an opportunity to derive the same important quantitative poroelastic properties of the tissue as stress relaxation but also the potential to avoid the difficulties and errors associated with the estimation of strain along the axes perpendicular to the imaging axis, thus offering opportunities for easier clinical translation.

Keywords: poroelasticity, stress relaxation, strain relaxation, ultrasound, material properties, interstitial fluid, permeability

INTRODUCTION

Tissue physiology [1, 2] and the nature of the time-dependent response to a sustained compression [3, 4] both indicate that soft tissue is poroelastic. Poroelastic strain imaging measures the time-varying and spatially varying deformation of the elastic matrix as the tissue fluid flows out of the compressed region of any soft tissue that is subjected to sustained compression. Ultrasound tracking of the images of the echo strain over time shows that, when a constant axial strain is applied on the tissue, the dilatation (volumetric strain) decreases slowly from the boundaries of a sample toward its center. An ability to image and measure such a tissue behavior is likely to have diverse clinical applications, including the assessment of lymphoedema, the diagnosis of cancer, and the monitoring of the tissue response to various treatments.

Soft tissue is multiphase in nature. As a result, in contrast to linear viscoelastic models, poroelastic models describe more closely the deformation that soft tissue experiences when subjected to a sustained compressive force. The resulting local volume reduction (equal to the amount of fluid that has been forced away from the compressed volume [5]) allows the spatially varying and time-varying strain to be related to particular biomechanical properties, namely the permeability k , Poisson's ratio ν , and Young's modulus E , that describe the microstructure of a poroelastic material. For practical measurement and imaging, ultrasound echo-tracking techniques employed in ultrasound elastography [6] may be used to track the tissue displacement over time in response to the applied force.

Previously published studies in experimental poroelasticity have used a mathematical model of poroelasticity and a finite element analysis to predict the strain evolution inside a poroelastic cylinder subjected to a stress relaxation compression under unconfined boundary conditions [7, 8]. Quantitative images of the confounded material properties, aggregate modulus and permeability $H_A k$, and of Poisson's ratio ν were produced in phantoms [9]. Clinical poroelasticity studies have also been conducted by using stress relaxation conditions [10, 11]. The poroelastic study of a homogeneous material using stress relaxation conditions is conducted *via* time-varying strain ratio imaging, which requires special measures to try to enhance the lateral strain estimation because, when the compressor plates

are frictionless, axial strain does not vary. These measures are not entirely successful and result in strain ratio images that have a poor signal-to-noise ratio and, to date, have been achieved clinically only in two dimensions [11], even though a preliminary three-dimensional (3D) work has been done in phantoms [12]. In specific clinical applications where heterogeneity in poroelastic properties exists, the observation of stress-relaxation *via* time-varying axial strain imaging only may yield useful information [10, 13], but the interpretation of the resulting images is complicated, and the results are not quantitative for the tissue poroelastic characteristics. Strain-relaxation (creep) experiments, however, may take advantage of time-varying axial strain, which is estimated with a good signal-to-noise ratio and has been very useful for the study of the tissue viscoelastic characteristics, thus producing, for example, diagnostically useful relaxation-time images of breast tumors [14].

However, as strain relaxation (creep) and stress relaxation offer different advantages and disadvantages in their application to ultrasound poroelasticity imaging, in this paper, we investigate for the first time whether a strain relaxation compression experiment provides the same quantitative and qualitative information about the poroelastic nature, behavior, and properties of the studied sample as that seen in Berry et al. [8] for the stress-relaxation experiment. We use analytical theory and finite element modeling (FEM) to demonstrate the qualitative equivalence of the dilatation propagation seen for strain relaxation and stress relaxation, and that the fitting of the analytical model predictions to the strain-relaxation FEM results allows the recovery of images of the poroelastic material properties as it did for the stress-relaxation study in Berry et al. [8].

In the absence of the ability to accurately estimate all (three) components of strain, a strain relaxation compression may be preferable clinically because it allows the relaxation to be followed when imaging is such that an accurate strain estimation is confined to an axial direction of the applied and sustained force. The use of strain relaxation may offer more suitable options for clinical translation, since it may avoid the difficulties and errors associated with the ultrasound measurement of lateral and elevational strain needed for observing stress relaxation.

MATERIALS AND METHODS

Based on the fundamentals of the biphasic theory of poroelasticity, the stages that a cylindrical unconfined poroelastic sample is going through during the relaxation should not be dependent on the type of compression but only on the biomechanical and geometrical properties of the sample and the magnitude of the external mechanical stimuli [16]. The three main stages of poroelastic response as illustrated and described by Berry et al. [8] may be categorized as follows: (1) The elastic stage: a sudden lateral expansion without a change in the volume of a sample immediately after the application of the compression. (2) The transient or biphasic stage: the radial tensile stress in the porous matrix of the sample is decaying, while the hydrostatic pressure gradient induces the fluid flow across the boundary of the sample. (3) The steady-state stage: as time increases, the material of the sample tends toward the equilibrium stage where the fluid flow has ceased and the fluid pressure and radial stress are zero.

The fluid flows radially in an outward direction. Initially, only fluid at the boundary of the cylinder flows because only there does a pressure gradient exist. This pressure gradient, and therefore the annulus of moving fluid, moves slowly toward the center, creating either the creep or the stress relaxation along the direction of axial compression. In creep, the deformation changes over time in the axial, radial, and circumferential directions, whereas in stress relaxation, the axial strain remains constant but the radial strain and circumferential strain vary.

The hypothesis tested in this work was that, by sustaining either an external force or an equivalent axial strain on identical poroelastic specimens during an unconfined compression, the poroelastic effects are equivalent to the extent that, with appropriate data processing, poroelastic property information that is solely determined by the interstitial fluid flow within the solid matrix may be extracted.

Theoretical Analysis

Poroelasticity

A perfectly elastic material deforms instantly under an applied force and returns instantaneously to its original dimension immediately after the force is removed. In contrast with the fact that strain in a purely elastic material is time independent, viscoelastic materials exhibit a viscous (as well as elastic) behavior and are characterized by their time-dependent mechanical behavior under an applied mechanical stimulus [4, 17, 18].

Viscoelastic materials behave in some ways as if they are partly solid and partly fluid. The strain increases with time when the stress is constant, which is known as strain relaxation or creep. The stress decreases with time when the strain is constant, which is known as stress relaxation. Finally, mechanical energy is lost to the medium as heat, which is revealed by cyclic loading in a phenomenon known as hysteresis. The elastic moduli also depend on the rate of loading and the interval between the loading cycles and the number of previous loading cycles. The underlying physical mechanisms that are the causes of such behaviors in a given material may often be unknown, particularly in complex media such as biological tissues, and

indeed the mechanisms at work will often vary with the strain rate.

Poroelastic materials are truly partly solid and partly fluid. They have physically separated phases, for example, in the form of a sponge-like elastic matrix that contains space (pores) occupied by fluid, which is able to move through that space. Like viscoelastic materials, poroelastic materials demonstrate stress relaxation and strain relaxation although the mechanism is that given time the fluid will flow down the pressure gradient caused by applied stress. However, poroelastic materials exhibit time-dependent phenomena with features that are additional to the abovementioned features associated with viscoelastic materials. In particular, they experience volumetric strain (even when each of the components is incompressible) and the propagation of strain fronts [8, 9] described as follows.

For small strains, the equation that describes the time and spatial dependence of volumetric strain in a poroelastic material was established by Biot [19] and, with a modification that takes into account the fluid viscosity [20], is written as follows:

$$\nabla^2 \varepsilon = \frac{1}{H_A k \eta^{-1}} \frac{\partial \varepsilon}{\partial t} \quad (1)$$

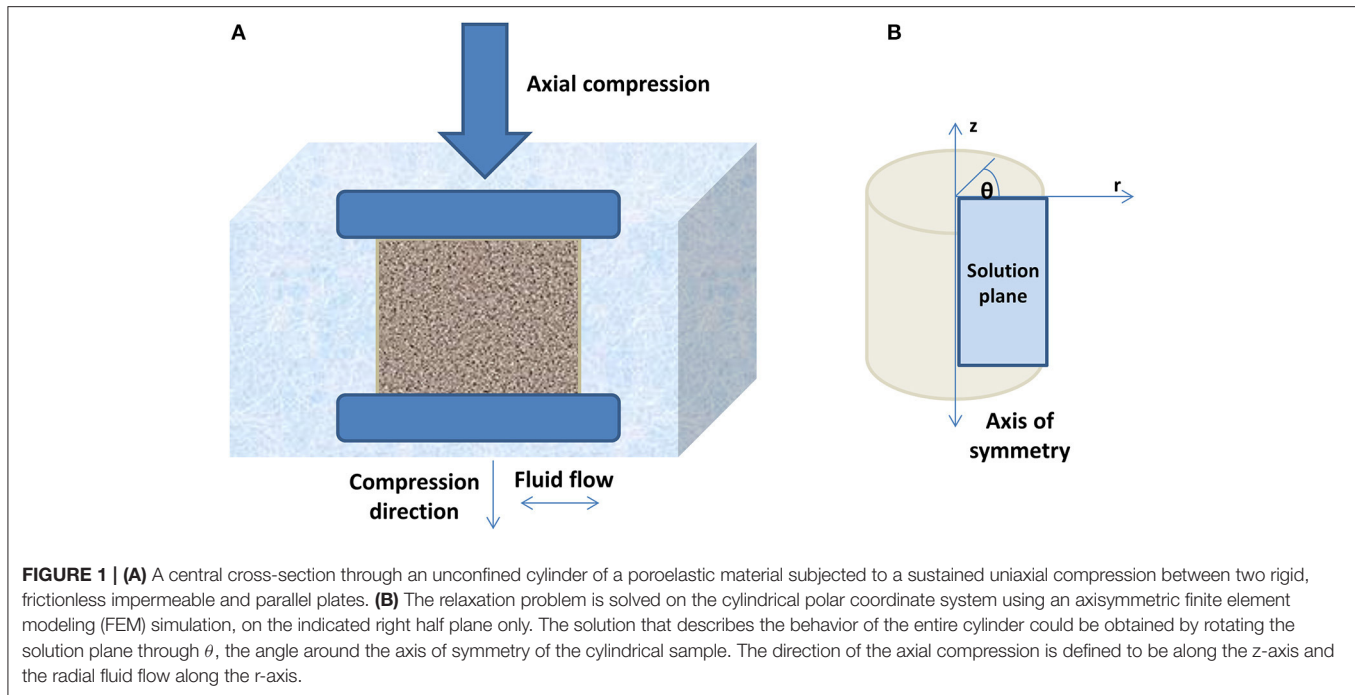
where ε is the strain, H_A the aggregate modulus, k the permeability, η the dynamic viscosity of the pore fluid, and t the time. The aggregate modulus H_A can be expressed in terms of the Young's modulus E and the Poisson's ratio ν . It is a measure of the stiffness of the composite material at equilibrium after all the fluid flow has stopped [16]. The Poisson's ratio is defined as the ratio of the lateral to axial strain and covers the range $0 \leq \nu \leq 0.5$, for linear, isotropic materials.

$$\nu = -\frac{\varepsilon_l}{\varepsilon_a} \quad (2)$$

$$H_A = \frac{E(1 - \nu)}{(1 + \nu)(1 - 2\nu)} \quad (3)$$

Permeability, as mentioned here, refers to the Darcy permeability, which measures the ability of a porous medium to allow fluid, such as interstitial fluid, to pass through its pores. It is a characteristic of the matrix of a material and connects the fluid velocity to the pressure gradient [2]. Permeability is defined in units of area that are directly related to the pore space cross-section perpendicularly to the direction of fluid flow. It depends solely on the characteristics of the porous structure of a material, namely, the pore size, density of pores, and interconnectivity of the porous network. For large deformations (assuming not to occur here), as the poroelastic material deforms, its permeability varies due to the ensuing microstructural changes. This usage of the term permeability is different from the other uses, such as when it describes the "leakiness" of the capillary vascular endothelium, although such phenomena do govern a fluid transport between the extravascular and intravascular compartments and therefore will influence the poroelastic behavior of tissues as described by multiscale models (e.g., [13, 21]).

The mechanical behavior of the Biot poroelastic model is controlled by three material properties: the permeability k of the solid matrix to the pore fluid, the dynamic viscosity η of the fluid flowing through the porous structure of a sample,



and the Poisson's ratio ν of the solid matrix and the aggregate modulus H_A .

A Poroelastic Sample Under Relaxation Compression

We consider here a homogeneous cylindrical poroelastic sample. Even though this geometry is unlikely to occur naturally in the body (apart from intervertebral discs), it suffices here purely to permit a comparison of the qualitative and quantitative information that may be obtained from the strain-relaxation and stress-relaxation experiments. If such a sample of radius a is axially compressed between two rigid impermeable compressor plates (**Figure 1A**), immediately after the application of a sustained compressive axial stress σ_0 or strain ε_0 (time $t = 0^+$), it behaves like an incompressible elastic solid and, as illustrated by Berry et al. [8], expands radially to conserve volume and experiences an increased average stress or pressure. For $t > 0$, the sample's response is directed by its poroelastic nature as follows [8, 16]. The pressure gradient between the sample and its surroundings drives an exudation of interstitial fluid at its unconfined (freely permeable) boundary ($r = a$). This radial fluid flow, which takes time, releases the pressure at that radial position and allows the radial elastic restoring force to pull the solid matrix toward the center (radial distance $r = 0$). In other words, stress, radial strain, and volumetric strain all relax at that radial position. As a result, the radial position of the pressure gradient moves inward ($r < a$) and the process continues, producing a front of relaxation, which travels radially inward until stability is reached with a reduced sample volume when all fluid that is going to leave the sample has escaped *via* its outer surface.

For the purposes of modeling the strain-relaxation loading problem, it is assumed that an infinitesimal axial compressive stress is applied instantaneously on the upper surface of the

sample and sustained thereafter:

$$\sigma_{zz}(t) = \begin{cases} 0 & t < 0 \\ -\sigma_0 & t \geq 0 \end{cases} \quad (4)$$

where σ_{zz} is the magnitude of the stress applied in the axial direction.

Assuming that no shear forces exist on the compression plates (i.e., no friction between the sample and the plates), the radially averaged radial strain inside the sample, which is equal to the global radial strain defined by the radial displacement of the curved cylindrical surface at $r = a$, is given as [16]:

$$\varepsilon_{rr}(a, t) = (\sigma_0) \left[\frac{\nu}{E} + \frac{(1-\nu^2)(1-2\nu)}{E} \sum_{n=1}^{\infty} \frac{4e^{\frac{-\alpha_n^2 H_A k \eta^{-1} t}{a^2}}}{\{9\alpha_n^2(1-\nu)^2 - 8(1+\nu)(1-2\nu)\}} \right] \quad (5)$$

Furthermore, the axial strain history at $r = a$ is given as follows [16]:

$$\varepsilon_{zz}(t) = -\frac{\sigma_0}{E} \left[1 - \sum_{n=1}^{\infty} \frac{(1-\nu^2)(1-2\nu)4e^{\frac{-\alpha_n^2 H_A k \eta^{-1} t}{a^2}}}{\{9\alpha_n^2(1-\nu)^2 - 8(1+\nu)(1-2\nu)\}} \right] \quad (6)$$

The evolution of the dilatation or volumetric strain, defined as the relative change in volume $\frac{\Delta V}{V}$ [22] within an element of a sample,

is given as [16]:

$$\varepsilon_v(r, t) = -\frac{\sigma_0}{E} \left[(1-2\nu) - \sum_{n=1}^{\infty} \frac{(1-2\nu^2)(1+\nu) \frac{8J_0(\alpha_n r)}{aJ_0(\alpha_n)} e^{-\frac{\alpha_n^2 H_A k \nu^{-1} t}{a^2}}}{9\alpha_n^2 (1-\nu)^2 - 8(1+\nu)(1-2\nu)} \right] \quad (7)$$

where α_n are the roots of the characteristic equation:

$$J_0(x) - \frac{4(1-2\nu)}{3(1-\nu)} \frac{J_1(x)}{x} = 0 \quad (8)$$

where $J_0(x)$ and $J_1(x)$ are the Bessel functions of the first kind of order 0 and 1, respectively, and ν is the Poisson's ratio.

For the purposes of modeling the stress-relaxation loading problem, an infinitesimal compressive axial strain is assumed to have been applied instantaneously on the upper surface of the sample and sustained thereafter:

$$\varepsilon_{zz}(t) = \begin{cases} 0 & t < 0 \\ -\varepsilon_0 & t \geq 0 \end{cases} \quad (9)$$

where ε_0 is the magnitude of the strain applied in the axial direction.

For all times, the radially averaged radial strain inside the sample is given as [16]:

$$\varepsilon_{rr}(a, t) = \varepsilon_0 \left[\nu + (1-2\nu)(1-\nu) \sum_{n=1}^{\infty} \frac{e^{-\frac{\beta_n^2 H_A k \nu^{-1} t}{a^2}}}{\beta_n^2 (1-\nu)^2 - (1-2\nu)} \right] \quad (10)$$

This equation for stress relaxation is equivalent to that for strain relaxation (Equation 5). The evolution of the radial strain field over radius and time is [8]:

$$\varepsilon_{rr}(r, t) = \varepsilon_0 \left[\nu + \sum_{n=1}^{\infty} \frac{J_0(\beta_n) - \left(\frac{1-2\nu}{1-\nu} \right) \left\{ \frac{J_0(r\beta_n)}{a} - \frac{J_1\left(\frac{r\beta_n}{a}\right)}{\frac{r\beta_n}{a}} + \frac{J_1(\beta_n)}{\beta_n} \right\}}{\left(\frac{1}{1-\nu} \right) J_0(\beta_n) - \beta_n J_1(\beta_n)} e^{-\frac{\beta_n^2 H_A k \nu^{-1} t}{a^2}} \right] \quad (11)$$

Finally, the radial and temporal variations of the dilatation or volumetric strain during stress relaxation are [8]:

$$\varepsilon_v(r, t) = \varepsilon_0 \left[1 - 2\nu - \sum_{n=1}^{\infty} \frac{2J_0(\beta_n) - \left(\frac{1-2\nu}{1-\nu} \right) \left\{ \frac{J_0(r\beta_n)}{a} + \frac{2J_1(\beta_n)}{\beta_n} \right\}}{\left(\frac{1}{1-\nu} \right) J_0(\beta_n) - \beta_n J_1(\beta_n)} e^{-\frac{\beta_n^2 H_A k \nu^{-1} t}{a^2}} \right] \quad (12)$$

where β_n are the roots of the characteristic equation:

$$J_1(x) - \frac{(1-\nu)}{(1-2\nu)} x J_0(x) = 0 \quad (13)$$

By setting $t \rightarrow \infty$, the steady-state dilatation is equal to:

$$\varepsilon_v(r, t) = \varepsilon_0 [(1-2\nu)] \quad (14)$$

regardless of whether a stress relaxation or strain relaxation compression is used.

In reaching this steady state, the dilatation equation describes diffusion-like propagations of volumetric strain relaxation in a radial direction across the whole volume of a cylinder, beginning at the outer boundary and ending when it reaches the center of a sample. This propagation was chosen to be a qualitative and quantitative metric of comparison of the two loading configurations. Time-varying images showing this propagation of a volumetric strain relaxation were shown from the finite element simulations in Berry et al. [8] and confirmed experimentally in tissue mimicking poroelastic phantoms in Berry et al. [9]. In addition, the accuracy and precision of the poroelastic material characteristics, $H_A k$ and ν , recovered by fitting the predictions of the above equations to the results of the experiments simulated by FEM (see below), were used for further quantitative comparison of the utility of the two loading configurations.

Finite Element Modeling

A commercial FEM program, MARC Mentat[®] (MSC Software Ltd., Frimley, UK), as used for poroelastic simulations by Fromageau et al. [12, 15], was employed to predict the response of an unconfined cylindrical sample to an externally applied stress relaxation or strain relaxation compression using a structural and diffusion-type simulation. The cylindrical geometry was used for the reasons provided at the beginning of the Section entitled The Poroelastic Sample Under Relaxation Compression. In both strain and stress-relaxation scenarios, the material of a sample was simulated to be a poroelastic fluid–solid mixture that obeys the consolidation and biphasic theories [16, 19]. The sample was compressed along its axis of symmetry between two frictionless, rigid, impermeable, flat, parallel surfaces while immersed in a fluid with a viscosity equal to that of water at room temperature. The axisymmetric relaxation problem was solved in cylindrical polar coordinates, for the right half of the z - r solution plane (Figure 1B).

The FEM mesh was composed of 1,000 elements (type 33 MARC Mentat[®]) and 29,209 nodes, arranged in consecutive rows with a constant density throughout the homogeneous cylindrical model. The height of the simulated cylinder was set equal to 30 mm and its radius equal to 25 mm, similar to that employed in the ultrasound strain imaging of poroelastic cylinders undergoing the stress-relaxation experiments of Berry et al. [9]. The poroelasticity of the model was satisfied by setting the porosity equal to 0.5 and the permeability of the simulated biphasic sample to the pore fluid equal to $6.4 \times 10^{-16} \text{ m}^2$. The permeability value was chosen to reflect the value expected in healthy soft tissue [13]. Other material properties are shown in Table 1. Fluid flow was prevented along the z -axis but allowed radially, conditions which allowed a good match to be achieved between the results from simulation and experiment in a previous study of poroelastic cylinders [8, 9]. The time step for sampling the temporal evolution of the strain field was 0.5 s.

Strain relaxation was simulated by loading the upper surface of a sample with a compressive axial stress of 4 kPa, corresponding to an axial force of 8 N uniformly distributed across the sample

TABLE 1 | The material properties specified for the simulated poroelastic sample.

Young's Modulus (Pa)	Poisson's ratio	Viscosity η of the pore fluid (Pa s)	Permeability (m^2)
8.00E+04	0.3	1.00E-03	6.40E-16

As a result, the aggregate modulus H_A given by Equation (3) equals $8.9\text{e}+4$ Pa.

cross-section, allowing the upper surface to move while ensuring that the bottom plate was stationary.

Stress relaxation was simulated by moving the upper compressor plate along the z-axis, to apply a displacement to the upper surface of a sample with a magnitude equal to 3% of the height of a sample.

The axial stress or strain was sustained for 180 min. As frictionless boundaries had been assumed, the resulting axial and volumetric deformations of the sample were independent of the z-axis component of their location.

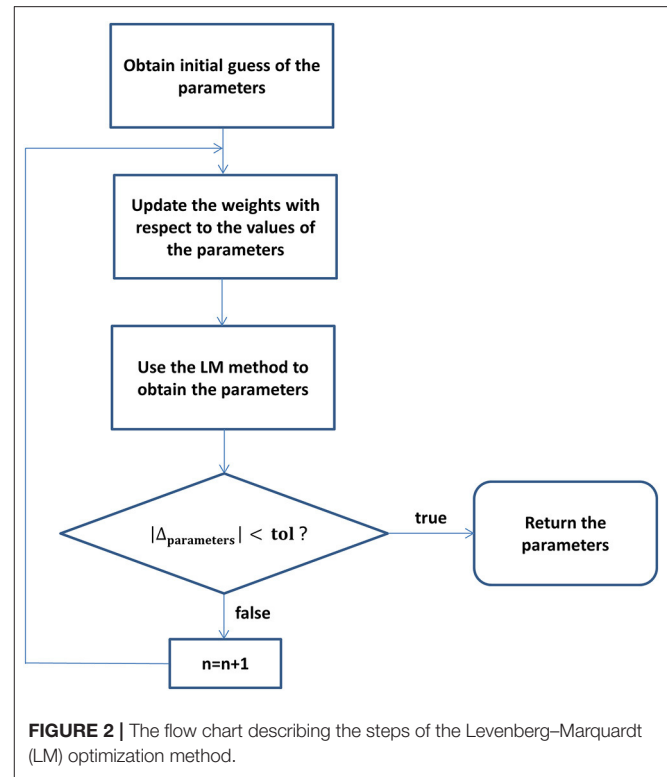
All strain components were simulated for both strain-relaxation and stress-relaxation experiments. Using the strain components, the volumetric strain was calculated so that the poroelastic sample behavior in strain relaxation could be compared with that in stress relaxation. Inserting the material properties (Table 1) into the strain equations of poroelasticity [Equations (5)–(7) and (10)–(12)] the deformation of the poroelastic sample was also analytically modeled during stress relaxation or strain relaxation for the same boundary conditions, time, and the applied compression as that employed in the FEM.

Estimation of Poroelastic Characteristics of a Material

The method previously employed by Berry et al. [8, 9], for estimating the poroelastic characteristics of the material of a cylinder under stress-relaxation loading, was applied again here, but also for strain-relaxation loading. By examining the poroelastic equations, it is straight forward to observe that not only do all equations share the same exponential mathematical form but also, importantly, their exponential decay is determined by the same time constant $\frac{s_n^2}{t_r}$, where s_n (α_n , β_n) are the eigenvalues of the characteristic equations (Equations 8 and 13) and t_r is the characteristic poroelastic relaxation time. This is the characteristic strain diffusion time of the porous matrix, determined as defined below by the square of the length of the maximum fluid path (which in this case is equal to the radius of a cylinder), the aggregate modulus H_A (Equation 3), the permeability k of the matrix, and the viscosity η of the pore fluid.

The parameterized form of the mathematical equations that describe the imaged strain in a poroelastic relaxation experiment may be written as follows:

$$\text{strain}(r, t) = P_i \left[A_i + \sum_{n=1}^{\infty} B_i e^{-\frac{s_n^2}{t_r} t} \right] \quad (15)$$

**FIGURE 2** | The flow chart describing the steps of the Levenberg–Marquardt (LM) optimization method.

The constants of Equation (15) that are related to the volumetric strain ε_v in a strain relaxation compression experiment (Equation 7) are equal to:

$$\begin{aligned}
 P_1 &= -\frac{\sigma_0}{E} \\
 A_1 &= (1 - 2\nu) \\
 B_1 &= -\frac{(1 - 2\nu^2)(1 + \nu) \frac{8J_0(\alpha_n r)}{aJ_0(\alpha_n)}}{\left\{ 9\alpha_n^2 (1 - \nu)^2 - 8(1 + \nu)(1 - 2\nu) \right\}} \\
 t_r &= \frac{a^2}{H_A k \eta^{-1}}
 \end{aligned}$$

and $s_n = \alpha_n$, the roots of the characteristic equation (Equation 8).

On the other hand, the constants related to the volumetric strain ε_v in a stress relaxation compression experiment (Equation 12) are equal to:

$$\begin{aligned}
 P_2 &= \varepsilon_0 \\
 A_2 &= 1 - 2\nu \\
 B_2 &= -\frac{2J_0(\beta_n) - \left(\frac{1-2\nu}{1-\nu} \right) \left\{ \frac{J_0(r\beta_n)}{a} + \frac{2J_1(\beta_n)}{\beta_n} \right\}}{\left(\frac{1}{1-\nu} \right) J_0(\beta_n) - \beta_n J_1(\beta_n)} \\
 t_r &= \frac{a^2}{H_A k \eta^{-1}}
 \end{aligned}$$

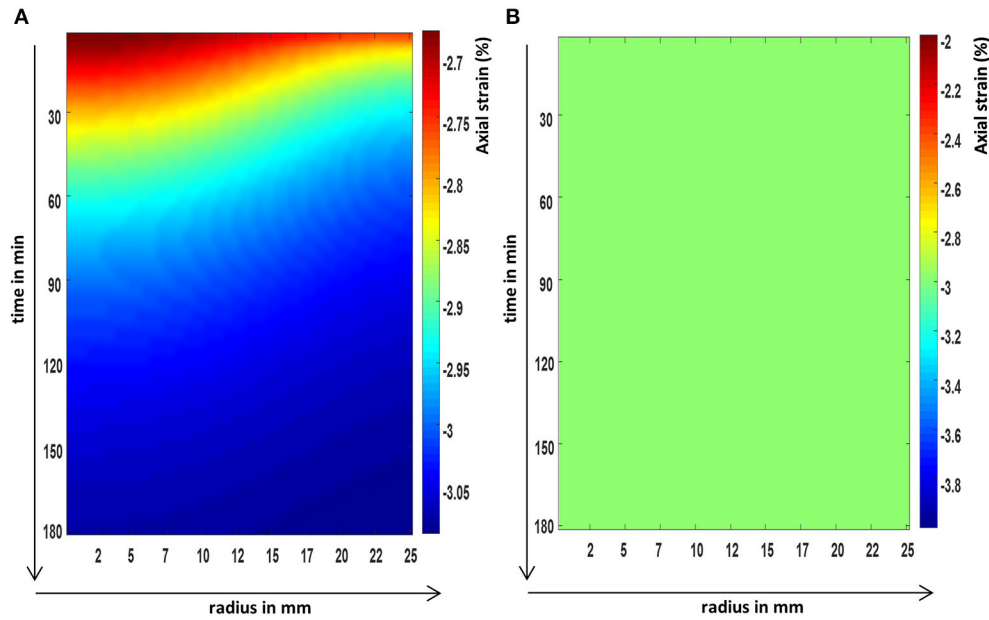


FIGURE 3 | The FEM predictions of the axial strain evolution over time, at the axial center, and across the radius of a cylindrical poroelastic sample subjected to a constant axial stress (A) and an equivalent constant axial strain (B).

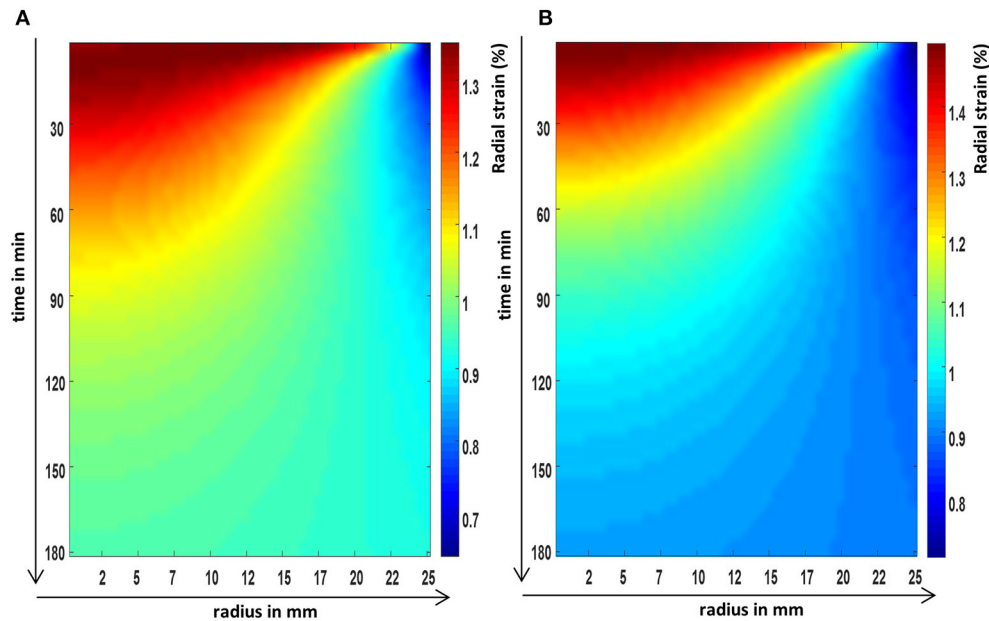


FIGURE 4 | The FEM predictions of the radial strain evolution over time, at the axial center, and across the radius of a cylindrical poroelastic sample subjected to a constant axial stress (A) and an equivalent constant axial strain (B).

and $s_n = \beta_n$, the roots of the characteristic equation (Equation 13).

For the cylindrical poroelastic phantom specified in **Figure 1** and **Table 1**, the applied axial stress σ_0 or strain ε_0 , the radius of the sample a , and the roots of the characteristic equations s_n are known. Thus, the parameters, determined by the poroelastic characteristics of the material from which the phantom is made,

recovered by fitting the appropriate equation mentioned above to the strain data acquired during stress relaxation or strain-relaxation are:

$$\begin{aligned} p_1 &= \nu \\ p_2 &= p_{21} * p_{22} = H_A * k \end{aligned} \quad (16)$$

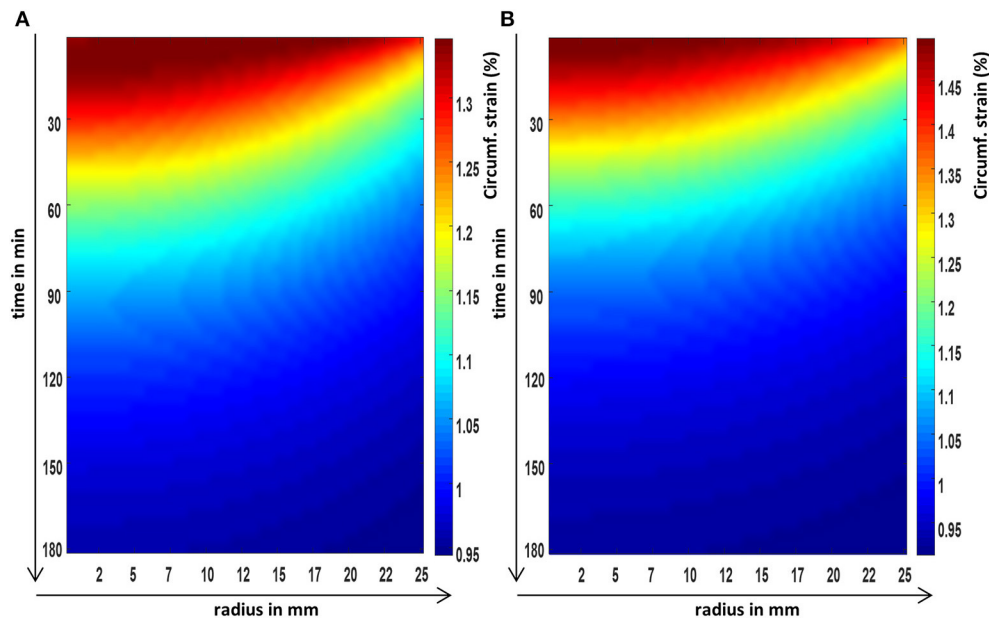


FIGURE 5 | The FEM predictions of the circumferential strain evolution over time, at the axial center, and across the radius of a cylindrical poroelastic sample subjected to a constant axial stress **(A)** and an equivalent constant axial strain **(B)**.

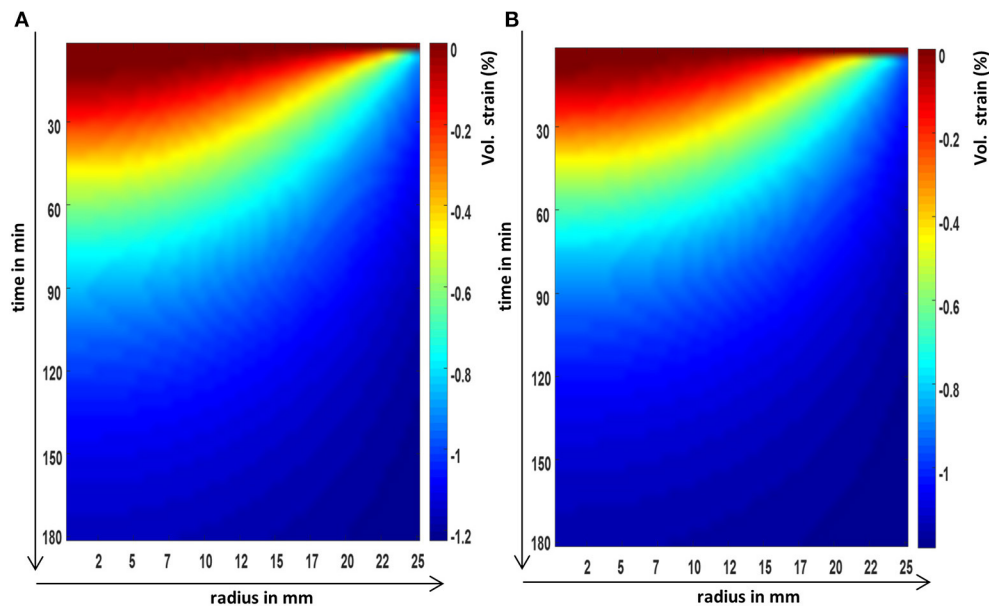


FIGURE 6 | The FEM predictions of the evolution of the volumetric strain or dilatation over time, at the axial center, and across the radius of a cylindrical poroelastic sample subjected to a constant axial stress **(A)** and an equivalent constant axial strain **(B)**.

There is only one equation in which the parameters p_{21} and p_{22} are employed for the relaxation time $t_r = \frac{a^2}{H_A k \eta^{-1}}$. Thus, it is impossible to separate the parameters p_{21} and p_{22} directly by model fitting without compromising the uniqueness of the solution.

The Levenberg–Marquardt (LM) algorithm that is available in the Matlab[®] (Mathworks Inc., Natick, MA, USA) optimization

toolbox was employed in this work to fit the above poroelastic equations to the FEM-predicted volumetric strain data. The goodness of fit was computed on 10 by 10 matrices of the simulated strain data produced by a forward analytical modeling using the poroelastic volumetric strain equations for strain relaxation (Equation 7) or stress relaxation (Equation 12). The flow chart (**Figure 2**) describes the LM algorithm steps.

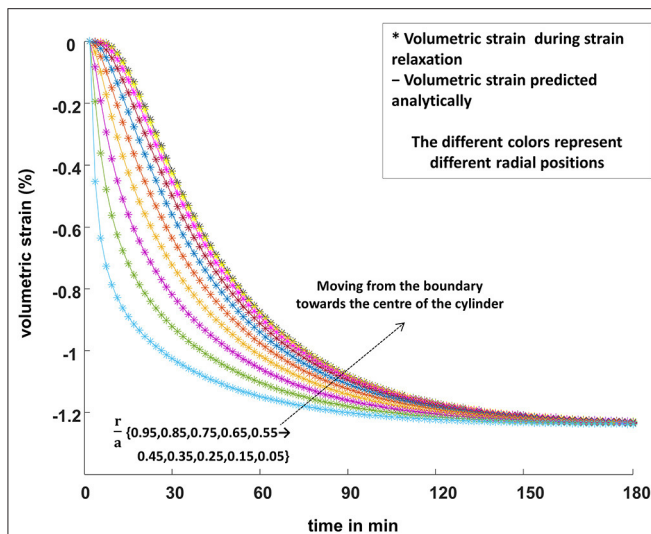


FIGURE 7 | The FEM predictions of the spatially varying volumetric strain ("*" symbols) inside a cylindrical poroelastic sample subjected to a constant compressive axial force, undergoing strain relaxation, are plotted along with the analytical predictions (Equation 7; solid lines). The volumetric strain decreases more rapidly for large values of the radial position r , where the pore fluid first leaves the sample, than for positions closer to the center of the cylinder, where the fluid contained in the pores is the last to leave its original location. The different colors represent different radial positions, r/a : cyan 0.95, lime 0.85, purple 0.75, yellow 0.65, orange 0.55, blue 0.45, brown 0.35, magenta 0.25, yellow 0.15, and olive 0.05.

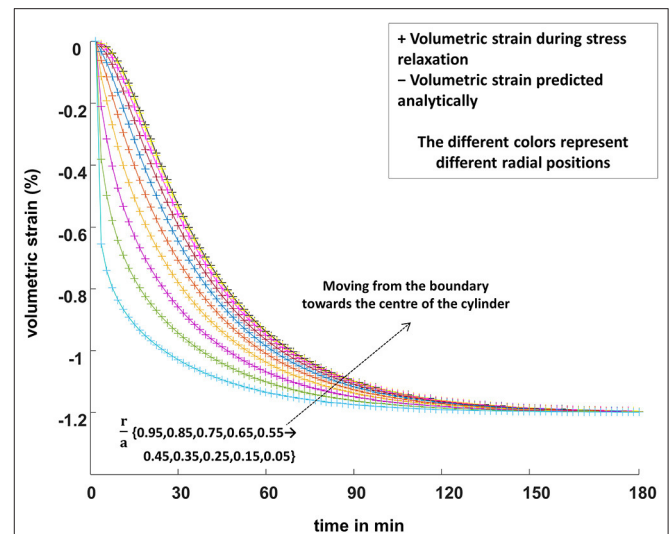


FIGURE 8 | The FEM predictions of the spatially varying volumetric strain ("+" symbols) inside a cylindrical poroelastic sample subjected to a constant compressive axial strain, undergoing stress relaxation, are plotted along with the analytical predictions (Equation 12; solid lines). Similar to the strain-relaxation case in **Figure 7**, the volumetric strain decreases most quickly at the larger values of the radial position r where the fluid path out of the sample is shorter. The different colors represent different radial positions, r/a : cyan 0.95, lime 0.85, purple 0.75, yellow 0.65, orange 0.55, blue 0.45, brown 0.35, magenta 0.25, yellow 0.15, and olive 0.05.

A small percentage of random, uncorrelated noise with a flat probability density function (white noise) was added to the simulated strain data to mimic the random noise that exists in experimentally acquired data sets. Computationally, the noise was created by randomly varying each strain pixel within a range equal to $\pm 3\%$ of the respective strain value. The flat probability density function was chosen, instead of another function such as a Gaussian, to render the results unbiased with respect to the noise properties of any particular ultrasound acquisition system.

Starting from an arbitrary initial guess of the material properties, $p_1 = [0.05 - 0.2]$, $p_2 = [1 - 7] \times 10^{-10}$ Pa m², the LM algorithm attempted to converge on the values that result in the best fit to the strain vs. time curves. The criteria used to assess data fitting were (a) the final value of the minimization cost function after an iteration had been stopped, and (b) the accuracy and precision of the estimated values of the material properties by comparing them with the predefined values (**Table 1**).

RESULTS

Previously published studies in the area of experimental poroelasticity measurement using ultrasound have combined the mathematical models of poroelasticity with a finite element analysis to predict and validate the strain evolution inside a poroelastic cylinder subjected to a stress relaxation compression under unconfined boundary conditions [7, 8]. In this work, the analysis was extended to compare the results with those for

an unconfined strain relaxation compression. The two types of compression induce distinct fluid-flow-related effects along the axial, radial, and circumferential axes.

In order to qualitatively and quantitatively compare the strain relaxation with the stress-relaxation (of a poroelastic material subjected to an unconfined compression), an objective metric is necessary. For this purpose, the fluid-induced internal strain behavior was simulated for both types of relaxation and the results are shown in the figures. The diffusion-like propagation of the volumetric strain across the whole volume of the cylinder, and the accuracy, precision, and appearances of the images of the poroelastic material characteristics, H_{AK} and ν , recovered by fitting the predicted time-varying strain to the measured time-varying strain, were used as the qualitative and quantitative metrics of comparison of the two loading configurations.

Comparison of Two Types of Poroelastic Relaxation Behavior Using Finite Element Analysis and Analytical Modeling

The strain across each polar coordinate was modeled by using a finite element analysis. The behavior of the deformation of a poroelastic cylinder was determined by subjecting it to an axial stress and an equivalent axial strain sustained for 3 h. **Figures 3–6** show the evolution of the axial, radial, circumferential, and volumetric strains, respectively, at the axial center and across the radius of a poroelastic sample subjected to a constant axial stress (strain relaxation, left figure) and a constant axial strain (stress relaxation, right figure), determined by using a finite element

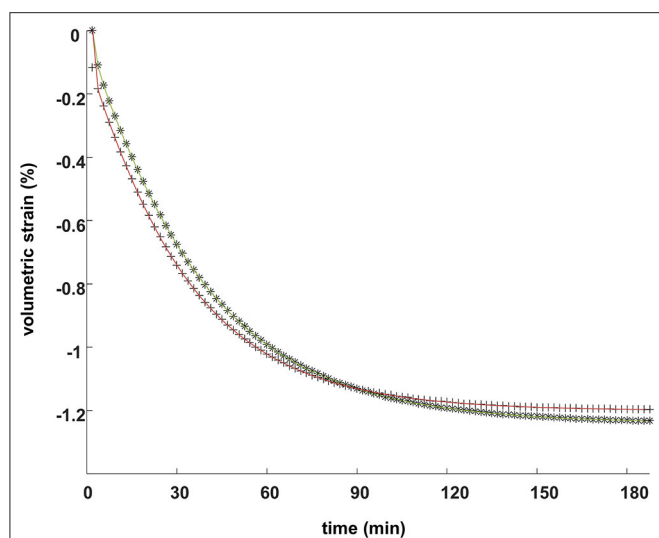


FIGURE 9 | The FEM predictions (plotted symbols) of the volumetric strain behavior over time, spatially averaged across all radial positions, compared with the analytical predictions of Equations (7) and (12) (solid lines), in the strain relaxation (olive lines and stars) and the stress relaxation (brown lines and crosses) compressions of a sample.

analysis. The volumetric strain is the sum of the axial strain, the radial strain, and the circumferential strain.

In **Figures 7 and 8**, for strain relaxation and stress relaxation, respectively, the FEM predictions (plotted symbols) and the analytical predictions (solid lines) of the time dependence of the volumetric strain inside the simulated biphasic cylinder are plotted throughout the radial extent of the phantom. The boundary conditions were the same for the FEM and the analytical calculations. Specifically, **Figure 7** shows the spatial and temporal dependence of the volumetric strain inside a phantom in a strain relaxation compression. **Figure 8** displays the volumetric strain behavior of the same phantom during stress relaxation.

Figure 9 compares the volumetric strain behavior, averaged across the whole solution plane, computed by using an FEM and analytically, for the two different types of axial compression.

Equivalence of Strain Relaxation and Stress Relaxation for Using an Inverse Method to Determine the Poroelastic Material Properties

Table 2 shows the values of the extracted material properties averaged over the whole region of interest inside a poroelastic cylinder undergoing strain relaxation or stress relaxation. The poroelastic properties (p_1 and p_2) were estimated by applying an LM inverse method to the strain-relaxation and stress-relaxation strain data, produced directly from the equations of poroelasticity, for 10 realizations of the algorithm, employing different initial parameters and white noise each time. The estimated values and the true parameter values that were predefined for the simulated poroelastic phantom may be

observed in **Table 1**. **Table 2** shows the values for the cost function that is a goodness-of-fit parameter.

Figure 10 displays, for both the strain and the stress relaxation compressions, the material property and the cost function images that were derived by applying an LM inverse method to fit the analytically predicted time-varying volumetric strain to the FEM-measured time-varying volumetric strain at each of the 10×10 pixel locations inside a poroelastic cylinder.

DISCUSSION

In this work, the fluid-induced strain within a poroelastic phantom in a typical *in vitro* experimental relaxation scenario was predicted by using both analytical and finite element methodologies. Furthermore, biomechanical material property images were created by recovering the poroelastic parameters from the simulated strain data using an LM inverse method. In all simulations, the experimental design, biomechanical properties, and geometry of a sample were identical.

The central question that was being asked was, does a strain relaxation compression experiment provide the same quantitative and qualitative information about the poroelastic nature, behavior, and properties of the studied sample as a stress-relaxation experiment?

In order to compare the two loading configurations, both relaxation experiments were simulated identically, with the only difference being that in the strain relaxation, a constant axial force was applied and in the stress relaxation an axial strain was held constant. All strain components were simulated for both strain relaxation and stress relaxation. The volumetric strain was selected as an unbiased metric of comparison of the poroelastic sample behavior in the two different types of relaxation.

There was a good agreement between the phantom dilatations obtained using the strain relaxation and stress relaxation, and this result was independent of the duration of the relaxation (**Figure 6**).

The pixel-by-pixel dilatation data obtained by the FEM, from the boundary to the center of the simulated phantom, were in excellent agreement with the analytical predictions of the equations of poroelasticity for the same biomechanical properties and boundary conditions as simulated by FEM (**Figures 7, 8**).

The relative ability to use stress relaxation and strain relaxation experiments to recover the poroelastic properties of the simulated phantom was assessed by fitting the volumetric strain produced directly from the equations of poroelasticity to the “measured” data generated by the FEM simulation. The LM inverse method successfully converged close to the correct values of the material properties for both hypothetical strain and stress-relaxation experiments, independent of the poor initial guess of the material properties and the random noise added in the simulated strain data. The LM algorithm located the minima of the cost functions and converged precisely and accurately to the parameter p_1 (Poisson’s ratio) and the parameter p_2 (product of the aggregate modulus and permeability), with respective mean values and SDs displayed in **Table 1**, just as well for the strain relaxation loading as it did for the stress relaxation

TABLE 2 | The performance of the inverse method was tested by using the strain data generated from FEM simulation of a poroelectric phantom undergoing a stress or strain relaxation compression, with predefined biomechanical properties.

Biomechanical parameter or performance index	Preset value	Estimated parameter from strain relaxation imaging	Estimated parameter from stress relaxation imaging
Poisson's ratio	0.3	0.2984 ± 0.0034	0.3072 ± 0.002
Product of aggregate modulus and Permeability (Pa m ²)	5.7×10^{-11}	$(5.708 \pm 0.144) \times 10^{-11}$	$(5.711 \pm 0.121) \times 10^{-11}$
Cost function, sum of squared errors (arbitrary units)	–	$(7.071 \pm 0.0986) \times 10^{-4}$	$(2.781 \pm 0.0199) \times 10^{-4}$

Means and SDs are given for 10 estimates, using different initial guesses for the values of the parameters and different random noise values each time.

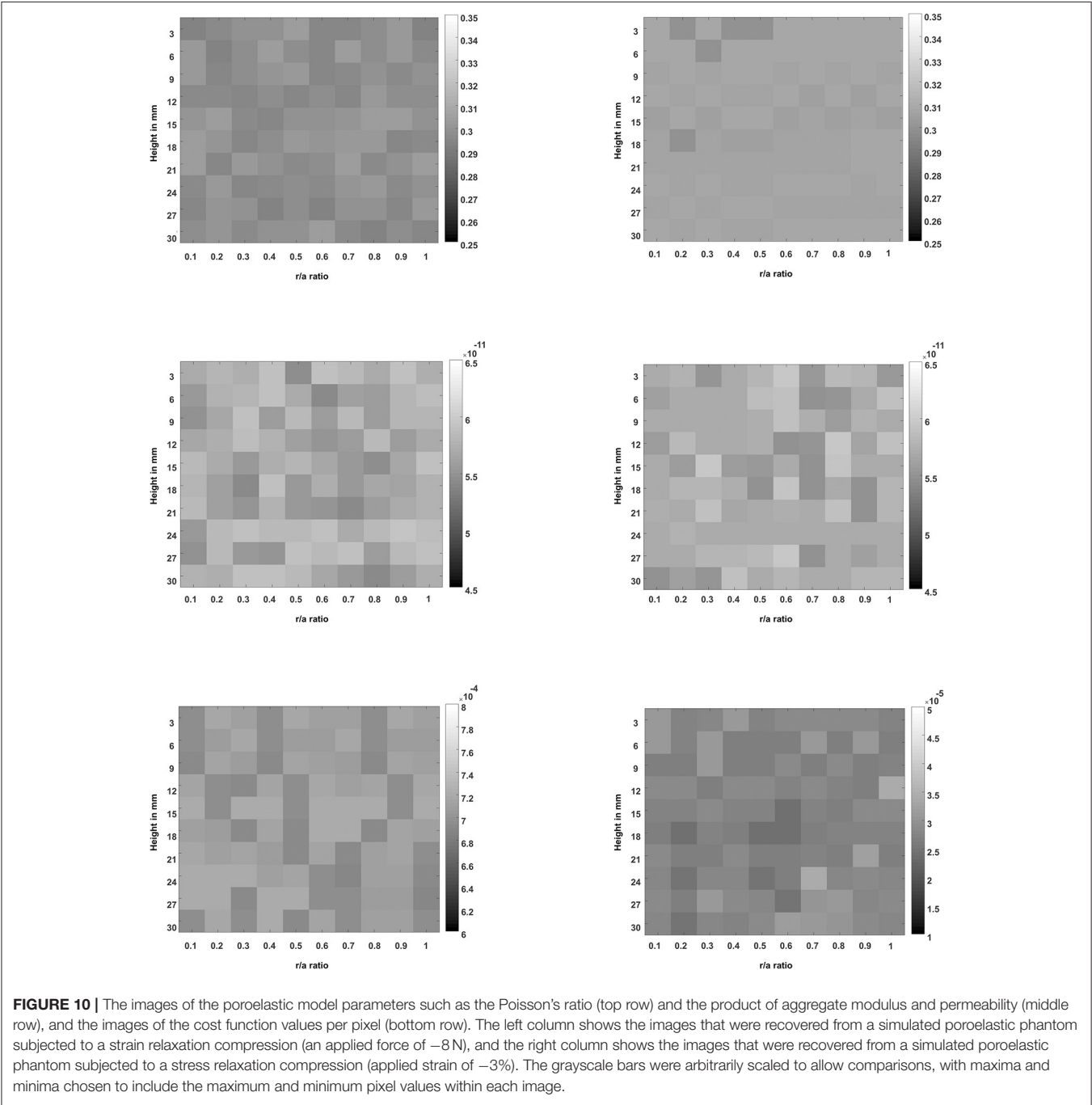


FIGURE 10 | The images of the poroelectric model parameters such as the Poisson's ratio (top row) and the product of aggregate modulus and permeability (middle row), and the images of the cost function values per pixel (bottom row). The left column shows the images that were recovered from a simulated poroelectric phantom subjected to a strain relaxation compression (an applied force of -8 N), and the right column shows the images that were recovered from a simulated poroelectric phantom subjected to a stress relaxation compression (applied strain of -3%). The grayscale bars were arbitrarily scaled to allow comparisons, with maxima and minima chosen to include the maximum and minimum pixel values within each image.

loading. Images of the local values of the Poisson's ratio and the product of the aggregate modulus and permeability were created, again demonstrating equal capability and performance for the strain relaxation loading as for stress relaxation loading. For the simulation of homogeneous media, the Poisson's ratio and aggregate modulus maps in **Figure 10** illustrate a desirable absence of spatial bias in the solution to which the inversion method converged. They also indicate the level of variation to be expected, for the noise level employed in this simulation. Finally, they illustrate the capability to produce the quantitative images of these novel biomechanical properties, which for biological tissues should show spatial variation that may be expected to highlight differences for different tissues and spatially localized pathology.

However, it should be noted that the determination of the characteristics of a poroelastic material by fitting a time-varying strain at a point to the analytical predictions is applicable only to the conditions to which the analytical theory applies, i.e., in this case to the cylindrical samples of homogeneous materials where the biomechanical properties are not varying significantly over time and space. The implication of this assumption is that to apply this method in the laboratory, homogenous cylindrical phantoms of controlled mechanical properties need to be made and tested under strictly monitored experimental conditions. Moreover, to extend the application of the method *in vivo*, the inverse method should be further developed to account for tissue inhomogeneities, non-linearity, anisotropy, and viscoelasticity of the solid matrix, a variation in tissue permeability due to the different types of fluid flow and widely varying (i.e., non-cylindrical) boundary conditions, including the varying levels of confinement provided by surrounding tissues. Also not considered is the *in vivo* feasibility of applying and tracking tissue deformations for the long periods that may be necessary. Nevertheless, the positive results of previous *in vivo* studies involving various types of loading and strain measurement [10, 11, 14], provide substantial encouragement that our findings concerning the strain-relaxation loading should be applicable for poroelastography *in vivo*.

The noise model employed in the simulation, which was spatially uncorrelated and has a uniformly distributed probability density function, may not reflect the correlations and distribution of noise in ultrasonically generated estimates of strain, which may also be anisotropic showing the largest variance in the lateral direction. A further limitation is that only a single noise level was simulated rather than a range of signal-to-noise ratios. However, these limitations should not impact negatively on the main finding that, for quantitative poroelastography, strain relaxation (which may have practical advantages as described below) represents a viable alternative loading condition to stress relaxation. In fact, the use of a more accurate noise model with a greater strain estimation variance in the lateral direction is likely to have further emphasized the benefits of strain relaxation, which can take advantage of a greater precision with which axial strain can be measured. Nevertheless, further work would be helpful to understand whether there are differences between the two approaches in terms of sensitivity to noise.

By studying the strain inside a cylindrical phantom, it has been found from these simulations that either a constant axial

stress or strain may be used for quantitative poroelastography because they generate a similar and predictable temporal and spatial variation of internal strain when applied to the same phantom. Although a cylindrical geometry is unlikely to occur naturally in the body (apart from, perhaps, intervertebral discs), it is sufficient here to allow a qualitative and quantitative comparison of the experiments of strain relaxation and stress relaxation. If an inversion method for recovering the quantitative values and the images of the characteristics of a poroelastic material was to be applied *in vivo*, however, sample geometries other than cylindrical geometries may need to be modeled. For such purposes, analytical modeling is likely to be too complex and a more feasible approach may be to extract parameters from the simulations that best fit the ultrasound-measured spatially varying and time-varying strain. The input data for such simulations, regarding the organ (such as breast, liver, and brain) boundary shape, for example, could be obtained from 3D medical images such as those obtained using MRI, x-ray CT, or ultrasound. In such future work, however, it would also be necessary to extend the modeling to predict the behavior under conditions of localized compression (indentation) of large organs [15].

The reader should note though that the creep (strain relaxation) poroelastic results simulated herein may not be easily replicated experimentally using a rigid compressor because the compressor may not permit the radially varying axial strain shown in **Figure 3A** in all axial locations. Sridhar et al. [23] and Qiu et al. [14] performed creep experiments using a rigid compressor for the purposes of studying a viscoelastic behavior and did not report behavior such as that shown in **Figure 3A**, although they were not specifically looking for such effects. For the optimal laboratory replication of the strain-relaxation results shown here, a much more sophisticated non-rigid way of applying the constant axial stress would be ideal, possibly by using an indenter array or a fluid compressor. Alternatively, a rigid compressor may yield useful results for some axial locations, or a more complete understanding of the axial variation of the time-varying and radially varying strain may allow more general use of a rigid compressor.

The stress relaxation experiments are relatively easily implemented in a laboratory using a conventional rigid compressor to sustain an axial strain on the top surface of a poroelastic sample. Indeed, Berry [24], using an FEM and experiments *in vitro*, reported a poroelastic strain response during stress relaxation that was like that shown in **Figures 3B–6B**. Strain relaxation, on the other hand, could be advantageous if one considers potential clinical imaging setups.

If 3D ultrasound methods for accurately estimating all three components of strain and their temporal evolution were available, the dilatation of the poroelastic sample could be imaged during the relaxation [12]. This would be an ideal echo imaging system for poroelastography because it could quantify the change in the volume of the poroelastic sample, which would be equal to the fluid that has flowed out of the matrix during relaxation. If only one accurately estimated component of strain is available (i.e., along the ultrasound beam axis), which is typically the case for two-dimensional (2D) or 3D ultrasound imaging, then

a strain-relaxation (creep) methodology may have advantages compared with a stress-relaxation method, since it exhibits a time-varying axial strain whereas for stress relaxation it does not (compare Equation 6 with Equation 9, and **Figures 3A,B**). Importantly for clinical applications, if axial strain alone could be employed, it would allow the ultrasound probe to be used for both compression and imaging, avoiding the errors and difficulty of lateral and elevational strain estimation.

It was shown in the FEM images (**Figures 3–5**) that all poroelastic strain components vary spatially and all components contribute to the local dilatation information (**Figure 6A**) during strain (creep) relaxation. In a stress relaxation case, as axial strain is fixed, only the radial and circumferential strain components (**Figures 4B** and **5B**) contribute to the local dilatation information (**Figure 6B**). Thus, the mathematical formulation of each compression problem should be considered before selecting the most appropriate experimental design.

Overall, an important potential advantage of the creep methodology is the possibility to derive useful information related to the biomechanical nature of a sample by imaging the time evolution of the axial strain only, avoiding the technical difficulties and errors associated with the volumetric or even axial-to-lateral strain ratio calculation.

CONCLUSIONS

Based on both the FEM and analytical results, it is evident that the diffusion-like strain behavior during the relaxation of an unconfined poroelastic sample under compressive loading is determined entirely by its elastic constants (aggregate modulus, Poisson's ratio, and Young's modulus), and by the permeability of its matrix to the pore fluid and the radial flow path length. Regardless of whether the type of sustained compression employed elicited strain or stress relaxation, the simulated relaxations of a poroelastic sample with interstitial permeability and Poisson's ratio similar to a healthy tissue, resulted in a similar magnitude of volumetric strain at equilibrium. Similar spatially varying and time-varying dilatations of a poroelastic cylinder were observed in a relaxation compression, independent of the type of loading, as long as other boundary conditions remained unchanged. Therefore, both unconfined compression

methodologies permit the study of the poroelastic deformation of a sample. Most importantly, either of the unconfined compression configurations could be applied experimentally to obtain the qualitative and quantitative information related to the biomechanical nature of a chosen poroelastic sample. For eventual clinical application of the method for recovering the quantitative poroelastic parameters and images, it will be necessary to consider sample geometries other than the cylindrical geometry with, for example, an inversion by fitting the predictions of simulations to the ultrasound measurements of the spatially varying and time-varying strain.

This work shows that both unconfined compression relaxation methodologies could in principle be applicable *in vitro* and *in vivo* and strain relaxation provides several potential advantages due to the fact that the strain variation could be imaged by axial displacement tracking with the ultrasound beam aligned along the axis of compression, which bypasses the need for the estimation of lateral strain and elevational strain and significantly simplifies the setup for *in vivo* scanning and compression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

MT performed the computations and analyzed the data under the supervision of JF, JB, and NdS. JF and JB assisted with the analysis and JF helped to carry out the FEM simulations. MT wrote the manuscript in consultation with JF, NdS, and JB. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Elasticity of the Achilles Tendon in Individuals With and Without Plantar Fasciitis: A Shear Wave Elastography Study

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The elastic properties of the Achilles tendon (AT) are altered in local injury or other diseases and in response to changes in mechanical load. Recently, elastography has been used to evaluate variations in tendon elastic properties, mainly among healthy individuals or athletes. Therefore, this study evaluated the biomechanical changes in ATs in individuals with and without plantar fasciitis (PF). The purposes were as follows: (1) to evaluate the passive stiffness of three regions of the AT which defined as 0 (AT0 cm), 3 (AT3 cm), and 6 cm (AT6 cm) above the calcaneal tuberosity in participants with and without PF, (2) to investigate the interplay between the passive stiffness in patients with PF and pain, (3) to detect optimal cut-off points of stiffness of the AT in assessing individuals with chronic PF, and (4) to determine the correlation between the plantar fascia thickness (PFT) and pain. This cross-sectional study included 40 participants (mean age = 51 ± 13 years). When the ankle was in a relaxed position, patients with PF experienced increased passive stiffness in AT0 cm ($p = 0.006$) and AT3 cm ($P = 0.003$), but not in the neutral position. Significant correlations were observed between pain and stiffness of AT (AT0 cm $r = 0.489$, $P = 0.029$; AT3 cm $r = 0.487$, $P = 0.030$; AT6 cm $r = 0.471$, $P = 0.036$), but not in the PFT ($P = 0.557$). Optimal cut-off stiffness was AT (452 kPa) in the relaxed ankle position. The plantar fascia of patients with PF was significantly thicker than that of the controls ($P < 0.001$). Findings from the present study demonstrate that tendon stiffness is a good indicator of the clinical situation of patients with PF. Monitoring passive tendon stiffness may provide additional information to assess severity of the condition and guide therapeutic. The treatment programs for PF should also be tailored to the distal AT, as conventional therapy might not be targeted to tight tendons.

Keywords: elasticity, Achilles tendon, plantar fasciitis, shear wave elastography, rehabilitation

INTRODUCTION

Plantar fasciitis (PF) is a common disorder of the foot, with an incidence of approximately 1% (Siriphorn and Eksakulkla, 2020). Although PF pain can be alleviated by conservative management, such as a variety of stretching methods, including the Achilles tendon (AT) and plantar fascia, relapse can easily occur. It is generally accepted that the most common causes of PF include

tightness of the AT and/or plantar fascia (Nakale et al., 2018). From the perspective of anatomy, the AT is connected to the plantar fascia via the highly regular aligned calcaneal trabeculae of the posterior calcaneus (Zwirner et al., 2020). These published findings all demonstrate a close functional relationship between the AT and plantar fascia. However, the biomechanical effects of AT on plantar fascia have not yet been fully addressed. A better understanding of the pathological changes in the AT of patients with PF will not only enable rehabilitative physicians to better diagnose ankle joint function, but also assist therapists with formulating more effective and targeted treatment programs.

The AT is one of the strongest tendons in the body. It is the elastic structure that connects the triceps surae muscle to the plantar fascia, serves as the main plantarflexing mechanism of the ankle joint, and transmits force based on the amount of stretch endured (Zwirner et al., 2020). Tendon stiffness is a critical determinant of proper muscle force transmission and movement generation (Matijevich et al., 2018). The plantar fascia serves as the primary arch-supporting structure of the foot and tolerates high tension during standing, running, and jumping (Thierfelder et al., 2018). Previous studies have demonstrated that the increased tension of the AT resulting from intense muscle contraction was coupled with the increased strain on the plantar fascia (Cheung et al., 2006). In addition, laboratory testing of the relationship between tensile force in the AT and plantar fascia strain was conducted using eight cadaver lower extremities and revealed that increased AT tension was associated with increased plantar fascia strain (Carlson et al., 2000). Thus, the excessive force on the AT may result in PF, as it alters the normal biomechanical properties of the ankle. However, musculoskeletal simulations or autopsy observations have extensive application for clinical investigations and biomechanical research, and the accuracy of these observations may not be sensitive enough to provide direct quantification of tendon tension, compared to direct measurements of individuals. Therefore, musculoskeletal simulations or autopsy observations cannot accurately reflect biomechanical changes of the tendon, especially its elastic properties. Therefore, reliable and effective imaging techniques are critical for the quantification and assessment of biomechanical changes in patients with PF.

Shear wave elastography (SWE) is an ultrasonography elastography technology known for fast, accurate, and non-invasive evaluation of tissue stiffness (Zhou et al., 2019a,b; Liu et al., 2020). It generates shear waves through an acoustic radiation force impulse, which propagates through the surrounding tissue and traces the wave back to provide biomechanical information about the tissue of the measured object (Zhou et al., 2019a). It has been widely used for muscles, ligaments, and tendons (Zhou et al., 2019a,b; Liu et al., 2020). Our previous studies also demonstrate that SWE is a reliable and valid imaging technique to assess the elastic properties of tendons and muscle (Zhou et al., 2019b). As described, SWE provides an opportunity to evaluate varieties in the AT and plantar fascia thickness during passive ankle stretching in individuals with PF (Zhou et al., 2019a,b; Liu et al., 2020). Additionally, the AT is particularly amenable to SWE assessment given its relatively superficial anatomic positioning. Although alterations in the

elastic properties and thickness have been well demonstrated in healthy individuals or individuals with AT pathology, data regarding the biomechanical changes in measurements with SWE in individuals with PF are more limited. To the best of our knowledge, the biomechanical effects of the AT on the plantar fascia have not been fully addressed. As of yet, no study has examined the biomechanical properties of tendon tension in individuals with and without PF, and the relationship between tension and pain.

Therefore, we used Young's modulus measurements as an index of tendon elastic properties. The purposes of this study were fourfold: (1) to evaluate the passive stiffness of different regions of the AT in participants with and without PF, (2) to investigate the interplay between the passive stiffness in patients with PF and pain, (3) to detect optimal cut-off points of stiffness of the AT to assess chronic PF, and (4) to determine the correlation between the plantar fascia thickness (PFT) and pain.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Human Subject Ethics Committee of the Luoyang Orthopaedic Hospital of Henan Province (NO: KY2019-001-01). The experimental procedures were explained to each participant prior to participation. All participants provided written informed consent prior to the experiment, and all study procedures adhered to the principles of the Declaration of Helsinki.

Participants

This study comprised of 40 participants, 20 patients with PF and 20 asymptomatic, aged 32–69 years (mean age 51 ± 13 years for asymptomatic participants and mean age 51 ± 13 years for patients with PF. There were 10 males and 10 females in each group). The inclusion criteria for PF were as follows: (1) plantar heel tenderness, (2) pain symptoms longer than 6 months, (3) morning pain with the first few steps out of bed, (4) pain score $\geq 3/10$ using a visual analog scale (VAS), and (5) the more painful leg of patients with bilateral PF was included (BlaBolgla et al., 2015). The exclusion criteria were as follows: (1) neuromuscular disease, musculoskeletal injury of the lower limb, tendon rupture, or anomaly on ultrasound; (2) any previous foot or ankle pathology, such as infections, fracture, or surgery; (3) received any treatment about for the foot or ankle; and (4) need for chronic analgesics due to another condition. The inclusion criteria of 20 age-matched and sex-matched asymptomatic participants were as follows: healthy, no plantar heel tenderness, and meet the exclusion criteria of PF group.

Demography Characteristics

Basic information about the participants was recorded, including age, sex, height, weight, BMI, VAS score, and duration of pain.

Equipment and Parameter Setting

The Young's modulus of the AT was quantified using an ultrasound shear wave elastography system (SuperSonic Imagine,

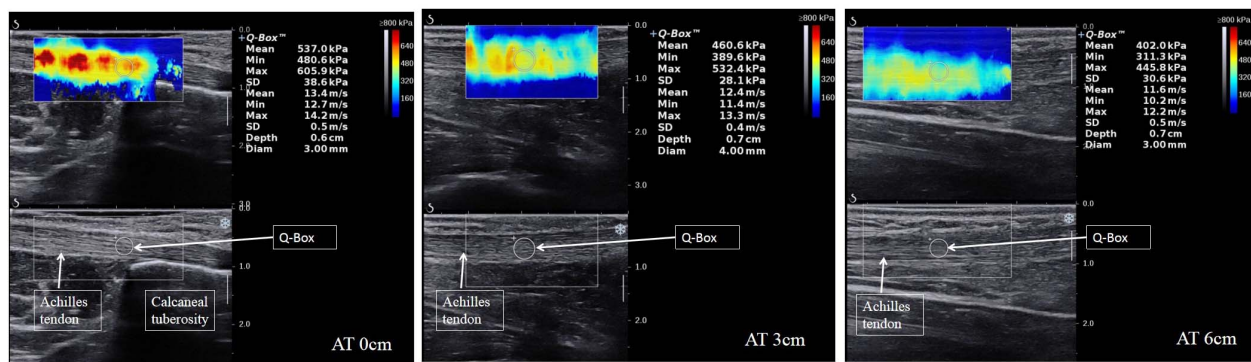


FIGURE 1 | Longitudinal shear wave elastography images of the three regions of the AT in patients with PF show the values of the Young's modulus at a relaxed ankle position. The Q-Box™ is shown on the right of each scale sonogram.

Aix-en Provence, France) with a 40 mm linear array transducer (2–10 MHz, SL10-2). The settings were set as follows (Zhou et al., 2019a,b; Liu et al., 2020): the initial condition was standard musculoskeletal mode. The opacity was 85%. The elastic modulus of the AT was 0–800 kPa, and the preset of B-scan ultrasound was adjusted to a depth of 0–2.5 cm. In the SWE examination, the Q-box diameter of AT was defined as the thickness of the AT (Liu et al., 2020). The color scale used in the Young's modulus (in kPa) showed the lowest values in blue and the highest values in red.

Examination Procedures

Participants were placed in a prone position with their feet fully relaxed and placed over the edge of the examination bed. The Young's modulus of the AT was examined on the painful leg when the ankle was passively “relaxed” or neutrally placed (90°). Leg-matched asymptomatic participants were examined as controls. It is noteworthy that for the relaxed position of the ankle participants took what they perceived to be a “relaxed” ankle position, that is, the feet were maintained in a naturally hanging position, while the neutral (90°) position was fixed by a customized and movable ankle foot orthosis. The angle of ankle of PF group included as the angle of “relaxed” position was tested by a manual goniometer (Sammons Preston, Royan, Canada), and it was used as the relaxation angle of age-matched and gender matched asymptomatic group. As AT has unique twisted structure and anatomical composition, there are some differences in biomechanical characteristics among different positions of AT (Ballal et al., 2014). According to our previous studies, the three regions of AT were defined as 0, 3, and 6 cm above the calcaneal tuberosity (Liu et al., 2020). All corresponding skin surfaces were marked with a black pen. To avoid any effect of tendon fatigue (if any) on the elastic properties, the passive task at the relaxed position was performed before the passive task at the neutral position.

Achilles Tendon Measurement

As proposed previously (Zhou et al., 2019a,b; Liu et al., 2020), a large quantity of ultrasound gel was placed on the AT

surface and the transducer was placed at the marker point without applying pressure. Then, activated B-mode ultrasound was adjusted to display the superior-inferior borders of the AT under the longitudinal section. The images were frozen until the images in the region of interest were clear and stable. The Q-box was then placed to calculate the shear modules (Figure 1). This examination procedure was repeated three times in the same region, and the images and values were stored in the equipment.

Plantar Fascia Thickness Measurement

Participants were placed in the prone position as mentioned above, and a 40 mm linear array transducer (SL10-2, Supersonic Imagine, France) was used to measure the sagittal plantar fascia thickness (PFT). The transducer was placed over the surface closest to the medial foot, and then the PFT was measured at the anterior margin of the calcaneus and stored for offline analyses (Figure 2; Gamba et al., 2018; Gatz et al., 2020; Petrofsky et al., 2020).

Statistical Analyses

G*Power 3.0.10 software was used to determine the required number of participants. According to the results of a previous pilot study, an estimated effect size of 0.3, was set as 0.05, and the power was 0.85; therefore, the total sample size was 30 participants (i.e., 15 per group). To avoid issues due to dropouts or missing data, the number was increased to 40 (i.e., 20 per group).

The data were analyzed using SPSS version 22.0 software (IBM Corporation, Armonk, NY, United States) and expressed as mean $\pm$ standard deviation. The normality of distributions was assessed using the Shapiro-Wilk test. Our data were normally distributed (all $p > 0.05$). Independent t -tests were performed for demographic data, such as age, weight, and height. Multivariate analysis of covariance (MANCOVA) tests were performed to compare the Young's modulus of the ATs (AT0 cm, AT3 cm, and AT6 cm) at different ankle angles (relaxed or neutral) on the painful side of patients with PF and the dominant side of healthy controls, with variables with significant group differences as covariates. When the level of significance was reached, *post hoc*



FIGURE 2 | Individual example of a longitudinal sonogram of the PFT. The PFT was assessed at the anterior margin of the calcaneus. **(A)** The PFT of a healthy participant. **(B)** The PFT of a patient with PF.

analyses were performed using univariate analysis of covariance tests. Pearson correlation analysis (r) was used to examine the correlation among the main variables (Young's modulus of ATs), duration of pain and pain, and to examine the correlation between secondary variables (PFT) and pain. Receiver operating characteristic (ROC) curve analysis was performed to calculate the cut-off point of the elastic properties of the ATs that displayed significant group differences. For all tests, statistical significance was set at $p < 0.05$.

RESULTS

Demographic Data

Twenty-two participants reported heel pain at the medial plantar and clinical diagnosis suggested the presence of PF, and two candidates declined to participate. Twenty-three asymptomatic participants were assessed for study eligibility, and three did not meet the inclusion criteria. The age, height, weight, and

BMI in both groups, VAS score, and duration of heel pain for symptomatic participants are summarized in **Table 1**. There were no significant differences in age, height, weight, or BMI ($P > 0.05$). However, the PFT of the PF group (0.5 ± 0.8 cm) was significantly thicker than that of the healthy group (0.3 ± 0.1 cm) (by 66.67%; $P < 0.001$). The angle of “relaxed” position of the ankle joint was 23 ± 2 degrees, which was significant difference with the neutral (90°) position ($P < 0.001$).

Stiffness of the AT Between PF Group and Healthy Group

The mean elastic property values of the AT in the PF and healthy groups are summarized in **Table 2**. In the relaxed position, the elastic property values of the AT0 cm and AT3 cm of the PF group were significantly higher than those of the healthy group ($13 \pm 20\%$, $P = 0.006$; $10 \pm 14\%$, $p = 0.003$, respectively). There was no significant difference at the AT6 cm. No between-group differences were observed in the three regions of AT measured in the neutral position ($p > 0.05$).

Correlations Among ATs Stiffness, PFT and Pain

The correlations among PFT, elastic properties of ATs, and pain scores are showed in **Figure 3**. When the ankle was in a relaxed position, significant correlations were detected between the pain score and the elastic properties of AT0 cm ($r = 0.489$, $p = 0.029$), the Young's modulus of AT3 cm ($r = 0.487$, $p = 0.030$), and the Young's modulus of AT6 cm ($r = 0.471$, $p = 0.036$). There was no significant correlation between pain score and elastic properties of the ATs in the neutral position ($p > 0.05$). In addition, there was no significant correlation between the pain score and PFT ($r = 0.146$, $P = 0.557$).

TABLE 1 | Demographic data of subjects.

	PF group ($n = 20$)	Healthy group ($n = 20$)	P -values
Age (years)	51 ± 13	51 ± 13	0.941
Weight (kg)	66 ± 12	66 ± 11	0.340
Height (cm)	166 ± 8	166 ± 9	0.970
Body Mass Index (kg/m ²)	25 ± 2	24 ± 3	0.136
Thickness of plantar fascia (cm)	0.5 ± 0.8	0.3 ± 0.1	0.000*
Duration of heel pain (months)	11 ± 5		
VAS score	4 ± 1		

Values are mean $\pm$ SD; Healthy subjects: Female 10 (ages 36–69 years), Male 10 (ages 33–62 years); Patients with plantar fasciitis: Female 10 (ages 32–69 years), Male 10 (ages 35–69 years).

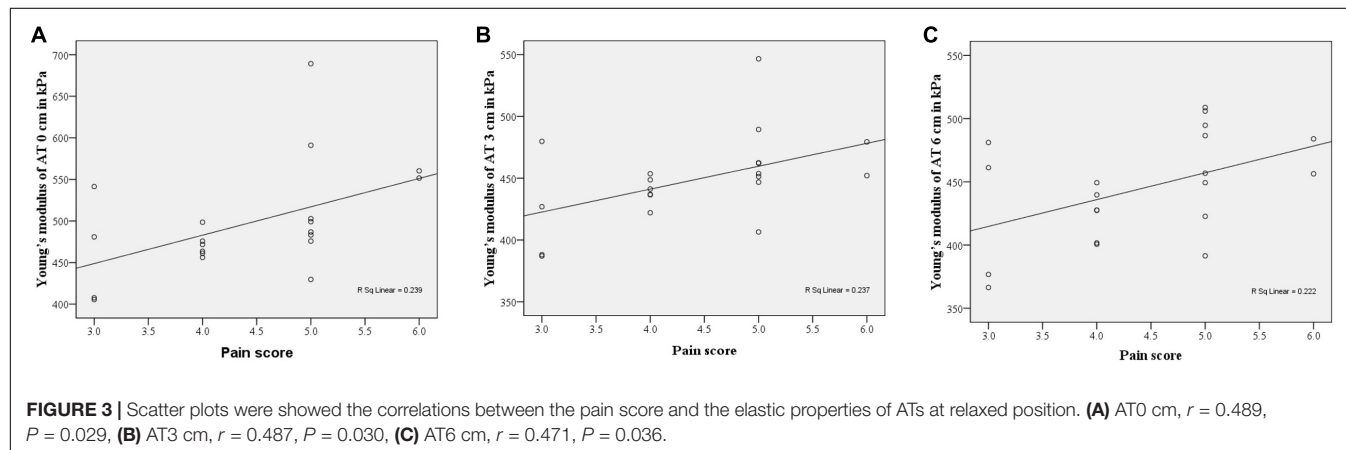
* Denotes significant difference between groups ($p < 0.05$).

TABLE 2 | Comparisons of Young's modulus of the ATs on the painful side of patients with PF and the control subjects.

Variables elastic modulus (kPa)			Healthy group	PF group	Percentage change (%)	P-values
Relaxed position	AT	0 cm	445 ± 43	497 ± 66	13 ± 20	0.006*
		3 cm	411 ± 39	449 ± 36	10 ± 14	0.003*
		6 cm	437 ± 39	444 ± 42	3 ± 17	0.565
Neutral position	AT	0 cm	574 ± 57	610 ± 86	7 ± 19	0.133
		3 cm	561 ± 59	601 ± 70	8 ± 17	0.241
		6 cm	568 ± 48	596 ± 42	6 ± 12	0.257

AT, Achilles tendon; AT0 cm, Position of the Achilles tendon at 0 cm above the calcaneal tuberosity; AT3 cm, Position of the Achilles tendon at 3 cm above the calcaneal tuberosity; AT6 cm, Position of the Achilles tendon at 6 cm above the calcaneal tuberosity.

*Denotes significant difference between groups ($p < 0.05$).



Correlations Among ATs Stiffness and the Duration of Heel Pain

The correlations among elastic properties of ATs and the duration of heel pain are summarized in **Table 3**. There was no significant correlation between ATs stiffness and the duration of heel pain (all P -values > 0.05).

Optimal Cut-Off Point in Assessing Chronic PF Patients With SWE

The optimal cut-off point for the elastic properties of ATs for assessing individuals with chronic PF was calculated using ROC curves (**Supplementary Figure 1**). When considering the ankle in the relaxed position, the AUC of AT0 cm was 0.755 ($p = 0.006$), and that of AT3 cm was 0.755 ($p = 0.006$). Youden's index revealed that the Young's modulus of AT (AT0 cm:

452 kPa, sensitivity = 0.85, specificity = 0.40; AT3 cm: 417 kPa, sensitivity = 0.85, specificity = 0.40) at the relaxed position of the ankle in assessing individuals at risk for chronic PF. The AUCs for the rest of the tested regions were not statistically significant and were included in the neutral position.

DISCUSSION

Taking advantage of SWE, this study evaluated the biomechanical changes in the AT of individuals with and without PF. We observed that the PF group exhibited higher distal AT (AT0 cm and AT3 cm) stiffness in the relaxed position of the ankle. Moreover, significant correlations were detected between pain score and the elastic properties of AT in the relaxed position of the ankle, and no significant correlation between ATs stiffness and the duration of heel pain. Furthermore, the optimal cut-off points of the elastic properties of ATs were determined in the relaxed position of the ankle, which may help to assess individuals with chronic PF.

Stiffness of the AT Between Individuals With and Without PF

This is the first study to compare the elastic properties of ATs between individuals with PF and healthy controls. As proposed in a recent study, the measurement of passive elastic properties could be advocated as a preventive, diagnostic, and prognostic instrument in individuals with PF. Our results indicate that

TABLE 3 | Correlations among Young's modulus of AT and the duration of heel pain.

Variables			r	p
Relaxation position	AT	0 cm	0.053	0.824
		3 cm	0.007	0.977
		6 cm	0.176	0.459
Neutral position (90°)	AT	0 cm	0.206	0.382
		3 cm	0.059	0.806
		6 cm	0.224	0.342

when the ankle was in a relaxed position, the passive elastic properties of the AT0 cm and AT3 cm of patients with PF were stiffer than those of healthy individuals, but this was not observed for the AT6 cm. Interestingly, there were no significant differences in the passive elastic properties of the ATs between the PF and healthy groups when the ankle was in a neutral position. These findings could be interpreted by the fasciopathy patterns of the histological alterations of PF. In the degenerative processes, matrix degradation, fibroblastic hypertrophy, and collagen breakdown result in a lack of plantar fascia stiffness and contribute to PF (Schillizzi et al., 2020). The stiffer plantar fascia could reduce the length and excitation of the AT, resulting in increased stiffness of the AT (Ostermann et al., 2020). The increased stiffness in the AT could reduce the efficiency of stabilization and plantar flexion of the ankle, and could ultimately compromise the plantar fascia further (Ostermann et al., 2020). Recently, various stretching methods, including both the AT and plantar fascia, have been proven to be effective in mitigating pain in patients with PF (Engkananuwat et al., 2018). From the perspective of biomechanics, increasing strain on the plantar fascia was coupled with increased tension on the AT, and the plantar fascia showed a twofold larger straining effect (Cheung et al., 2006). Reduction of the AT force may decrease the plantar fascia load (Chen et al., 2015). Moreover, previous studies have demonstrated that stretching can decrease tendon stiffness (Zhou et al., 2019a,b; Liu et al., 2020). Therefore, lengthening or tension relief of the AT, especially in participants with tight AT, may be beneficial in terms of plantar fascia stress relief (Cheng et al., 2008). However, the passive elastic properties of ATs were not significantly different between the PF group and the healthy group when the ankle was in a neutral position. These results could be explained by the similar passive tension of AT engendered by the stretching force in individuals with or without PF. Further investigations are needed to verify this, as well as the change in AT stiffness in patients with PF before and after treatment (e.g., extracorporeal shock wave therapy, stretching).

Our results also demonstrated that the percentage change in the AT0 cm (11.50%) stiffness of the participants with PF was significantly higher than that of AT3 cm (9.23%). The disparity in different regions of AT may be due to the different regions of AT experiencing different strains and compressive loads and exhibit distinct histological alterations in PF (Ballal et al., 2014). For example, higher amounts of cartilage cells and cartilage matrix proteins were produced in AT0 cm, which could result in a stiffer tendon structure (Maffulli et al., 2006; Bah et al., 2016), whereas the increase in abnormal tenocytes and type III collagen in AT3 cm could reduce the ability of tendon tissue to resist tensile forces (Ooi et al., 2015). In addition, considering the rotatory anatomy and multiple muscles of the AT, it can be assumed that a difference in AT composition has an impact on the non-uniform behavior of the different regions in the AT (Winnicki et al., 2020). Furthermore, AT0 cm is directly attached to the calcaneus so that it suffers more load-bearing strain than other regions (Zwirner et al., 2020). In conclusion, the stiffness was higher at AT0 cm than at other locations in patients with PF.

Correlations Among Stiffness, PFT, and Pain

We observed significant correlations between pain and AT stiffness when the ankle was in a relaxed position. This means that the increased stiffness of the AT is associated with greater intensity of pain. Pearce et al. (2021) found that there was a strong correlation between the severity of heel pain and gastrocnemius tightness in patients with PF (Pearce et al., 2021). The tension of AT is closely related to plantar fascia loading (Schneider et al., 2018). In addition, anatomical studies have shown that a continuation of fibers connects the gastrocnemius via the AT to the plantar fascia (Ballal et al., 2014). Furthermore, previous studies have shown that the success of AT stress relief for treatment of PF, such as stretching. In summary, clinically, reducing the tension or stiffness of AT is very important to improve the outcomes of patients with PF. The stiffness of the AT may play a role in guiding therapeutic interventions in patients with PF. Notably, the degree of plantar fascia thinning in response to a determinate treatment is usually the principal variable evaluated in previous studies but remains somewhat controversial. Mahowald et al. (2011) observed a good correlation between decreased pain and reduced thickness of plantar fascia for 30 patients after receiving various treatments and concluded that changes in the thickness of the plantar fascia are a reliable indicator of the efficacy of treatment protocols for PF. In addition, McMillan et al. (2012) reported a moderate correlation between pain and thickness of the plantar fascia. However, in another study (Maki et al., 2017) the thickness of the plantar fascia did not vary after extracorporeal shock wave therapy (ESWT). On MRI findings before ESWT, the mean thickness of the PF was 4 ± 2 mm, and after ESWT, the thickness was 5 ± 1 mm. Gamba et al. (2018) also found that the PFT in patients with PF should not be used in treatment planning, as the PFT did not correlate with AOFAS, pain, or any item of the SF-36. Therefore, changes in PFT are not a reliable indicator of the efficacy of treatment protocols for PF. The findings of the present study showed that the stiffness of the AT is an important reference parameter in treatment management, as we found a strong correlation between pain and AT stiffness. SWE can provide quantitative data to monitor the efficacy of rehabilitation and treatment protocols. More importantly, this result suggests that releasing AT stiffness is recommended for the treatment of patients with PF. Further studies are needed to establish whether this suggestion is related to clinical benefits.

Our results demonstrate that the plantar fascia is significantly thicker among individuals with PF than healthy participants (0.5 ± 0.8 cm vs. 0.3 ± 0.1 cm), and there was no correlation between PFT and pain, indicating that PFT is not a good clinical indicator of treatment response. The findings of this study are consistent with recent reports. For example, Gatz et al. (2020) reported that the mean PFT of symptomatic participants (0.4 ± 0.1 cm) was thicker than that of asymptomatic participants (0.3 ± 0.1 cm). Petrofsky et al. (2020) also demonstrated a significantly thicker PFT in their PF group compared to controls. In addition, Gamba et al. (2018) observed that the PFT in

patients with PF was not associated with its clinical impact, as the PFT was not associated with AOFAS, pain score, or any item of the SF-36. Moreover, although the findings of Alotaibi et al. (2020) indicate that PFT significantly decreased after treatment, no significant correlation was observed between PFT and changes in pain after 4 weeks of treatment. Therefore, PFT could be used as a diagnostic predictor, but not as an indicator of treatment response in PF.

Correlations Among ATs Stiffness and the Duration of Heel Pain

This study also found that there was no significant correlation between ATs stiffness and the duration of heel pain. Our results are similar to previous studies. Chen et al. (2013) found that there was no significant correlation between symptom duration and pain in patients with plantar fasciitis. This may be related to the self-healing of plantar fascia. Under overload due to multiple causes, the morphological characteristics of the plantar fascia are modified by the process of inflammation (Lareau et al., 2014). In this process, the plantar fascia is swollen and thickened, and pain intensity increases. This could still be reversed by conservative treatment (Fabrikant and Park, 2011; Luffy et al., 2018). However, the inflammation becomes chronic with ultrastructural changes in the plantar fascia if there is no response to treatment and the inflammation process will stop and leave a thickened and degenerated fascia. The pain threshold increases during this entire process, resulting in the illusion of pain relief, and the VAS score decreases (Pomares et al., 2011). In addition, the plantar fascia is capable of self-healing, just as the measure of rest is taken for the affected foot to allow time for healing (Luffy et al., 2018). Clinically, the assessment of the severity of PF mainly depends on clinical symptoms, and imaging diagnosis mainly includes MRI and ultrasound. In addition, some validated scales will be used to evaluate the foot and ankle function and activity, such as foot and ankle ability measure and foot function index (Martin et al., 2014). Therefore, the assessment of the severity of PF needs to be combined with multiple examination findings and clinical symptoms.

Optimal Cut-Off Point in Assessing Chronic PF Patients With SWE

As discussed above, the increase in the passive elastic properties of the AT may be due to an overload of the plantar fascia associated with sports participation. Preventive methods include a variety of stretching methods, including AT, plantar fascia, or silicone insoles. Ultrasound measurements, using both the SWE and grayscale to assess the morphology of the plantar fascia and the AT stiffness, may provide additional information to assess severity of the condition and guide therapeutic interventions in patients with PF. Given this, we calculated the initial cut-off stiffness of the AT (452 kPa, sensitivity = 0.85) at the relaxed position of the ankle to assess chronic PF. This also suggests that individuals with a passive stiffness of the AT greater than 452 kPa may have a higher risk of plantar fascia injury. It is worth noting that the results of ROC curves showed that AUC has

relatively low specificity for patients with chronic PF. This may be related to the different activity patterns and exercise intensity between the PF group and healthy group. According to previous studies, the more activity or exercise intensity, the greater the stiffness of AT (Engkananuwat et al., 2018; Schneider et al., 2018; Zhou et al., 2019a,b; Liu et al., 2020; Siriphorn and Eksakulka, 2020).

Limitations

The limitations of this study are that electromyography was not used to monitor the gastrocnemius muscle and soleus muscle activity during SWE measures and ensure these muscles were not firing. However, all participants were verbally instructed to stay relaxed, and no signs of muscle contraction were observed on the gray-scale image. Based on this, we are confident that while their lower limb were fully supported, patients or subjects remained in a passive state during passive dorsiflexion. In addition, we did not estimate the correlation between AT stiffness and plantar fascia stiffness in patients with PF. Furthermore, we did not use longitudinal follow-up to observe how AT stiffness and plantar fascia thickness changed during treatment, and whether they were affected by interventions such as stretching. Further studies should be conducted to investigate these factors.

CONCLUSION

The present study is the first to assess the relationship between heel pain severity and AT stiffness in patients with PF. Patients with PF had significantly greater AT stiffness than healthy participants. The increase in passive stiffness of distal AT is associated with pain, and initial cut-off points of distal AT stiffness at the ankle relaxed position may provide additional information to assess severity of the condition and guide therapeutic. The treatment programs for PF should also be tailored to the distal AT, as conventional therapy might not be targeted to tight tendons.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Datasets are available on request. Requests to access these datasets should be directed to reha_jp@163.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Human Subject Ethics Committee of the Luoyang Orthopedic Hospital of Henan Province. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WP and ZZ designed the experiments. JZ and YL carried out the experiments and collection data. WP and JZ analyzed the experimental results and drafted the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2021.686631/full#supplementary-material>

Supplementary Figure 1 | The ROC curves for the AT0 cm and AT3 cm in the relaxed position. The AUC of AT0 cm was 0.755 ($P = 0.006$), and that of AT3 cm was 0.755 ($P = 0.006$).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Machine Learning in Model-free Mechanical Property Imaging: Novel Integration of Physics With the Constrained Optimization Process

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The Autoprogressive Method (AutoP) is a fundamentally different approach to solving the inverse problem in quasi-static ultrasonic elastography (QUSE). By exploiting the nonlinear adaptability of artificial neural networks and physical constraints imposed through finite element analysis, AutoP is able to build patient specific soft-computational material models from a relatively sparse set of force-displacement measurement data. Physics-guided, data-driven models offer a new path to the discovery of mechanical properties most effective for diagnostic imaging. AutoP was originally applied to modeling mechanical properties of materials in geotechnical and civil engineering applications. The method was later adapted to reconstructing maps of linear-elastic material properties for cancer imaging applications. Previous articles describing AutoP focused on high-level concepts to explain the mechanisms driving the training process. In this review, we focus on AutoP as applied to QUSE to present a more thorough explanation of the ways in which the method fundamentally differs from classic model-based and other machine learning approaches. We build intuition for the method through analogy to conventional optimization methods and explore how maps of stresses and strains are extracted from force-displacement measurements in a model-free way. In addition, we discuss a physics-based regularization term unique to AutoP that illuminates the comparison to typical optimization procedures. The insights gained from our hybrid inverse method will hopefully inspire others to explore combinations of rigorous mathematical techniques and conservation principles with the power of machine learning to solve difficult inverse problems.

Keywords: Inverse problem, elasticity, ultrasound, finite element analysis, neural network constitutive model

1 INTRODUCTION

Soft tissues are complex structures that exhibit non-linear, time-dependent elastic properties. Variations in mechanical properties can provide information pertaining to tissue health, including detecting and diagnosing lesions of the breast [6, 34], liver [26, 47, 56], and thyroid [9, 10, 54], monitoring thermal lesions during ablation therapy [41, 53], and characterizing

atherosclerotic plaques [2, 3, 11]. Conventional medical imaging modalities are insensitive to this mechanical contrast. Elasticity imaging methods have been developed to fill this niche and directly evaluate the mechanical properties of soft tissue.

Elasticity imaging can be largely grouped into quasi-static or dynamic methods based on the type of mechanical stimulus applied to induce tissue motion. Comprehensive reviews of the various techniques can be found in several articles [33, 35, 38, 39, 46, 52]. We focus our discussion on quasi-static ultrasound elastography (QUSE), wherein local tissue displacements are estimated from RF echo frames acquired as an ultrasound probe is slowly pressed into the tissue surface. Quasi-static loading allows time for the force stimulus to propagate through the entire tissue section. The resulting force-displacement measurements at points provide an enormous amount of information pertaining to tissue material properties throughout the contiguous volume and at boundaries.

Estimating material properties from force-displacement measurements constitutes the inverse problem in QUSE. The majority of quantitative QUSE approaches are built upon model-based optimization methods. While effective, model-based methods are prone to modeling errors that can lead to artifacts in reconstructed images of pre-selected material property distributions. More importantly, mathematically defined constitutive models are inherently limited in their ability to capture material behavior. This limitation could preclude discovery of clinically relevant soft tissue material properties.

Data-driven inverse methods circumvent modeling errors by removing the constitutive model assumption and building a soft-computational model of material behavior from measurement data. The Autoprogressive Method (AutoP) is one such method. Unlike other data-driven approaches, AutoP combines physical modeling through finite element analysis (FEA) with artificial neural networks (ANNs) to extract the stress-strain relationship embedded within force-displacement measurements. In the context of elasticity imaging, ANNs characterizing the stress-strain behavior of soft tissues can be interrogated to infer material parameters that best summarize the learned mechanical properties.

Many of the research articles that employed AutoP have described the training process, but none have directly compared and contrasted its operation with the more typical model-based approaches. In this review, we aim to more thoroughly examine the operating principles behind AutoP to better highlight the fundamental differences when compared to model-based inverse methods. Of particular importance is the manner in which physical principles are directly exploited in AutoP to generate increasingly accurate estimates of stress and strain distributions from force-displacement measurements under arbitrary geometry and loading. We believe the working principles of AutoP can be applied as a data-driven, physics-guided alternative to other difficult boundary value inverse problems.

Section 2 reviews model-based inverse methods in QUSE to establish a basis for comparison. We focus on iterative methods utilizing FEA as the forward solver to keep the discussion concise and comparable to AutoP. **Section 3** reviews AutoP and builds intuition of its operation by drawing parallels to typical optimization methods. We also cover a novel neural network

architecture developed for QUSE and a regularization term uniquely suited for AutoP. Challenges with non-linear viscoelasticity imaging and potential approaches with alternative ANN structures are briefly discussed in **Section 4**. Data acquisition and displacement estimation details are omitted from our discussions, but are available in [24, 25]. Even though such details are important in practice and will affect the efficacy of QUSE methods, we wish to compare the operation of AutoP and model-based methods at a more conceptual level.

2 MODEL-BASED INVERSE METHODS

Reconstructing a map of material properties from force-displacement measurements is an ill-posed inverse problem.¹ By combining a prior assumption of the underlying mechanical behavior with a system model incorporating physical principles, model-based inverse methods reduce the solution space and estimate parameter values of a pre-selected constitutive model. The goal of QUSE methods is to find the set of spatially distributed material parameters $\theta(x)$ that minimize the difference between displacements $U(x)$ computed in a forward problem and measured displacements $\tilde{U}(x)$,

$$\hat{\theta} = \operatorname{argmin}_{\theta \in \mathbb{R}} \int_{\Omega} \mathcal{C}(U, \tilde{U}) d\Omega, \quad (1)$$

where Ω denotes domain of the scan plane(s). The dependence of variables on position x is assumed and not explicitly stated to simplify notation. The cost function $\mathcal{C}$ in **Eq. 1** is often defined as the L_2 -norm of the difference between computed and measured displacements, augmented by a regularization term $\mathcal{R}(\theta)$ acting on the set of parameters,

$$\mathcal{C}(U, \tilde{U}) = \frac{1}{2} (U - \tilde{U})^2 + \alpha \mathcal{R}(\theta), \quad (2)$$

where α is a hyperparameter that controls how strongly the regularization term affects the solution.

2.1 Finite Element Analysis

In modern methods, the forward problem is usually solved through finite element analysis (FEA). A brief description of the FEA formulation for solid mechanics follows; more thorough treatments can be found in [12, 18]. The strong form of the governing equations for a solid continuum under quasi-static loading is

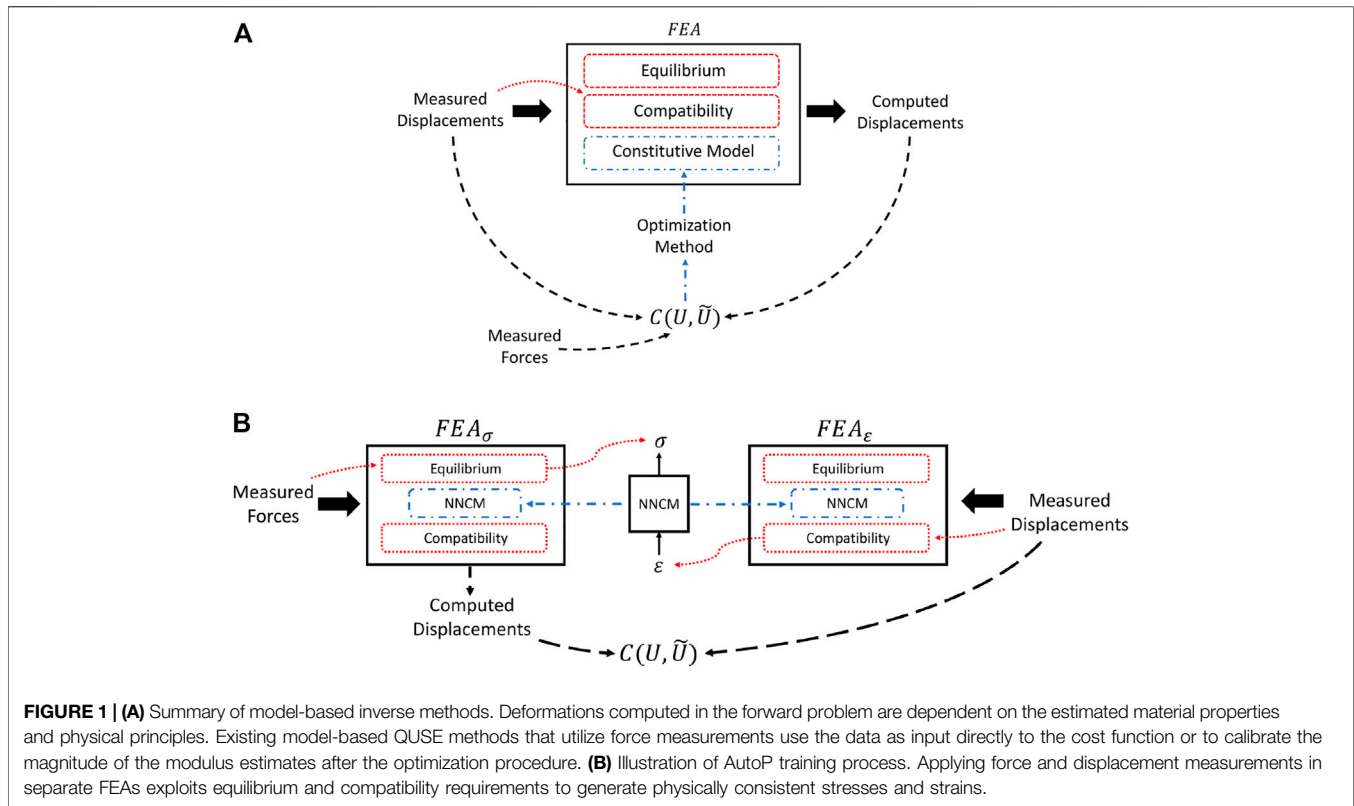
$$\nabla \cdot \sigma = 0 \text{ in } \Omega, \quad (3)$$

$$u = \tilde{u} \text{ on } \Gamma_D, \quad (4)$$

$$\sigma \cdot n = \tilde{p} \text{ on } \Gamma_N, \quad (5)$$

where σ denotes the stress tensor and u are displacements throughout the continuum Ω . Displacement boundary conditions (BCs) are represented by $\tilde{u}$ whereas surface traction BCs are denoted by $\tilde{p}$. In the latter two equations, Γ_D represents the set of boundary points over which displacements are measured, Γ_N is the set of boundary points corresponding to

¹Inverse problems and image science are expansive fields of study. Some foundational textbooks with comprehensive coverage of these topics are [7, 8].



measurements of surface tractions with surface normal n , and $\Gamma_D \cap \Gamma_N = \emptyset$.

The finite element formulation expresses the boundary-value problem as a system of equations,

$$[K(\theta)]U = P, \quad (6)$$

$$K(\theta) = \sum_{\Omega_e}^{N_e} B_e^T D_e(\theta) B_e d\Omega_e, \quad (7)$$

$$P = \sum_{\Omega_e}^{N_e} \int B_e^T \sigma_e d\Omega_e. \quad (8)$$

where N_e is the number of elements in the mesh, B_e is the strain-displacement matrix for a given element, and the summation symbols represent the assembly operation. The matrix $K(\theta)$ is determined directly from the material property matrix $D(\theta)$. Any displacement BCs are imposed in global displacement vector U . Likewise, force BCs are included in the global force vector P .² We chose to express P in Eq. 8 as a function of stress, instead of as a function of the surface traction BCs, to emphasize the relationship between stress and force. Generally, the solution for a mechanical system is found through FEA by discretizing the domain (i.e., “meshing”), applying load BCs in the global node force and displacement vectors, and solving for the remaining “free” nodal displacements and reaction forces.

²Only the linear form of the FEA is expressed in Eq. 6 for clarity. When the system includes non-linear materials or deformation, a Taylor expansion of Eq. 6 reformulates the problem in an iterative, incremental form.

FEA solutions of a mechanical system must satisfy both equilibrium and compatibility requirements. The former is specified by Eq. 8 and relates stresses to forces. Compatibility, on the other hand, relates displacements to strains and, in simple terms, precludes elements in the mesh from overlapping or separating during deformation. These physical principles combined with object geometry and a valid constitutive model determine the set of admissible deformations under loading (Figure 1A). The importance of these principles in solving the inverse problem will be made clear in the following sections.

2.2 QUSE as an Optimization Problem

Gradient or Hessian-based optimization techniques are typically applied to solve Eq. 1, wherein the parameter estimates are iteratively updated through the gradient of the error computed in Eq. 2. The solution process for QUSE can be generalized to five steps:

Algorithm 1 | Optimization-based QUSE Inverse Method.

- [1] Apply displacement BCs at the mesh boundary
- [2] Compute the displacements over the whole scan plane through FEA
- [3] Compare the computed displacements to the measured values (i.e., evaluate Eq. 2)
- [4] Update the parameter estimates based on the error
- [5] If solution converges, exit, otherwise return to [1].

The diagram in Figure 1A provides a simplified illustration of the role of FEA within an optimization-based solution method while simultaneously highlighting the fact that the cost function

directly affects estimated parameter values. Optimization methods often have intuitive geometric interpretations that aid in understanding the solution procedure. For example, consider the method proposed by Kallel and Bertrand [29]. Define the operator $\mathcal{T}(E) = U$ as the solution of a FEA where the material is assumed to be linear-elastic with spatial distribution of Young's modulus $E(x)$. The goal is to find $E(x)$ that minimizes the cost function

$$\mathcal{C}(E) = \frac{1}{2} \|\tilde{U} - \mathcal{T}(E)\|_2^2 \quad (9)$$

$$\hat{E} = \underset{E \in \mathbb{R}^+}{\operatorname{argmin}} [\mathcal{C}(E)], \quad (10)$$

where $\|\cdot\|_2$ is the L_2 -norm and $\mathbb{R}^+$ represents the set of positive-valued real numbers.

Adopting an iterative, Hessian-based solution procedure with k indicating the current iteration, the Young's modulus values can be found,

$$E_{k+1} = E_k + \Delta E_k \quad (11)$$

$$\Delta E_k = \overbrace{(J_k^T J_k)^{-1}}^H J_k^T \Delta U_k \quad (12)$$

$$\Delta U_k = \tilde{U} - \mathcal{T}(E_k) \quad (13)$$

$$J_k = \frac{\partial \mathcal{T}(E)}{\partial E} \quad (14)$$

Geometrically, the curvature of the forward operator (the Hessian H), guides the Young's modulus update in **Eq. 12**. A perhaps more intuitive geometric interpretation is achieved by setting the Hessian equal to the negative identity matrix $H = -I$, changing the update **Eq. 11** to

$$E_{k+1} = E_k - J_k^T \Delta U_k. \quad (15)$$

In this form, the operator gradient forming the sensitivity matrix J_k coupled with the displacement error directs the iterative parameter updates. Iterative QUSE methods typically follow a Hessian or gradient-based optimization procedure. A review of relevant methods can be found in [13, 36] **Algorithm 1**. We will show in **Section 3** how displacement errors also influence the material properties learned by ANNs trained in AutoP, albeit indirectly.

QUSE methods utilizing only displacement measurements can provide quantitative parameter estimates up to a multiplicative constant, unless the parameter value(s) is known for some portion of the boundary or interior [4, 5]. That is, through compatibility requirements embedded within FEA, at best the distribution of relative parameter values can be inferred from displacement BCs. Incorporating measurements of surface force or boundary stresses can provide the additional information necessary to estimate parameter values exactly [51]. This can be seen directly from **Eqs. 5, 8**: if no values of reaction forces corresponding to displacement BCs are known, there exists no means to calibrate the magnitude of forces in P . In short, both equilibrium and compatibility requirements affect the solution in model-based QUSE but are not directly exploited.

Force and displacement measurements coupled with FEA are insufficient for overcoming the ill-posed nature of the inverse

problem, hence the inclusion of a regularization term in **Eq. 2**. The choice of regularization depends on some presumed property of the solution; e.g., Tikhonov regularization is founded in energy arguments, L_1 -norm to promote sparse solutions, or total variation to encourage smooth solutions [43]. Although effective and often necessary, the form of regularization will significantly alter the solution in model-based methods and requires skill in choosing appropriately. We will introduce a regularization term in **Section 3.1** that is unique to AutoP and arises from physical principles, removing operator judgment from its use.

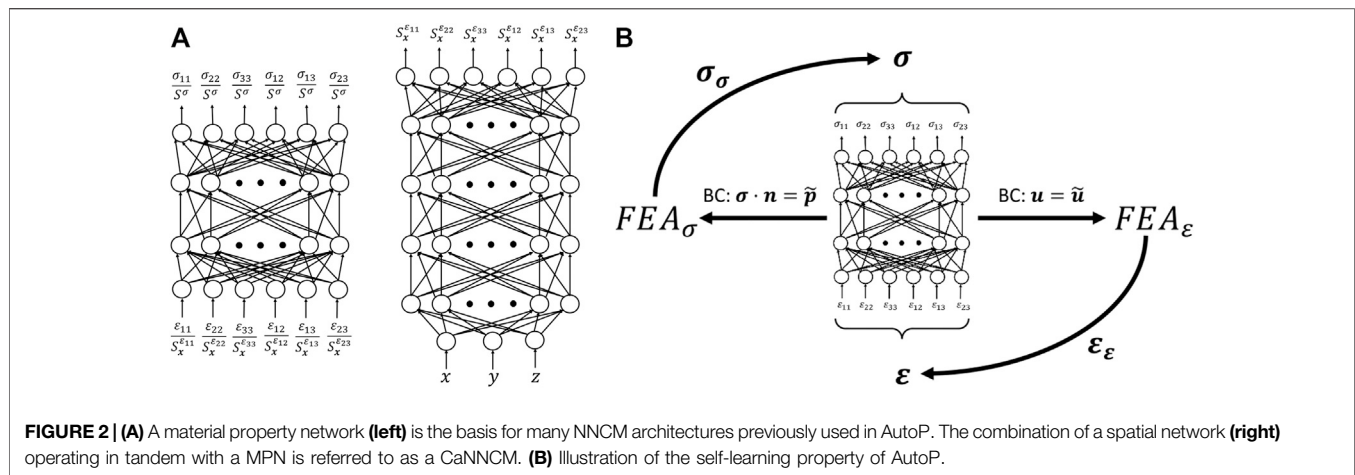
Model-based QUSE methods are effective when the applied load and true tissue material properties match the selected constitutive model and loading geometry. Choosing the wrong constitutive model can cause artifacts in the resulting elasticity images that corrupts or discards clinically relevant diagnostic information not described by the selected model parameters. The limited flexibility of model-based methods makes them ineffective at *discovering* material properties. Overcoming limitations of model-based methods and inferring mechanical behavior from measurement data without constraints imposed by constitutive model assumptions may allow investigators to find new clinically relevant material properties.

3 THE AUTOPROGRESSIVE METHOD

Contrary to model-based inverse methods, the goal of AutoP is to describe the mechanical behavior of a material non-parametrically. By replacing the classic mathematically defined constitutive model with an artificial neural network, any arbitrary stress-strain relationship can be captured. This formulation replaces parameters in **Eq. 1** with the connection weights of one or more ANNs³. The resulting, trained ANN describing the stress-strain behavior is referred to as a neural network constitutive model (NNCM). One example of a NNCM architecture is shown in **Figure 2A** (left). The NNCM displayed is a feed-forward, fully connected ANN that accepts a strain vector as input and returns a stress vector at the output. Alternative network architectures have been proposed by other investigators, but this form is the basis for most AutoP applications thus far. Even considering the various NNCM architectures utilized within AutoP, to the best of our knowledge all have comprised at most two hidden layers with up to a few dozen nodes each. Compared to deep-learning methods, NNCMs are very small ANNs.

Existing efforts to build model-free descriptions of material behavior typically rely on experimental test data acquired from a sample in a well-defined loading scenario, such as strip extensimetry, to obtain stress-strain data from force-displacement measurements. Recent investigations in data-driven mechanics use this type of measurement data directly (i.e., no NNCM) to solve computational mechanics problems [31,

³Node biases would also comprise the set of parameters if included in the network architecture.



32, 37, 50]. Alternatively, stress-strain measurement data can be used to train NNCMs for later use in computational problems (see reviews [14, 15, 44, 55]). More recently, researchers have developed physics-informed deep neural networks (PINNs) that embed physical laws into the cost function used in backpropagation-based training [40].

Some measure of success has been achieved with each of these methods; however, the resulting soft-computational constitutive model, in whichever applicable form, is fully defined by the measurement data acquired from a homogeneous sample. That is, the models are trained once and deployed. When used for inference against input data not within the domain of the training set, the solution becomes unreliable, analogous to modeling errors in model-based inverse methods. Furthermore, these methods rely on the existence of stress-strain training data. Unfortunately, the complexity of soft tissues precludes excision of geometrically precise, homogeneous samples for measurement in well-defined loading scenarios. Nor do *ex vivo* load tests provide a good measure of soft tissue properties *in vivo*. In short, current data-driven methods fail to extract relevant information from the available force-displacement measurements acquired in a normal clinical setting.

The Autoprogressive Method [17] circumvents limitations with model-based and other data-driven methods through a tight coupling of NNCMs with FEA and knowledge of both internal and external object geometry. It has been successfully used to build soft-computational mechanical models of many different materials in a multitude of loading scenarios, including soils [19, 20], concrete [27, 28], steel [30, 57], red blood cells [48, 49], and the cornea [16]. AutoP has even been applied to thermal constitutive models [1]. More recently, we have been developing an adaptation of AutoP for QUSE using both boundary and interior displacement measurements [22]. The caveat that internal geometry must be known precludes most clinical imaging applications and is addressed by introducing a new type of NNCM, discussed in Section 3.1.

AutoP is a method for generating information from measurements. When applied to inverse problems in continuum mechanics, the goal is to recover the stress and

strain fields induced by measured forces and displacements in a model-free way. Replacing the mathematical constitutive model with an ANN is paramount to provide the flexibility inherent to said networks, but alone is not a sufficient strategy. The two key components that make AutoP work are 1) the application of force and displacement BCs in separate FEAs— FEA_σ and FEA_ϵ —to obtain physically consistent estimates of stresses and strains through known physical principles and 2) NNCMs participate in the generation of their own training data by acting as the material model in the assembly of the system of equations in Eq. 6. The former point is analogous to model-based inverse methods wherein the parameter estimates in the current iteration affect the displacements computed in the forward problem. For clarity, the FEA equations can be rewritten as

$$\sigma^{NN} = NN(\epsilon), \quad (16)$$

$$D^{NN} = \frac{\partial \sigma^{NN}}{\partial \epsilon}, \quad (17)$$

$$K(\theta) = \sum_{e=1}^{N_e} \int_{\Omega_e} B_e^T D_e^{NN}(\theta) B_e d\Omega_e, \quad (18)$$

$$P = \sum_{e=1}^{N_e} \int_{\Omega_e} B_e^T \sigma_e^{NN} d\Omega_e. \quad (19)$$

We introduce the operator $NN(\cdot)$ to represent inference by the NNCM. The analytical expression for D^{NN} is derived in [21].

Imposing measured forces and displacements in separate FEAs exploits stress equilibrium and compatibility requirements. In FEA_σ , measured forces are applied as force BCs ($\sigma \cdot n = \tilde{p}$)⁴. Stress equilibrium, defined in Eq. 3, relates forces to stresses and we therefore claim the stresses σ_σ computed in FEA_σ are physically consistent approximations of the true stress field. In a separate FEA_ϵ , corresponding displacement measurements are applied as displacement BCs ($u = \tilde{u}$). Because compatibility requirements relate displacements to

⁴There exist certain geometric displacement BCs that are applied in all FEAs. For our examples, such BCs are the bottom surface of the scanned object being “pinned” and having zero displacement

Algorithm 2 | AutoP Training of NNCMs.

- [0] Linear-elastic NNCM pretraining
- [1] Apply force BCs in FEA_σ, compute σ_σ
- [2] Apply displacement BCs in FEA_ε, compute ε_ε
- [3] Train NNCM(s)
- [4] If displacement error converged, exit, otherwise return to [1].

strain, we claim the strains ε_ε computed in FEA_ε are physically consistent approximations of the true strain field. The AutoP training process is summarized in **Figure 1B** and can be broken into the five steps listed in **Algorithm 2**.

Linear-elastic pretraining is used only to provide NNCMs with a physically consistent starting state. Unlike model-based reconstruction methods that can be sensitive to parameter initialization [4], investigations with AutoP have demonstrated that linear-elastic pretraining does not constrain the mechanical properties learned by the NNCMs.

Convergence in AutoP is determined by comparing the values of a cost function to predefined limits. In most AutoP applications thus far, the cost function has assumed the form [17].

$$\Phi(\theta) = \|U - \tilde{U}\|_1. \quad (20)$$

Aside from the use of the L_1 -norm to diminish the effects of outliers, it is important to note that the magnitude of the cost function has no direct effect on the backpropagation-based training of the NNCMs. Even so, training the NNCMs with the stresses and strains computed in FEA_σ and FEA_ε, respectively, adjust the connection weights so that the material properties described by the NNCMs more accurately resemble the true material properties. We also note that because the cost function does not directly influence NNCM training, adjustments to $\Phi(\theta)$ do not significantly affect AutoP whereas altering the cost function in model-based inverse methods can significantly change the resulting parameter estimates.

It is not immediately clear why the combination of the stresses and strains from FEAs solved under separate, but conjugate, boundary conditions should lead the ANNs to the correct material properties. We will demonstrate that it is in fact an implicit displacement error that guides the learning process. Define u_σ to be the full set of displacements computed in the solution of FEA_σ, including any imposed BCs. Likewise, u_ε represents the displacements computed in the solution of FEA_ε. By definition of a NNCM, $\sigma^{NN} = NN(\varepsilon) = NN[f_u(u)]$, where $f_u(\cdot)$ is a function that receives a displacement vector as input and returns the corresponding strain vector. Let $\Delta u = u_\sigma - u_\varepsilon$. Thus, $u_\sigma = u_\varepsilon + \Delta u$ and therefore $\sigma_\sigma = NN[f_u(u_\varepsilon + \Delta u)]$. What does this mean? Inference by the NNCM using physically consistent strains ε_ε as input results in physically consistent stresses σ_σ only when the input is augmented by the displacement error.

To better understand this interaction, assume the NNCM describes a linear-elastic material so that $\sigma^{NN} \approx D^{NN} \cdot \varepsilon$ and deformation is infinitesimal, leading to

$f_u(u + \Delta u) = f_u(u) + f_u(\Delta u)$. We also denote the strains computed in FEA_σ as ε_σ while $\sigma_\varepsilon = NN(\varepsilon_\varepsilon)$ are the stresses output by the NNCM when strains computed in FEA_ε are supplied as input to the network. Training a NNCM with a backpropagation-based method consists of minimizing a cost function, in this case

$$\hat{R}_{\theta_m} = \operatorname{argmin}_{R_{\theta_m} \in \mathbb{R}} \frac{1}{2} \left\| \overline{\sigma_\sigma} - \sigma_\varepsilon(\varepsilon_\varepsilon; R_{\theta_m}) \right\|^2 \quad (21)$$

$$= \operatorname{argmin}_{R_{\theta_m} \in \mathbb{R}} \frac{1}{2} \left\| \overline{NN}(\varepsilon_\sigma) - NN(\varepsilon_\varepsilon; R_{\theta_m}) \right\|^2, \quad (22)$$

where R_{θ_m} denotes the NNCM connection weights and the overline is used to indicate a constant term. $\overline{NN}$ is the same NNCM used to solve FEA_σ and FEA_ε whereas $NN(\varepsilon_\varepsilon; \theta)$ changes each training iteration as the NNCM weights are updated. Applying the aforementioned linear approximations, **Eq. 22** can be expressed as

$$\hat{R}_{\theta_m} = \operatorname{argmin}_{R_{\theta_m} \in \mathbb{R}} \frac{1}{2} \left\| \overline{NN}[f_u(u_\varepsilon + \Delta u)] - NN[f_u(u_\varepsilon; R_{\theta_m})] \right\|^2 \quad (23)$$

$$\approx \operatorname{argmin}_{R_{\theta_m} \in \mathbb{R}} \frac{1}{2} \left\| \overline{D^{NN}} \cdot [f_u(u_\varepsilon + \Delta u)] - D^{NN}(R_{\theta_m}) \cdot [f_u(u_\varepsilon)] \right\|^2 \quad (24)$$

If we consider the first iteration of training when $\overline{D^{NN}} = D^{NN}(R_{\theta_m})$, the cost function further reduces to

$$\approx \operatorname{argmin}_{R_{\theta_m} \in \mathbb{R}} \frac{1}{2} \left\| D^{NN} \cdot f_u(\Delta u) \right\|^2, \quad (25)$$

and reverts back to **Eq. 24** after the first training iteration and the NNCM weights R_{θ_m} change.

The preceding argument does not imply NNCMs trained in AutoP are limited to learning linear-elastic material properties. Rather, the case of linear materials was chosen as an example to best highlight the effect of displacement error in NNCM training. We emphasize that, similar to model-based methods, the displacement error is a driving force in the solution of the inverse problem as approached through AutoP. However, in the case of AutoP, the effect of displacement error emerges from the inconsistency between stresses and strains computed in FEA_σ and FEA_ε. It is a characteristic that arises out of the equilibrium and compatibility requirements rather than an explicitly defined error measure to direct the parameter search.

Much like optimization-based methods, the parameter estimates in the current iteration—the weights of the ANNs—affect the solution of the forward problem. Moreover, the error when comparing the forward problem solution to experimental measurements affects the parameter updates. What sets AutoP apart from typical model-based and machine learning techniques is its self-learning paradigm guided by physical principles. When applied to QUSE, the result is a patient-specific soft-computational material model. **Figure 2B** aims to illustrate the self-learning property of AutoP. Imposing measurement data as BCs in separate FEAs forces the material

properties learned by the ANNs to satisfy physical principles and be self-consistent.

3.1 Cartesian NNCMs

The discussion of AutoP thus far has assumed that the finite element mesh conformed to both internal and external object geometry with a different NNCM assigned for each unique material. Eq. 18 implies this fact in that $D_e^{NN}(\theta)$ is constant over the entire domain of an element. The FEA solution procedure requires the appropriate NNCM to be selected for the element when evaluating the integral. This formulation of AutoP is not suitable for biomedical imaging applications because the internal tissue structure is normally not known *a priori* nor segmented into discrete regions with homogeneous material properties. As a consequence, the goal of AutoP must be adjusted to infer both material properties and geometry from the set of force-displacement measurements.

Two significant changes were made toward this goal. First, the structure of the NNCM was changed by adding a second network that interacted with the existing NNCM and was responsible for learning spatial information. We deemed these networks Cartesian neural network constitutive models (CaNNCMs) [23–25]. The CaNNCM architecture is illustrated in Figure 2A. To identify the individual subnetworks, we refer to the one which learns the “average” stress-strain relationship of the entire object as the material property network (MPN, Figure 2A left) and the network that maps a Cartesian coordinate input to a spatially varying value as the spatial network (SN, Figure 2A right). A thorough description of CaNNCM theory of operation can be found in [23, 24]. Unlike NNCMs previously utilized with AutoP, a single CaNNCM is capable of characterizing the heterogeneous material properties of an entire object. The FEA equations are adjusted slightly to accommodate this change:

$$K(\theta) = \sum_{e=1}^{N_e} \int_{\Omega_e} B_e^T D^{NN}(x, \theta) B_e d\Omega_e, \quad (26)$$

$$P = \sum_{e=1}^{N_e} \int_{\Omega_e} B_e^T \sigma^{NN}(x) d\Omega_e. \quad (27)$$

Spatial information is encoded within a set of spatial scaling values S_x^ϵ . The SN is responsible for learning the mapping $SN: x \rightarrow S_x^\epsilon$. Given a spatial location and strain vector, the SN provides the corresponding S_x^ϵ and the stress vector is then calculated as $\sigma^{NN}(\epsilon, x) = NN(\epsilon/S_x^\epsilon)$, where the division is element-wise and the operator $NN(\cdot)$ refers to the MPN. Determining the spatial values is not trivial, although they can be readily computed from the

stresses and strains already available in AutoP in conjunction with the MPN, as detailed in [23]. AutoP training must also be adjusted to accommodate CaNNCMs as described in Algorithm 3.

CaNNCMs work well in QUSE because information pertaining to the interior geometry can be gleaned from internal displacement measurements available through US imaging. In unpublished work, we found that CaNNCMs trained in AutoP using only boundary information were unable to recover internal structure away from the surface. Part of the shortcoming is due to the lack of surface force distribution measurements. Rather, the force measured experimentally is the sum of forces over the face of the US transducer that provides information about the mean stress distribution over the surface. Coupling these boundary data with prior knowledge of internal geometry is sufficient for a set of NNCMs to learn the heterogeneous material properties of an object. Therefore, in order for CaNNCMs to learn both material properties and geometry, some measurement data containing spatial information must be provided.

Interestingly, we found that incorporating internal displacements in FEA_e was insufficient for accurately estimating material property distributions, particularly when measurements were acquired by loading from a single direction [25]. The reason can be seen from Eq. 27. By making the stress term explicitly dependent on spatial position, additional force measurements populating P are required to ensure the internal resisting forces computed within the integral are correctly calibrated. Acquiring measurements from multiple sides and loading angles provides an immense amount of information for CaNNCMs at the cost of significantly increased FE modeling complexity and computational load.

An alternative strategy is to add a regularization term to augment the available information. Our solution was to add additional physical constraints during the calculation of S_x^ϵ in Step 4a of AutoP. To understand the functionality of the regularization term, we first summarize the detailed methods in [23]. The spatial values are computed by minimizing a cost function very similar to Eq. 21, with the primary difference being the parameters over which the function is minimized changes from the MPN connection weights to the spatial values,

$$\hat{S}_x^\epsilon = \underset{S_x^\epsilon \in \mathbb{R}}{\operatorname{argmin}} \frac{1}{2} \left\| \bar{\sigma}_\sigma - \sigma_\epsilon(\epsilon_\epsilon/S_x^\epsilon; R_{\theta_m}) \right\|^2 \quad (28)$$

The purpose of the spatial values is to further reduce the error in Eq. 21 by storing the information not captured by the spatially independent MPN weights.

Implementing Eq. 28 in AutoP as defined will lead to erroneous material property distribution estimates. Perhaps the most intuitive explanation starts by noting the CaNNCM is pretrained as a homogeneous, linear-elastic material. Then, when computing spatial values in Step 4a, the S_x^ϵ found as the solution of Eq. 28 map a heterogeneous strain field to a homogeneous stress field because the surface force BCs applied in FEA_σ contain no spatial information.

Algorithm 3 | AutoP Training of CaNNCMs.

- [0] Pretrain CaNNCM as homogeneous, linear-elastic material
- [1] Apply force BCs in FEA_σ , compute σ_σ
- [2] Apply displacement BCs in FEA_ϵ , compute ϵ_ϵ
- [3] Train MPN
- [4a] Update S_x^ϵ
- [4b] Train SN
- [5] If displacement error converged, exit, otherwise back to [1].

We introduced σ -matching regularization to inject spatial information into **Eq. 28** by imposing the physical constraint that conjugate force-displacement BCs imposed in separate FEAs should produce the same stress fields,

$$\mathcal{R}_\sigma = \left| \overline{\sigma}_\sigma - \overline{\sigma}_\varepsilon \right| \cdot \text{sign}(\overline{\sigma}_\sigma). \quad (29)$$

Note that the stress terms are constant, hence the multiplication by $\text{sign}(\overline{\sigma}_\sigma)$ to account for the lack of gradient information. We again chose the L_1 -norm to reduce the effects of outliers. If the same linear approximations from **Eq. 21** are applied to the σ -matching term, we can show that the displacement error is influencing the result:

$$\mathcal{R}_\sigma = \left| \overline{NN}(\varepsilon_\sigma) - \overline{NN}(\varepsilon_\varepsilon) \right| \cdot \text{sign}(\overline{\sigma}_\sigma), \quad (30)$$

$$\approx \left| \overline{D}^{NN} \cdot \mathbf{f}_u(u_\varepsilon + \Delta u) - \overline{D}^{NN} \cdot \mathbf{f}_u(u_\varepsilon) \right| \cdot \text{sign}(\overline{\sigma}_\sigma), \quad (31)$$

$$\approx \left| \overline{D}^{NN} \cdot \mathbf{f}_u(\Delta u) \right| \cdot \text{sign}(\overline{\sigma}_\sigma). \quad (32)$$

Adding the regularization term to the cost function for computing $\mathbf{S}_x^\varepsilon$ with free parameter β_σ to control its influence on the solution, **Eq. 28** becomes

$$\hat{\mathbf{S}}_x^\varepsilon = \underset{\mathbf{S}_x^\varepsilon \in \mathbb{R}}{\text{argmin}} \left\| \frac{1}{2} \left| \overline{\sigma}_\sigma - \sigma_\varepsilon(\varepsilon_\varepsilon / \mathbf{S}_x^\varepsilon; R_{\theta_m}) \right| + \beta_\sigma \mathcal{R}_\sigma \right\|^2. \quad (33)$$

A significant difference between regularization terms typically used in model-based inverse methods and 33) is the appearance of σ -matching within the L_2 -norm. Because it is constant, simply adding the σ -matching term to the existing cost function would have no effect on the computed spatial values. Adding σ -matching as we have in **Eq. 33** can be interpreted as adjusting the (constant) stresses $\overline{\sigma}_\sigma$ computed in FEA_σ with spatial information encoded within displacement measurements through the displacement error.

We demonstrated in [25] the significant improvement in material property estimates when σ -matching was incorporated into AutoP training, even with a relatively sparse set of force-displacement measurements. Contrary to typical forms of regularization, σ -matching is based on a physical principle that will always be true, not a prior assumption about the property the solution should exhibit. Furthermore, it is uniquely suited to AutoP because of the way equilibrium and compatibility requirements are exploited by FEA_σ and FEA_ε . Other constraints on physical properties will likely be incorporated into AutoP as CaNNCMs are further developed for imaging the non-linear and time-dependent properties of soft tissues.

4 NON-LINEAR ELASTICITY IMAGING WITH AUTOP

The preceding discussion of NNCMs and AutoP was limited to objects under loads inducing small deformation. Mathematically, the strain tensor in the limit of infinitesimal deformation is expressed as $\boldsymbol{\varepsilon} = \frac{1}{2}(\nabla \mathbf{u} + \nabla \mathbf{u}^T)$. Under this linear kinematic assumption, appropriate when induced strains are less than

5%, elastic solids can be adequately described by a linear-elastic constitutive model.

Probing the non-linear mechanical properties of deep structures in QUSE requires application of large loads to sufficiently deform distal tissues. In these scenarios, a large deformation framework must be invoked wherein the strain tensor includes geometrically non-linear terms. As an example, one potential definition of geometrically non-linear strain is $\boldsymbol{\varepsilon} = \frac{1}{2}(\nabla \mathbf{u} + \nabla \mathbf{u}^T + \nabla \mathbf{u}^T \nabla \mathbf{u})$. Application of large compressive loads coupled with the non-linear and time-dependent mechanical behavior of soft tissues implies that one or two linear-elastic parameters are ineffective for clinical QUSE. More importantly, the complex non-linear and viscoelastic properties of soft tissues may provide a significant amount of diagnostic information [42], reinforcing the value of reconstructing elasticity images beyond shear modulus.

The problem then arises of selecting an appropriate non-linear and/or viscoelastic constitutive model that describes tissue behavior and maximizes the diagnostic information in QUSE images. Complexity is further increased when considering the likelihood that different tissues or tissue states (i.e., healthy tissue, benign lesions, and malignant tumors) are best described by different material models. Current model-based approaches assume homogeneity of the underlying constitutive model for the entire imaged tissue section and estimate the spatial variation of the corresponding model parameters. There exists no model-based QUSE inverse method capable of *discovering* the set of constitutive models—and estimating values of the corresponding parameters—that adequately describes a heterogeneous group of tissues. The combination of AutoP with CaNNCMs can fill this void.

AutoP has been applied to build soft-computational models of complex materials undergoing small and large deformation when the total geometry was known (e.g. [19, 27, 28, 30, 45, 58]). To achieve non-linear, time-dependent material property imaging with AutoP, CaNNCMs must be modified to 1) accommodate geometrically non-linear deformation to facilitate non-linear elasticity imaging and 2) incorporate additional stress-strain state information as network input to properly capture a heterogeneous distribution of complex mechanical behaviors. Prior successes with AutoP lead us to believe there exists an adaptation to the CaNNCM architecture and/or AutoP that will accomplish both tasks.

The difficulty of this problem can be understood by comparing the information required for a NNCM to compute a stress vector for materials of varying complexity. For linear-elastic materials, the NNCM output is defined as $\sigma^{NN} = NN(\varepsilon)$. Non-linear, viscoelastic materials require time information to fully define the stress-strain response, $\sigma^{NN} = NN(\varepsilon, t)$. It is further complicated for objects under large deformation because of the need to account for differences in Lagrangian and Eulerian descriptions of stress and strain. It is clear the NNCM inputs must be changed. Our current work is toward the goal of identifying a NNCM architecture capable of learning the stress-strain response of hyperelastic materials.

Projecting further, adjustments to the CaNNCM architecture will also be necessary to characterize a heterogeneous distribution of non-linear, viscoelastic materials. If the operating principle of linear-elastic CaNNCMs is retained, the stress-strain response would take the form $\sigma^{NN} = NN(\epsilon/S_x^e, t)$, where spatial information is again encoded within the spatial scaling values S_x^e . Less obvious from this relationship is the necessity of stress-strain state information at the spatial network input to account for the non-linear and time-dependent stress responses. This requirement can be understood by expanding on the explanation provided in [24] for the operation of spatial scaling values. When all materials are linear-elastic, the stiffness matrix D^{NN} (recall its use in Eq. 18) computed from the weights of the MPN is constant. The role of S_x^e is to vary the effective “stiffness” described by D^{NN} based on position. Because the materials considered were linear-elastic, the modified stiffness matrix is independent of time and state of deformation, meaning S_x^e is constant at x . Conversely, D^{NN} computed from a non-linear MPN will not be constant, nor will the rate at which it changes be the same at all points. Therefore, values of S_x^e will need to change based not only on position, but also the current (or previous) stress-strain states.

5 DISCUSSION

Quantitative elasticity imaging is a challenging problem. Much of the difficulty arises from the limited measurement data that can be acquired during a clinical US imaging exam. Model-based inverse methods address the ill-posed inverse problem by constraining the solutions through geometric and constitutive model assumptions, well-defined force loads, and application of regularization to the solution. While effective, deviation of measurement data from the model assumptions can introduce large artifacts in the reconstructed material property images, potentially corrupting diagnostic information, and the regularization method is almost never grounded in physical laws. Furthermore, parameters associated with the pre-selected constitutive model may only capture a portion of the tissue mechanical properties, missing information that could be important to clinical decision making.

AutoP and NNCMs are a fundamentally different approach to the inverse problem in elasticity imaging. Rather than constraining the solution through model assumptions, AutoP extracts material property information from force-displacement measurements through a combination of physical modeling and machine learning. In this review, we presented AutoP in a new way to highlight similarities of the procedure with well-known optimization methods. Specifically, we demonstrated how the principles of stress equilibrium and strain compatibility are exploited through application of measurement data as BCs in separate FEAs, from which naturally arises an implicit displacement error that drives NNCM training.

The primary benefit of AutoP over model-based inverse methods is the potential to *discover* 3-D mechanical properties of soft tissues *in vivo*. Full computational modeling of applied

force loads means measurements need not be acquired in a constrained manner; e.g., uniaxial compression by a large platen. Nor is 3-D displacement data necessary to reconstruct material properties throughout a volume, as we demonstrated in [25]. However, the use of arbitrary force-displacement measurements as input to AutoP significantly increases the computational load. Furthermore, we expect intelligent sampling strategies will need to be developed in order to maximize the information content of measurement data and reduce the computational burden of AutoP. Good sampling strategies will likely be essential for effectively imaging non-linear, time-dependent material properties.

One particularly exciting prospect of CaNNCMs and AutoP is the ability to identify mechanical properties from a heterogeneous distribution of material types. Classic model-based inverse methods must first assume the form of the constitutive model for the entire tissue section. The solution is the spatial variation of corresponding parameters that best fits the measurement data. AutoP reverses this order to first build a non-parametric, soft-computational model that describes the spatially varying material properties encoded in force-displacement data. Parameter estimation occurs *after* relevant information has been extracted from measurements. Successfully developing this capability would represent a paradigm shift in not only QUSE, but inverse identification of material properties in general.

AutoP is at its core a method to solve boundary value inverse problems. It is naturally suited for elasticity imaging because the physical principles of stress equilibrium and strain compatibility are built into the forward model. These principles relate the available force-displacement measurement data to the desired stress and strain distributions. By combining the physical principles through forward modeling with the flexibility of ANNs, virtually any type of continuum material can be described non-parametrically. AutoP could be applicable to other inverse problems with well-defined physical laws relating measurement data to the desired quantities. Nevertheless, AutoP is just one example of integrating physical modeling with machine learning to build powerful methods of solving inverse problems.

AUTHOR CONTRIBUTIONS

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Effect of Post-mortem Interval and Perfusion on the Biophysical Properties of *ex vivo* Liver Tissue Investigated Longitudinally by MRE and DWI

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Structural changes of soft tissues on the cellular level can be characterized by histopathology, but not longitudinally in the same tissue. Alterations of cellular structures and tissue matrix are associated with changes in biophysical properties which can be monitored longitudinally by quantitative diffusion-weighted imaging (DWI) and magnetic resonance elastography (MRE). In this work, DWI and MRE examinations were performed in a 0.5-Tesla compact scanner to investigate longitudinal changes in water diffusivity, stiffness and viscosity of *ex-vivo* rat livers for up to 20 h post-mortem (pm). The effect of blood on biophysical parameters was examined in 13 non-perfused livers (containing blood, NPLs) and 14 perfused livers (blood washed out, PLs). Changes in cell shape, cell packing and cell wall integrity were characterized histologically. In all acquisitions, NPLs presented with higher shear-wave speed (*c*), higher shear-wave penetration rate (*a*) and smaller apparent-diffusion-coefficients (ADCs) than PL. Time-resolved analysis revealed three distinct phases: (i) an initial phase (up to 2 h pm) with markedly increased *c* and *a* and reduced ADCs; (ii) an extended phase with relatively stable values; and (iii) a degradation phase characterized by significant increases in *a* (10 h pm in NPLs and PLs) and ADCs (10 h pm in NPLs, 13 h pm in PLs). Histology revealed changes in cell shape and packing along with decreased cell wall integrity, indicating tissue degradation in NPLs and PLs 10 h pm. Taken together, our results demonstrate that the biophysical properties of fresh liver tissue rapidly change within 2 h pm, which seems to be an effect of both cytotoxic edema and vascular blood content.

Several hours later, disruption of cell walls resulted in higher water diffusivity and wave penetration. These results reveal the individual contributions of vascular components and cellular integrity to liver elastography and provide a biophysical, imaging-based fingerprint of liver tissue degradation.

Keywords: liver stiffness, viscosity, perfusion, degradation, magnetic resonance elastography (MRE), diffusion-weighted imaging (DWI), water diffusivity, post-mortem interval

INTRODUCTION

Changes in cellular and extracellular matrix (ECM) architecture associated with pathological or physiological processes affect the biophysical tissue properties that are visualized by medical imaging. However, the exact link between microarchitectural changes and the corresponding biophysical manifestations on the macroscopic imaging level is still elusive. Especially for the liver, where imaging of viscoelastic tissue properties by elastography has become a standard clinical procedure, a deeper understanding of the structure elements that determine the changes in macroscopic imaging properties is urgently needed (Mueller, 2020; Hudert et al., 2021).

Changes of the tissue's microarchitecture can be caused by either pathological factors (such as inflammation, fibrosis, etc.) or physiological factors (such as post-mortem cellular apoptosis, blood perfusion status, etc.). Post-mortem (pm) degeneration of biological tissues is a process that involves alterations of tissue composition and structure on many levels. The degree of *ex vivo* decomposition is determined by many factors, including the tissue type, post-mortem interval, temperature, and humidity (Oka, 1920; Otto et al., 1981; Milroy, 1999; Tomita et al., 2004). Earlier studies investigating the time course of tissue decomposition from biochemical and morphological perspectives have revealed changes in macro- and microstructures (Benda et al., 1957; Masshoff et al., 1964; Riede et al., 1976), glycogen concentration (Popper and Wozasek, 1932; Hertz, 1933; Nunley et al., 1973; Calder and Geddes, 1990), pH level and fat content (Shima, 1922; Sinapius, 1963; Donaldson and Lamont, 2013). In view of the structural and functional changes involved in the post-mortem degeneration process, serial characterization of the biophysical properties of *ex vivo* tissues by imaging with simultaneous histological validation could help us elucidate the link between imaging parameters and microscopic tissue structure.

Magnetic resonance imaging (MRI) has been employed to investigate the morphological and biophysical properties of *ex vivo* tissue specimens (Schneiderman et al., 2005; Tempel-Brami et al., 2015; Nebelung et al., 2016; Wolfram et al., 2020). In the context of tissue degradation, water diffusivity measured by diffusion-weighted imaging (DWI) has been revealed as a potential marker of post-mortem tissue alterations (Arthurs et al., 2015; Keller et al., 2018; Sapienza et al., 2019). Three studies investigating *ex vivo* brain tissue *in situ* have also demonstrated biomechanical properties quantified by magnetic resonance elastography (MRE) to be sensitive to instantaneous cerebral structural and functional changes occurring after death (Vappou et al., 2008; Weickenmeier et al., 2018; Bertalan et al., 2020).

Although published studies provide imaging data on *ex vivo* tissue, most publications report results obtained at a single point in time. To our knowledge, data from systematic MRI studies of tissue without fixation over a long post-mortem interval (>10 h) are very limited (Keller et al., 2018; Sapienza et al., 2019). The recently introduced compact MRI device with MRE capability is an excellent tool for performing repeated examinations for the longitudinal investigation of *ex vivo* tissue samples (Braun et al., 2018; de Schellenberger et al., 2019; Sauer et al., 2019; Everwien et al., 2020; Garczyńska et al., 2020). The cylindrical sample tube utilized by this device ensures well-defined boundary conditions, which is beneficial for MRE analysis, and this technique has been shown to provide consistent values of stiffness and viscosity (Ipek-Ugay et al., 2015; Braun et al., 2018; Garczyńska et al., 2020).

In the present study, we used this compact MRI/MRE device to longitudinally investigate changes in the biophysical properties of *ex vivo* liver samples harvested from rats in a post-mortem interval of up to 20 h. In addition to the effect of the duration of the pm interval, we investigated the possible effect of the presence of blood on tissue decomposition by comparing the biophysical features of perfused and non-perfused liver samples. This was done because tissue perfusion is a common sample preparation step for histopathological analysis in science, especially for immunohistology (Scouten et al., 2006). Moreover, our imaging findings were supported by histological findings. Our foremost aim using this setup was to further the understanding of the underlying microscopic structural changes that manifest as macroscopic imaging features. Specifically, we quantified the apparent water diffusion coefficient (ADC) by DWI as well as stiffness-related shear wave speed (*c*) and viscosity-related shear wave penetration rate (*a*) by MRE in order to provide a specific fingerprint of the biophysical alterations of perfused and non-perfused liver tissue over time after death.

MATERIALS AND METHODS

All procedures involving animals were approved by the local authority (Landesamt für Gesundheit und Soziales Berlin, Reg. No. T0212/19) and were performed according to animal welfare regulations and institutional guidelines.

Sample Preparation

Livers were harvested from young adult female Wistar rats (Forschungseinrichtungen für Experimentelle Medizin, FEM, Berlin, Germany; Janvier Labs, Le Genest-Saint-Isle, France). All rats were kept in the same animal facility under standard housing

conditions for at least 3 days before imaging. In total, 41 rats were sacrificed and livers were harvested and randomly selected for two groups: (i) non-perfused liver (NPL, $n_{\text{total}} = 20$ with $n = 13$ for the MRE/DWI investigations and $n = 7$ for histology) and (ii)

perfused liver (PL, $n_{\text{total}} = 21$ with $n = 14$ for the MRE/DWI investigations and $n = 7$ for histology).

To harvest the livers, the rats were first anesthetized with an overdose of isoflurane vapor. For the NPL group,

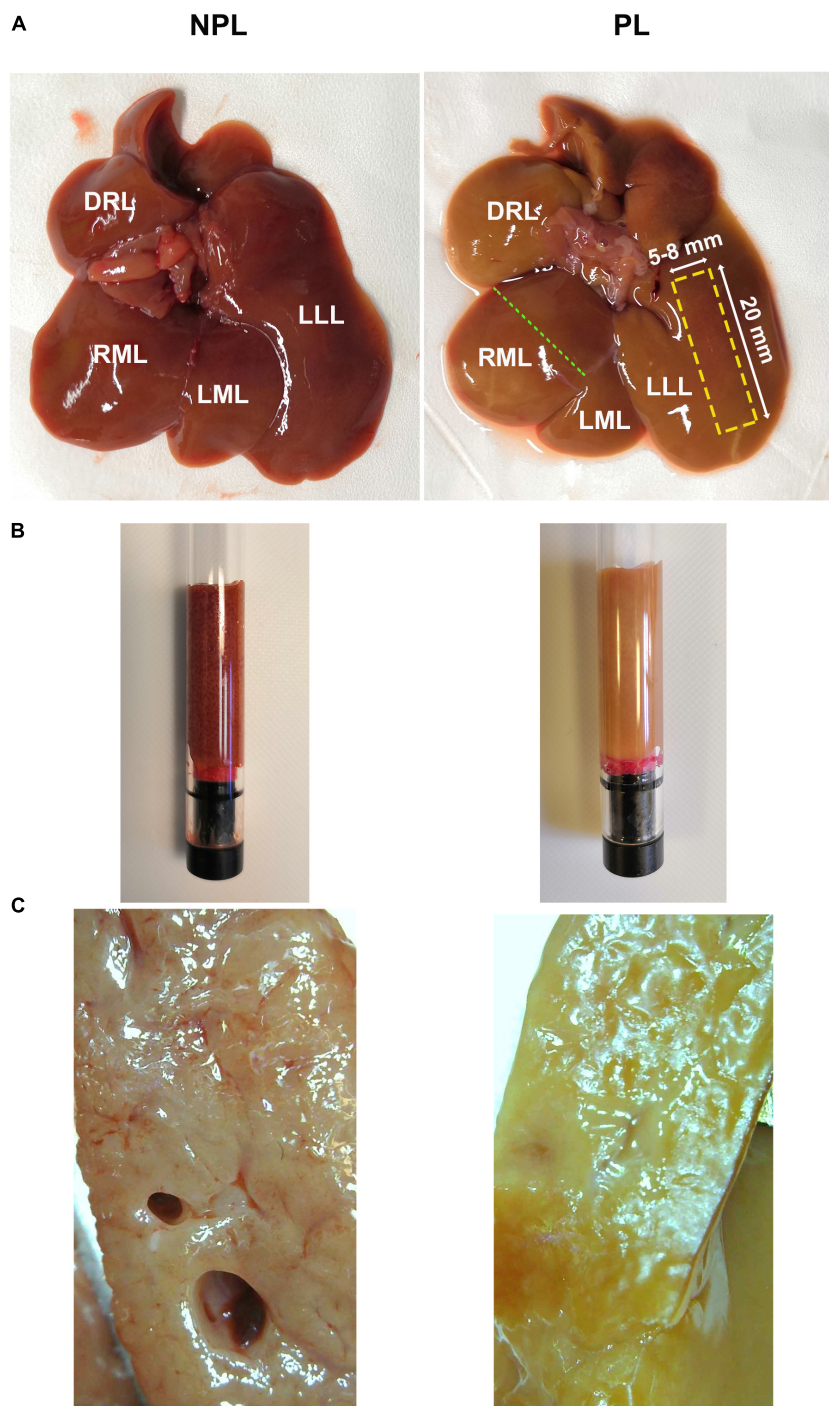


FIGURE 1 | (A) Photograph of fresh non-perfused livers (NPLs, left) and perfused livers (PLs, right); a rectangular area marked by a dashed yellow line indicates the site from which the sample for MRI was taken. The green dashed line indicates the position of the cross-sectional area shown in **(C)**. **(B)** Sample tubes containing liver tissue obtained from NPL and PL for DWI and MRE. **(C)** cross-sectional area of the liver showing more blood in the NPL sample (left) than in the PL sample (right). NPL, non-perfused liver; PL, perfused liver; LLL, left lateral lobe; LML, left middle lobe; RML, right middle lobe; DRL, dorsal right lobe.

the rats were decapitated with a rodent guillotine and the livers were removed without further processing. For the PL group, anesthesia was followed by exsanguinations, and the liver was perfused *in situ*. For liver perfusion, the portal vein (PV) was cannulated (20G Vasofix Safety IV Catheter, B. Braun, Melsungen, Germany) and the inferior vena cava (IVC) was severed and used as outflow. Blood was washed out using a peristaltic pump (MEDOREX TBE/200 62-4-3-3, MDX Biotechnik International, Nörten-Hardenberg, Germany), and circulating cells were removed from the liver by phosphate-buffered saline solution (PBS Tablets, Gibco, Thermo Fisher Scientific, Paisley, United Kingdom). Perfusion was stopped upon the observation of a non-decoloring liver with clear outflow. Average perfusion time was 13 min at a perfusion rate of approx. 40 ml/min and a total perfusate consumption of ~700 ml.

For MR imaging, a cylindrical sample (8 mm Ø, 20 mm height) was cut out from the left lateral lobe of the liver and directly transferred into a glass tube, which was then inserted into a compact MRI device (Pure Devices GmbH, Würzburg, Germany) for imaging. Sample preparation time was 19 min for NPLs and 32 min for PLs (13 min extra for perfusion). As a result, MRI started 19 min pm for NPLs and 32 min pm for PLs.

Magnetic Resonance Elastography (MRE) and Diffusion-Weighted Imaging (DWI)

All imaging examinations were performed in a 0.5-T compact MRI device as mentioned above. For MRE measurements, an external gradient amplifier (DC 600, Pure Devices GmbH, Würzburg, Germany) and a piezo-actuator (PAHL60/20 Piezosystem Jena, Jena, Germany) coupled to the sample tube were implemented to the MRI device to provide mechanical vibrations. Details of the MRE setup are provided in Braun et al. (2018); de Schellenberger et al. (2019); Sauer et al. (2019); Everwien et al. (2020) and Garczyńska et al. (2020).

Imaging parameters for MRE and DWI were the same as described in Garczyńska et al. (2020). For MRE, one axial 3 mm slice with an in-plane resolution of 150 μm was acquired using

a mechanical driving frequency of 800 Hz. The tissue deflection generated by the mechanical vibration was 0.67 μm . Eight time steps were recorded over a wave cycle within an acquisition time of 3 min. For DWI, the same slice with an in-plane resolution of 600 μm was acquired using seven b -values (50, 175, 300, 425, 550, 675, and 800 s/mm^2), which took a total of 5 min. Data from MRE and DWI were repeatedly acquired in an interleaved manner up to 15 h pm. MRE was discontinued thereafter due to visible liquid accumulation between the liver tissue and the sample tube wall, which compromised contact and effectively hindered transfer of mechanical vibrations. Conversely, DWI acquisition, which does not require wave propagation, was continued until 20 h after death. To minimize the possible influence of temperature (Bertalan et al., 2019), the sample was kept at a constant temperature of 30°C throughout the imaging process by a built-in temperature control unit of the MRI scanner.

All imaging data were postprocessed using algorithms written in MATLAB (R2019b, The Mathworks Inc., Natick, MA, United States) as described previously in Braun et al. (2018) and de Schellenberger et al. (2019). MRE-based shear wave speed (c in m/s) and shear wave penetration rate (a in m/s) were derived using a Bessel function fit. c is a surrogate marker of tissue stiffness which can be converted to stiffness in kPa, assuming the elastic model, by ρc^2 with ρ being the material's density of 1,000 kg/m^3 . a is inversely correlated to viscous damping of shear waves. For DWI, maps of apparent diffusion coefficient (ADC), which quantifies water diffusivity, were generated with mono-exponential fitting using all seven b -values.

Histology

For histology, 14 additional rats (7 per group) were used, and liver tissue specimens from the left lateral lobe were investigated at three time points: fresh (immediately after death for NPLs and 13 min pm for PLs), 2 h pm, and 10 h pm. To ensure that the histologic features examined reflected the same intrinsic tissue properties of specimens subjected to MRE, all specimens assigned to histology were exposed to the same imaging protocols, including temperature and mechanical fluctuation. In order to

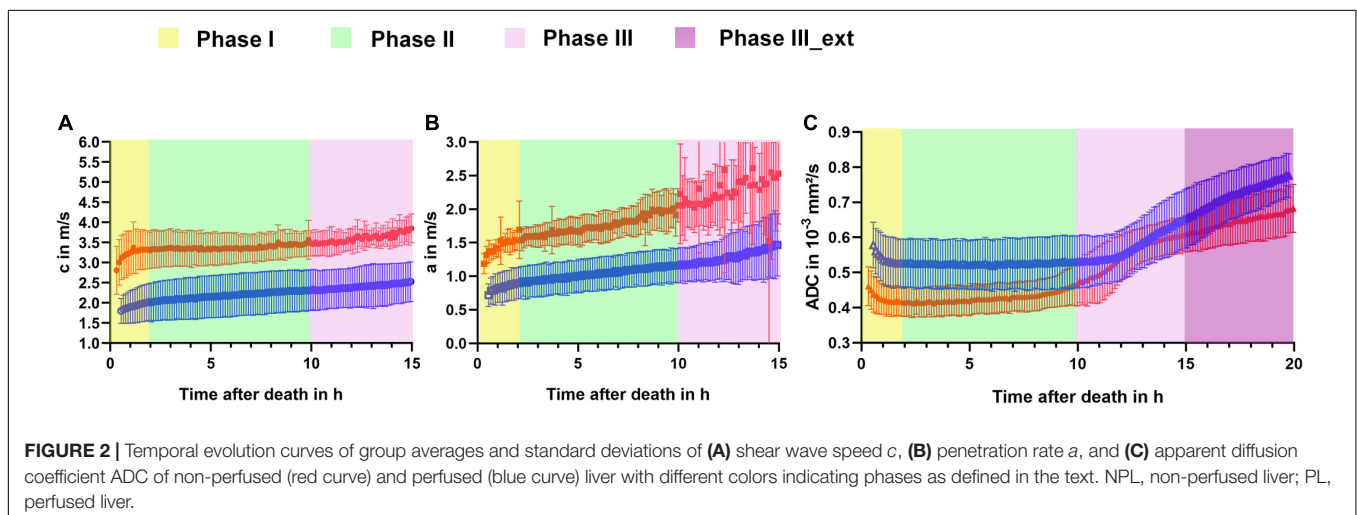


FIGURE 2 | Temporal evolution curves of group averages and standard deviations of (A) shear wave speed c , (B) penetration rate a , and (C) apparent diffusion coefficient ADC of non-perfused (red curve) and perfused (blue curve) liver with different colors indicating phases as defined in the text. NPL, non-perfused liver; PL, perfused liver.

minimize disturbance, each liver sample was divided into two portions separated in the sample tube by a small piece of foam earplug. After 1.5 h of MRI acquisition (2 h pm), the top liver portion was removed and prepared for histology while the bottom liver portion remained in the sample tube for another 8 h of MRI examinations and was finally removed for histology at 10 h pm. As the liver samples for histology were divided into 2 portions and were only imaged up to 10 h, the imaging data were not included in the final data analysis.

Tissue samples from all 3 time points were fixed in 4 % formaldehyde solution (Formalin Solution 4 %, J.T. Baker, Fisher Scientific, Avantor, Gliwice, Poland) at room temperature for 24 h. The fixed samples were processed as described earlier (Garczyńska et al., 2020). Paraffin sections of 2 μ m thickness were stained with hematoxylin and eosin (H&E; Mayer's Hemalum Solution, Merck, Darmstadt, Germany; Eosin Y solution, Sigma-Aldrich, Darmstadt, Germany).

Images of stained sections were taken with a BZ-X800 fluorescence microscope (KEYENCE DEUTSCHLAND GmbH, Neu-Isenburg, Germany). For each time point, two high-power fields per sample were analyzed. Hepatocytes were counted per field of view (FoV) at 40x magnification in H&E-stained sections using ImageJ software version 1.52v (Schneider et al., 2012). Histological analysis was performed in a blinded manner.

Statistical Analysis

Mixed analysis of variance (ANOVA) was performed to account for the effects and interactions of two factors present in our data- acquisition time (within-subject factor) and perfusion status (between-subject factor; non-perfused vs. perfused) for all imaging parameters.

Dynamic responses of water diffusivity over time as quantified by ADC were fitted using the exponential model:

$$ADC = ADC_0 + (ADC_p - ADC_0) \times (1 - \exp(-k \times t))$$

where ADC_0 and ADC_p are initial and plateau ADC values in mm^2/s , k is the rate constant in hours^{-1} , and t is acquisition time in hours.

Normality of the datasets was first assessed using the D'Agostino-Pearson normality test. Differences between groups were assessed using the unpaired t -test for data with normal distribution while the Mann-Whitney test was used for data without normal distribution.

P -values below 0.05 were considered statistically significant. Graphical and statistical analysis was performed with GraphPad Prism (GraphPad Prism 8.01. for Windows, GraphPad Software, San Diego, California, United States).¹

RESULTS

General Characterization

The difference in appearances between NPL and PL samples is shown in **Figure 1**. PLs appeared paler and contained less blood

TABLE 1 | Summary of variation rates of MRI-based parameters (in %/h) in different post-mortem phases for non-perfused (NPL) and perfused (PL) livers and pairwise comparison of phases.

	Phase I			Phase II			Phase III			Phase III_ext			Comparison of phases		
	NPL	PL	P	NPL	PL	P	NPL	PL	P	NPL	PL	P	NPL	PL	P
c	2.88 ± 2.82	4.45 ± 5.13	0.34	0.65 ± 0.86	1.58 ± 0.41	≤ 0.01	1.59 ± 1.91	1.59 ± 1.41	0.99	/	/	/	P1 vs. P2: $p \leq 0.05$	P1 vs. P2: $p \leq 0.05$	
in													P1 vs. P3: $p = 0.21$	P1 vs. P3: $p = 0.05$	
%/h													P2 vs. P3: $p = 0.12$	P2 vs. P3: $p = 0.98$	
a	4.99 ± 5.99	9.78 ± 3.94	≤ 0.05	2.85 ± 1.33	2.86 ± 0.66	0.98	4.09 ± 3.07	3.75 ± 2.40	0.75	/	/	/	P1 vs. P2: $p = 0.22$	P1 vs. P2: $p \leq 0.001$	
in													P1 vs. P3: $p = 0.64$	P1 vs. P3: $p \leq 0.001$	
%/h													P2 vs. P3: $p = 0.19$	P2 vs. P3: $p = 0.12$	
ADC	-2.75 ± 1.57	-4.95 ± 2.77	≤ 0.05	1.23 ± 1.17	0.09 ± 0.43	≤ 0.01	4.65 ± 0.94	3.50 ± 1.99	0.07	2.12 ± 0.69	3.26 ± 1.22	≤ 0.01	P1 vs. P2: $p \leq 0.001$	P1 vs. P2: $p \leq 0.001$	
in													P1 vs. P3: $p \leq 0.001$	P1 vs. P3: $p \leq 0.001$	
%/h													P2 vs. P3: $p \leq 0.001$	P2 vs. P3: $p \leq 0.001$	
													P1 vs. P3_ext: $p \leq 0.001$	P1 vs. P3_ext: $p \leq 0.001$	
													P2 vs. P3_ext: $p \leq 0.01$	P2 vs. P3_ext: $p \leq 0.001$	
													P3 vs. P3_ext: $p \leq 0.001$	P3 vs. P3_ext: $p = 0.69$	

P1 = Phase I, P2 = Phase II, P3 = Phase III, P3_ext = extended Phase III (DWI).

¹ www.graphpad.com

than NPLs. Note that, as a result of incomplete perfusion, residual blood was still visible in PLs.

Magnetic Resonance Elastography

Time evolution curves of group averages and standard deviations (SDs) of shear wave speed c and penetration rate a for NPLs and PLs are presented in **Figure 2**. Both c (2.81 ± 0.60 m/s) and a (1.19 ± 0.15 m/s) were initially higher in NPLs than PLs (c : 1.80 ± 0.31 m/s; a : 0.72 ± 0.17 m/s); the difference was 35.94% ($p \leq 0.001$) and 39.50% ($p \leq 0.001$), respectively. Based on the development of parameters over time, we identified three distinct phases: Phase I from the start of the first imaging experiment to 2 h pm; Phase II from 2 to 10 h pm; and Phase III from 10 to 15 h pm. In Phase I, an initial increase in both c and a was observed in both NPLs and PLs. While the increase in c did not differ significantly between the two groups, the increase in a was more pronounced in the PL group (c : 2.88 ± 2.82 %/h for NPLs vs. 4.45 ± 5.13 %/h for PLs, $p = 0.34$; a : 4.99 ± 5.99 %/h for NPLs vs. 9.78 ± 3.94 %/h for PLs, $p \leq 0.05$). In Phase II, both c and a were relatively stable over time in both groups. For c , a significantly higher increase was observed in PLs (NPLs: 0.65 ± 0.86 %/h vs. PLs: 1.58 ± 0.41 %/h, $p \leq 0.01$). The increase in a was similar in both groups with 2.85 ± 1.33 %/h in NPLs and 2.86 ± 0.66 %/h in PLs ($p = 0.98$). In Phase III, both c and a increased continuously over time without a significant difference between the two groups (c : 1.59 ± 1.91 %/h for NPLs vs. 1.59 ± 1.41 %/h for PLs, $p = 0.99$; a : 4.09 ± 3.07 % for NPLs vs. 3.75 ± 2.40 %/h for PLs, $p = 0.75$). Interestingly, the SD of a increased notably in both NPLs and PLs compared to Phases I and II (SD: NPLs: 0.13 ± 0.07 m/s, PLs: 0.06 ± 0.03 m/s for Phase I vs. NPLs: 0.17 ± 0.05 m/s, PLs: 0.08 ± 0.01 m/s for Phase II vs. NPLs: 0.39 ± 0.31 m/s, PLs: 0.11 ± 0.09 m/s for Phase III, all $p \leq 0.001$). The rate of parameters variation was calculated by comparing the two values at the beginning and the end of each phase taking the phase duration into consideration. The SD of each phase was obtained by averaging the SD of each

measurement point in the corresponding phase. All MRE results are presented in **Table 1**. The changes in MRE parameters over time are summarized in **Table 2**.

To analyze the influence of time after death and perfusion status on MRE parameters, mixed ANOVA analysis was performed separately for each of the three phases. First, in Phases I and II, the significantly higher values of c and a in NPLs than in PLs were found to be attributable to the factors time ($p \leq 0.001$) and perfusion status ($p \leq 0.001$) with a significant interaction between them (all $p \leq 0.01$). In Phase III, the two factors (i.e., time and perfusion status) continued to have a significant influence on both c and a , resulting in higher c and a values in NPLs than in PLs (all $p \leq 0.01$). Additionally, these two factors had independent effects on c and a with no significant interactions between them (c : $p = 0.90$; a : $p = 0.88$). All mixed ANOVA results are summarized in **Table 3**.

Diffusion-Weighted Imaging

As for MRE, we divided the period of DWI acquisition into different phases, based on group averaged apparent diffusion coefficient (ADC) curves over time as shown in **Figure 2C**. In addition to the three phases defined for MRE, an extended phase III, Phase III_ext, from 15 to 20 h pm was introduced due to the longer DWI acquisition interval. Based on initial ADC values, NPL samples demonstrated 26.08% lower water diffusivity than PL samples (NPLs: $0.46 \times 10^{-3} \pm 0.05$ mm²/s vs. PLs: $0.58 \times 10^{-3} \pm 0.07$ mm²/s, $p \leq 0.001$).

Contrary to MRE parameters, ADC values in Phase I decreased in both groups, and the decrease was more pronounced in the PL group (NPLs: -2.75 ± 1.57 %/h vs. PLs: -4.95 ± 2.77 %/h, $p \leq 0.05$). In Phase II, a 1.23 ± 1.17 %/h increase in ADC was observed in NPLs while the ADC was almost unchanged in PLs (0.09 ± 0.43 %/h, $p \leq 0.01$). A marked increase in ADC was found for both groups in Phase III and III_ext. While the increase was not significantly different between NPLs and PLs in Phase III (NPLs: 4.65 ± 0.94 %/h vs. PLs:

TABLE 2 | Main results of MRE, DWI and histological analysis in both non-perfused liver (NPL) and perfused liver (PL) samples at different phases and time points after death.

Method	Observation/parameter	Phase I		Phase II		Phase III	
		NPL	PL	NPL	PL	NPL	PL
MRE	c	↑	↑↑	↑	↑	↑	↑
	a	↑↑	↑↑↑	↑	↑	↑↑	↑↑
	ADC	↓	↓↓	↑	↔	↑↑	↑↑
		Fresh		2h post-mortem		10h post-mortem	
Histology	Hepatocyte organization	Tight	Loose	Loose, a few separated cells	Separated cells	Loose, separated cells	Separated cells
	Hepatocyte shape	Polygonal	Round	Round	Round + polygonal	Elongated	Elongated
	Cell wall visibility	High	High	Moderate	Moderate	Low	Moderate
	Glycogen amount	High	Low	Low	None	None	None

For the imaging parameters, relative changes are qualitatively indicated as increase (↑), decrease (↓), or unchanged (↔). The number of arrows indicates the degree of changes.

TABLE 3 | Summary of mixed model ANOVA results and initial values (means with SDs) in different phases during the acquisition interval in non-perfused liver (NPL) and perfused liver (PL) samples.

	Phase I			Phase II			Phase III			Phase III_ext		
	Initial value		Time Status Interaction	Initial value		Time Status Interaction	Initial value		Time Status Interaction	Initial value		Time Status Interaction
	NPL	PL		NPL	PL		NPL	PL		NPL	PL	
c	2.81 ± 0.60	1.80 ± 0.31	***	3.31 ± 0.45	2.03 ± 0.47	***	3.31 ± 0.45	2.32 ± 0.50	***	/	/	/
<i>in m/s</i>												
a	1.19 ± 0.15	0.72 ± 0.17	***	1.53 ± 0.15	0.90 ± 0.24	***	1.91 ± 0.31	1.16 ± 0.26	***	/	/	/
<i>in m/s</i>												
ADC	0.46 ± 0.05	0.58 ± 0.07	***	0.42 ± 0.04	0.53 ± 0.07	***	0.47 ± 0.06	0.53 ± 0.07	***	0.61 ± 0.05	0.65 ± 0.08	***
<i>in 10⁻³ mm²/s</i>												

NPLs (*n* = 13); PLs (*n* = 14); ns, non-significant; ***p* ≤ 0.01, ****p* ≤ 0.001.

3.50 ± 1.99%/h, *p* = 0.07), PLs exhibited a significantly higher increase than NPLs in Phase_III_ext (NPLs: 2.12 ± 0.69 %/h vs. PLs: 3.26 ± 1.22 %/h, *p* ≤ 0.01). All averaged ADC values are summarized in **Table 1**. Changes in ADC in the three phases are provided in **Table 2**.

Mixed ANOVA was also performed to assess the effects of perfusion status and time after death on ADC. In all four phases, time after death had a significant effect on ADC values between the NPL and PL groups (*p* ≤ 0.001) while the effect of perfusion status was only significant in Phases I, II, and III_ext (Phase I: *p* ≤ 0.001; Phases II and III_ext: *p* ≤ 0.01; Phase III: *p* = 0.24). The two factors considered in mixed ANOVA demonstrated significant interactions in all phases (*p* ≤ 0.001) except Phase I (*p* = 0.97). All results of mixed ANOVA are presented in **Table 3**.

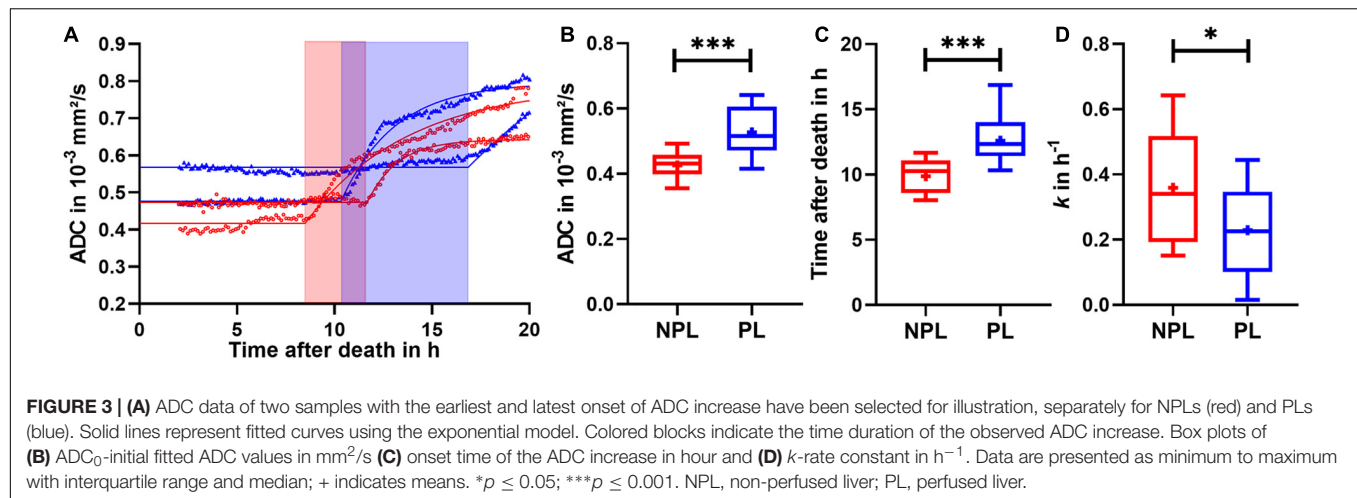
The ADC curves revealed a strong dynamic change during Phase III with different onset time between the NPL and PL groups. To better describe this change, exponential fitting as described in the Methods section was performed for each individual sample from Phase II to Phase III_ext.

In **Figure 3A**, the fitted curves for samples with the earliest and the latest onset of ADC increase in both NPLs and PLs are selectively shown. Comparison of the two groups showed the initial value, ADC₀, to be significantly higher in PLs than in NPLs (PLs: 0.53 × 10⁻³ ± 0.07 × 10⁻³ mm²/s vs. NPLs: 0.43 × 10⁻³ ± 0.04 × 10⁻³ mm²/s, *p* ≤ 0.001, **Figure 3B**). Secondly, the averaged onset time for the ADC increase in the NPL group was significantly earlier than in the PL group (NPLs: 9.88 ± 1.29 h vs. PLs: 12.59 ± 1.78 h, *p* ≤ 0.001, **Figure 3C**). Thirdly, rate constant *k*, which denotes the degree of ADC increase over time, was also significantly different between the NPL and PL groups (NPLs: 0.36 ± 0.17 h⁻¹ vs. PLs: 0.23 ± 0.13 h⁻¹, *p* ≤ 0.05, **Figure 3D**). The time range that covered the dynamic increase in ADC for all samples in the NPL group and the PL group was 3.19 and 6.53 h, respectively. All parameters obtained from model fitting were averaged for NPL and PL groups and are summarized in **Table 4**.

Histological Evaluation

Representative microscopic images of H&E-stained liver sections from PLs and NPLs at three time points are shown in **Figure 4**. The most prominent histological features of these samples can be described as follows: fresh NPL samples commonly displayed structural integrity with polygonal hepatocytes tightly organized in trabeculae. Additionally, a high amount of glycogen was present, seen as white spaces inside the cytoplasm of hepatocytes. Conversely, hepatocytes in fresh PL samples tended to appear roundish rather than polygonal. Although the trabecular arrangement was still visible, some hepatocytes already demonstrated detachment from the rest, resulting in a seemingly loosened packing. Furthermore, there was almost no glycogen visible in the fresh PL sample.

At 2 h pm, the histological appearance of the NPL sample was very similar to that of the fresh PL sample as described above, showing roundish hepatocytes which were loosely packed with no visible presence of glycogen. Comparatively in the PL sample, roundish hepatocytes displayed larger-scale separation and dissociation with almost no visible hepatocyte clusters. At



this time point, the nuclei appeared hyperchromatic in both NPL and PL samples, while the nuclei in the NPL sample additionally showed a slight size reduction.

At 10 h pm, the most prominent changes appearing in both the NPL and PL samples affected the shape and size of hepatocytes. In both groups, the formerly round hepatocytes observed at 2 h pm became elongated, taking on an ellipsoidal shape. Overall size of hepatocytes appeared to be smaller compared to 2 h pm. Additionally, cell walls appeared to become less distinct, making it harder to visually separate hepatocytes from one another. Furthermore, nuclei became smaller and exhibited signs of hyperchromasia and pyknosis. There was even evidence of karyorrhexis in the NPL sample. All histological findings are summarized in **Table 2**.

Hepatocytes per FoV at 40x magnification were counted at all three time points for both NPLs and PLs, and counts were further analyzed using the mixed ANOVA method. We found that the difference in cell counts between the NPL and PL groups was solely influenced by the time after death ($p \leq 0.01$) with no significant effect of perfusion status ($p = 0.98$, **Figure 5**). Mean cell counts for the two groups at the different time points were as follows: fresh, NPLs: 183 ± 41 vs. PLs: 189 ± 26 ; 2 h pm, NPLs: 189 ± 45 vs. PLs: 183 ± 24 ; 10 h pm, NPLs: 205 ± 56 vs. PLs: 204 ± 26 .

DISCUSSION

In this study, we investigated longitudinal variation of two biophysical properties, water diffusion and viscoelasticity,

of perfused (PLs) and non-perfused (NPLs) livers over a long post-mortem (pm) interval with a high sampling rate. Comparison of NPLs and PLs consistently revealed higher stiffness, better wave penetration, and lower water diffusivity in NPLs for all measurements. Furthermore, we observed a significant dependency of all imaging parameters in both groups on the time after death. These findings are summarized in **Table 2** and will be briefly discussed in the following paragraphs.

In both NPLs and PLs, the initial phase (Phase I) from immediately after death up to 2 h pm was characterized by the biological pattern of ischemia followed by hypoxia and anoxia, ultimately resulting in cytotoxic edema and hepatocytes swelling (Riede et al., 1976). It appears as if the enlarged hepatocytes created intracellular pressure and mechanical resistance (Bertalan et al., 2020) and formed a tightly packed and compact structure which mechanically manifested as increased stiffness and lower viscosity (i.e., higher wave penetration rate a). Furthermore, it is known from the literature that water mobility is restricted by cell walls, leading to diminished water diffusivity when extracellular water is shifted into the intracellular space (Le Bihan and Iima, 2015) e.g., during formation of cytotoxic edema. A similar finding of reduced ADC and increased stiffness was reported after induction of hypoxic-anoxic injury in the mouse brain (Bertalan et al., 2020). Arthurs et al. (2015) also observed a decrease in ADC values in the post-mortem liver. In addition to cytotoxic edema, blood coagulation could also contribute to higher stiffness and restricted water diffusivity (Berg, 1950; Clark et al., 1996; García-Manzano et al., 2001). The effect of coagulation should be more pronounced in NPLs than PLs; however, residual blood in PLs due to incomplete perfusion might also contribute to stiffening in NPLs. It is also worth mentioning that based on literature data, blood remains mostly fluid in the main vessels after sudden death (Mole, 1948; Berg, 1950; Haba et al., 1963; Takeichi et al., 1984, 1985, 1986; Jackowski et al., 2006; El alfy and Elhadidy, 2018), such as decapitation used in our study for the NPLs.

After the prominent changes observed in the initial phase, we identified a stable phase extending up to 10 h pm that was characterized by fairly small changes in stiffness, wave

TABLE 4 | Summary of parameters from model fitting of DWI results averaged for non-perfused liver (NPL) and perfused liver (PL) samples.

	ADC_0 —initial value in $10^{-3} mm^2/s$	T —onset time in h	K —rate constant in h^{-1}	ADC_p —plateau in $10^{-3} mm^2/s$
NPL	0.43 ± 0.04	9.88 ± 1.29	0.36 ± 0.17	0.68 ± 0.08
PL	0.53 ± 0.07	12.59 ± 1.78	0.23 ± 0.13	0.98 ± 0.35

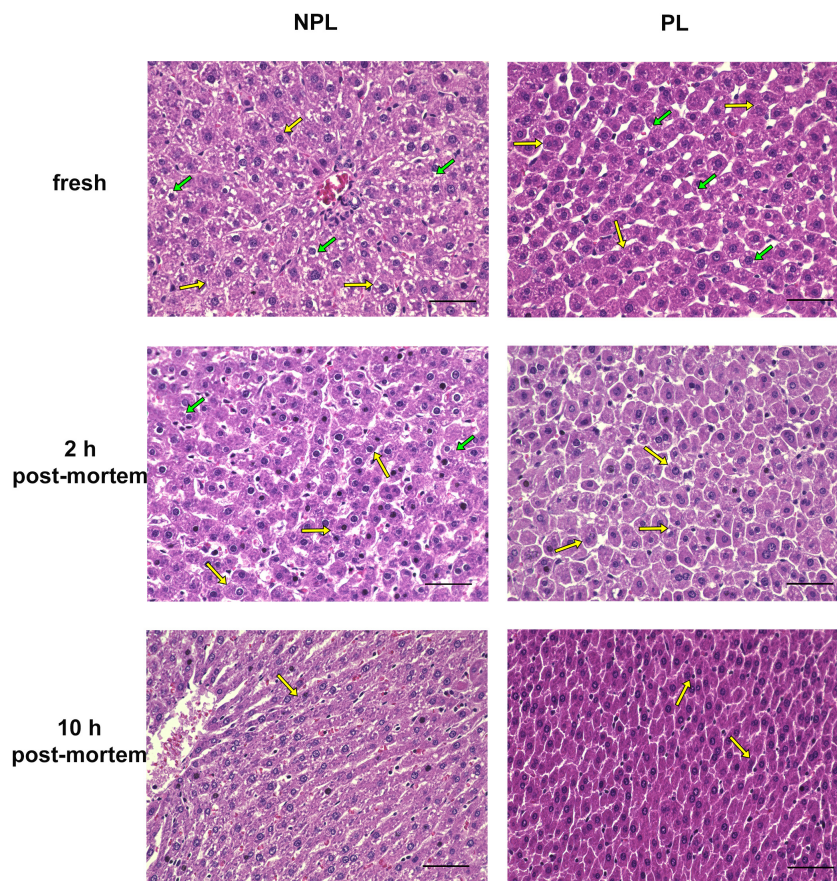


FIGURE 4 | Representative H&E-stained liver sections from NPLs and PLs collected immediately after death (fresh), 2 h post-mortem, and 10 h post-mortem. Scale bars correspond to 50 μ m. NPL, non-perfused liver; PL, perfused liver. Arrows indicate examples for: glycogen (green) and cell wall (yellow).

penetration and water diffusivity in both NPLs and PLs. In this phase, there were no biophysical signs of major disruption of tissue integrity or cell breakdown.

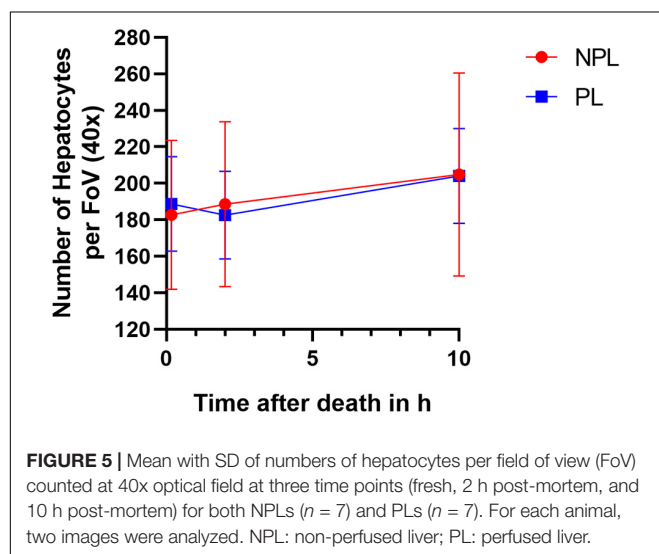


FIGURE 5 | Mean with SD of numbers of hepatocytes per field of view (FoV) counted at 40x optical field at three time points (fresh, 2 h post-mortem, and 10 h post-mortem) for both NPLs ($n = 7$) and PLs ($n = 7$). For each animal, two images were analyzed. NPL: non-perfused liver; PL: perfused liver.

After the stable phase, the penetration rate displayed a visible time dependency from 10 h to 15 h pm in both NPL and PL groups. Comparing histological images acquired at 2 h and 10 h pm, we suspected that the increase in penetration rate was a result of changes in cell shape with subsequent alteration in cell packing and reordering of the tissue lattice. In comparison to the fresh state, which was characterized by hepatocytes arranged in a tightly packed pattern, gradual elongation of hepatocytes over time seemed to create a more parallel-oriented packing arrangement. This arrangement of elongated hepatocytes appears to reduce intercellular friction, thus facilitating wave propagation. Similar to wave penetration rate, a significant increase in water diffusivity, indicating diminished restriction of water mobility, was observed from 10 h after death until the end of measurement. Considering that no decrease in stiffness was seen and cell walls were still visible in the histological images at 10 h pm, we hypothesize that the observed increase in water diffusivity is largely due to a gradual increase in cell membrane permeability rather than cell collapse and autolysis. Increased membrane permeability with a loss of membrane integrity is usually a process of tissue degradation which is followed by cell shrinkage and cell death (Majno et al., 1960; Kimura and Abe, 1994; Milroy, 1999; Yoon et al., 2002; Malhi et al., 2006;

Miller and Zachary, 2017; Yahia et al., 2018). Diffusivity started to increase earlier in NPLs, indicating faster tissue degradation in NPLs than PLs. Death induced anaerobic glycogenolysis during ischemia and anoxia produces a high amount of lactate, which leads to an acidic environment that is toxic to cells (Shima, 1922; Popper and Wozasek, 1932; Hertz, 1933; Nunley et al., 1973; Calder and Geddes, 1990; Donaldson and Lamont, 2013). Calder and Geddes (1990) reported the highest glycogen and lactate concentration in *ex vivo* livers of Wistar rats 30 min after death. Accumulation of lactate occurred in both NPLs and PLs in our study; however, due to perfusion, the high lactate-containing blood was replaced by PBS in PL samples, which apparently delayed the process of autolysis and tissue degeneration (Shima, 1922; Mukundan et al., 1986; George et al., 2016). We also found that the time window for observing such an increase in water diffusivity was narrower in NPLs than PLs, suggesting an inhomogeneity of residual blood remaining in PL vessels and leading to more heterogeneous tissue degradation in PLs. Additionally, the increase in cell membrane permeability and successive release of apoptogenic proteins and other small solutes might also function as intercellular lubricant that reduced friction and promoted wave propagation, as mentioned earlier. Since membrane permeability increases during tissue degradation, Phase III and the extended Phase III for DWI are considered to be degradation phases.

Despite the encouraging results, our study has limitations. First, no anticoagulant was used due to ethical restrictions in our study protocol. Therefore, we cannot rule out that some blood remained within the hepatic vessels. Second, the number of samples used for histological analysis was small so that the statistical power for cell counts was limited. Further studies investigating *ex vivo* tissue from other organs while controlling and varying factors such as medium osmolality, degree of cell hydration, and temperature levels are planned in order to gain deeper insights into autolysis and tissue decomposition and their biophysical manifestations.

To summarize, both MRE and DWI provided biophysical imaging parameters sensitive to structural alterations of rat livers during a cascade of biological events occurring after death. Throughout the 15-h pm interval investigated here, NPLs always showed higher stiffness, better penetration rate and lower water diffusivity than PLs. During this interval, three distinct phases were discernible - an initial phase with marked changes in biophysical properties up to 2 h pm, a stable phase up to 10 h pm, and a degradation phase characterized by significant increases in wave penetration and water diffusivity. As supported by histological results, these observed changes in biophysical parameters were closely related to variations in cell shape, cell packing patterns and cell wall integrity. Although obtained *ex vivo*, our results shed light on the individual contributions of vascular

components and cellular integrity to liver biomechanical properties that can be quantified by elastography. In future applications of elastography, our results might support the interpretation of the biophysical signature related to liver tissue degradation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Landesamt für Gesundheit und Soziales Berlin.

AUTHOR CONTRIBUTIONS

KG: conceptualization, data acquisition, investigation, formal analysis, data curation, writing—original draft, editing, and visualization. HT: software, validation, and writing—review and editing. AK: histological investigation, and writing, review and editing. SA, AH, ES, NB, and H-GH: writing—review and editing. JB: funding acquisition, resources, and methodology. IS: conceptualization, funding acquisition, resources, methodology, project administration, supervision, and critical revision of the manuscript. JG: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, visualization, supervision, writing—original draft, writing—review and editing, and critical revision of the manuscript. All authors fully qualify for authorship and have approved the final version of the manuscript.

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Fluids Alter Elasticity Measurements: Porous Wave Propagation Accounts for Shear Wave Dispersion in Elastography

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In shear wave elastography, rotational wave speeds are converted to elasticity measures using elastodynamic theory. The method has a wide range of applications and is the gold standard for non-invasive liver fibrosis detection. However, the observed shear wave dispersion of *in vivo* human liver shows a mismatch with purely elastic and visco-elastic wave propagation theory. In a laboratory phantom experiment we demonstrate that porosity and fluid viscosity need to be considered to properly convert shear wave speeds to elasticity in soft porous materials. We extend this conclusion to the clinical application of liver stiffness characterization by revisiting *in vivo* studies of liver elastography. To that end we compare Biot's theory of poro-visco-elastic wave propagation to Voigt's visco-elastic model. Our results suggest that accounting for dispersion due to fluid viscosity could improve shear wave imaging in the liver and other highly vascularized organs.

Keywords: liver characterization, shear wave elastography, poroelasticity, ultrasound imaging, viscoelasticity, biot theory, shear wave dispersion, voigt model

1 INTRODUCTION

Shear wave elastography is a fast, non-invasive method to estimate tissue elasticity *in vivo*. Its first clinical application has been the estimation of liver fibrosis, and it is more and more used as a replacement for liver puncture, the current gold standard. The method is quantitative through *in situ* measurement of the shear wave speed by ultrasonic or magnetic resonance imaging. An estimate of tissue elasticity is retrieved through inversion of the wave speed. Clinical applications as of today include ultrasonic 1D elasticity estimation (FibroScan, Echosens, Paris, France), ultrasonic 2D elasticity mapping (e.g., Aixplorer, SuperSonic Imagine, Aix-en-Provence, France; GE Logiq, Boston, United States; Canon Aplio, Canon Medical Systems, Tochigi, Japan) and magnetic resonance 2D and 3D elasticity mapping (e.g., General Electric/Philips/Siemens Healthcare). Linear elastic or visco-elastic theory is commonly assumed for the elasticity inversion. However, the order of magnitude of the shear modulus retrieved from elastography and other measurement methods, such as dynamic mechanical analysis (DMA), differs [1]. Furthermore, *ex vivo* and *in vivo* elastography measurements vary, making a standardization difficult [2, 3]. Finally, the observed *in vivo* shear wave velocity dispersion with frequency of the liver is badly predicted by visco-elastic theory, and recent *in vivo* studies [4, 5] challenge the commonly assumed correlation of viscosity with fibrosis [6]. As of today questions remain concerning the physical processes behind the observed dispersion. This ambiguity has led recent studies (e.g., [7]) to drop the rheological model in favor of a model-independent

dispersion slope as the “viscous” parameter. Our work attempts to explain the observed discrepancies between theory and *in vivo* elastography data by considering the liver as a porous biphasic medium, which is described by Biot theory, instead of a homogeneous visco-elastic solid. The introduction of a fluid phase introduces a new loss mechanism, the viscosity of the fluid, as well as a change in the effective density of the solid part. Proper physical modeling of the of the shear wave dispersion is key to a quantitative elasticity reconstruction and ultimately might reduce false positives and negatives in elastography liver characterization. We postulate that to properly account for the observed shear wave dispersion in the liver, porous theory needs to be taken into account. This hypothesis is based on our experimental results in a porous phantom and theoretical considerations which are compared to existing *in vivo* data. The paper is structured as follows:

In **Section 1.1** we report various approaches to model the liver as a porous material. Publications in elastography which regard the liver as a poro-elastic Biot medium are scarce and consequently the investigated literature spans includes from anatomy, dynamic mechanical analysis, surgery simulation, tissue engineering and numerical fluid dynamics. In **Section 3.1** we confirm the results of [8] who found that the shear wave dispersion in a soft porous material is described by poro-elastic wave propagation theory. This is unambiguously shown by comparing the shear wave dispersion in a soft porous phantom saturated by a Xanthan solution with the dispersion of the same phantom saturated by water. The observed results are exclusively explained by poro-visco-elastic theory, which is introduced as Biot’s theory in **Section 2.1.1**. Furthermore, a more intuitive interpretation of Biot’s theory is given in **Section 2.1.2** and the experimental results are compared to Biot’s model in **Section 3.1.1**. The experimental results are followed by analytic modeling of the liver as a porous material in **Section 3.2**. We use literature values for the poro-visco-elastic parameters (**Section 2.6.1**) to compare the theoretical dispersion of a model liver with previously reported *in vivo* shear wave dispersion measurements (**Section 4.2**). The analytic modeling shows that changes in the porous parameters can account for measured variations in shear wave speed that visco-elasticity fails to explain (**Section 3.2.1**, **Section 4.2.1**). We conclude by comparing Biot theory with commonly used rheological models in **Section 4.3.1**. Finally, we present challenges for future research in **Section 4.3** and a common conclusion in **Section 5**.

1.1 The Liver-A Sponge?

Recently, we have shown experimentally that Biot’s theory of poro-elastic wave propagation well predicts the shear wave dispersion in a melamine sponge [8]. In Biot’s theory of poro-elastic wave propagation shear wave dispersion is caused by the viscosity of the fluid saturating the connected pore space. Several organs that are of interest to elastography imaging have been described as porous materials, e.g., the lung, spleen, brain and liver. The liver is the foremost organ to be clinically imaged using shear wave elastography. Here, it is usually modeled as an elastic or visco-elastic solid. However, fresh liver grafts clearly show the

presence of at least one liquid phase: the blood. In fact, the liver is one of the blood-richest organs and has previously been depicted as a fluid-filled sponge by several research groups [9–11]. Together with other organs, such as the spleen, the liver serves as a specific blood reservoir of the body [12]. It is thus justified to regard it as a soft porous material saturated by a viscous fluid. Furthermore, blood saturation or liver perfusion and blood viscosity are not constants. Both vary among individuals as well as with time for each individual. Perfusion and viscosity are known to be dependent on many factors such as hydration, diet, health and physical fitness. Liver diseases do not only alter the elasticity of the cellular matrix of the liver but are known to affect the porous parameters, notably porosity and permeability, as well. These insights led different groups to numerically model the liver as a poro-elastic or poro-visco-elastic material in the last decade [1, 9, 13–19]. However, albeit early realization of interstitial fluid flow as an dissipative mechanism for acoustic waves [20], few attempts to link the shear wave dispersion in the liver to its porous and fluid properties have been made to our knowledge. Some recent studies investigated the influence of pore pressure on shear wave speed in the liver [21–23], and [24] describes a 2-parameter pore channel flow model for shear wave dispersion. The first group focuses on pressure effects. The second develops a model that incorporates flow in the constitutive equations, similar to Biot’s first porous theory, the theory of consolidation [25], and to earlier work in compression elastography [26]. Recently [27], model the liver as a hyper-poro-visco-elastic but focus on hyper-elastic deformation and the fluid pressure effect on the shear wave velocity by assuming low porosities and permeabilities. The effect of the viscous fluid on the dynamic wave propagation however, as developed by [28–31] and presented in **Section 2.1.1**, seems to have been overlooked by the ultrasound elastography community. Hence, the application of Biot theory to ultrasound shear wave elastography seems overdue.

2 MATERIALS AND METHODS

2.1 Porous Wave Propagation Theory

2.1.1 Biot Theory

A non-phenomenological theory that models porous wave propagation based on the physics of fluid-solid interaction is Biot’s theory of poro-elastic wave propagation [28, 29]. Biot’s shear wave dispersion is governed by the characteristics of the porous structure and the pore filling fluid. As a consequence, the expression for the wave speed differs significantly from that of a purely elastic monophasic wave propagation model. Instead of a single homogeneous density, the model takes into account the fluid density ρ_f and the density of the solid frame ρ_{fr} , which is expressed in terms of bulk density of the solid material ρ_s and the porosity ϕ as $\rho_{fr} = \rho_s (1 - \phi)$. The mean density is expressed as $\bar{\rho} = \rho_s (1 - \phi) + \rho_f \phi$. Furthermore, the model accounts for inertial and viscous coupling between the displacements of the two phases.

The consideration of viscous effects through the fluid viscosity η_f leads to the frequency dependence of the shear wave speed,

which is expressed in terms of wavenumber and angular frequency:

$$v_s = \frac{1}{\Re \sqrt{\left(\frac{\omega_s}{k_s}\right)^{-2}}} \quad (1)$$

$$\left(\frac{\omega_s}{k_s}\right)^{-2} = \frac{q\tilde{\rho} - \rho_{fl}^2}{q\mu_{fr}}, \quad (2)$$

where μ_{fr} is the shear modulus of the foam. The term q can be expressed in terms of the solid, fluid and porous parameters as:

$$q = \frac{\alpha\rho_{fl}}{\phi} + \frac{i\eta F(\zeta)}{\omega_s\kappa}, \quad (3)$$

where κ is permeability, α is tortuosity and $F(\zeta)$ expresses the frequency dependence of the viscosity, or the deviation from Poiseuille flow [32]. The permeability is measured in m^2 and is a measure of fluid transmission through a sample. It is defined as the proportionality constant in Darcy's law of the volumetric flow rate. The tortuosity takes into account the pore shape. It is generally assumed for cylindrical pores and is evaluated as the total length of the pore divided by the shortest path connecting its ends or in other words: "the ratio of the arc length of a curve relative to its end-to-end distance" [16]. It also accounts for the inertial coupling of the solid and fluid phase, where inertial coupling signifies that an accelerating solid drags parts of the surrounding fluid with it [33]. The solid moving in the fluid thus acquires an additional mass due to the deflection of the surrounding fluid volume, which can be seen as a renormalization process (see [34] 1.3.4). The virtual mass of the solid is thus $\rho_{virt} = \rho_{fr} + \rho_a = (1 - \phi)\rho_s + \rho_a$, where the additional or apparent mass is calculated as $\rho_a = (\alpha - 1)\phi\rho_{fl}$.

The derivation from first principles and a detailed description of Biot's theory are beyond the scope of this work and can be found in the cited references as well as several textbooks, e.g., [35–37]. In order to follow our reasoning we find it however helpful to give an intuitive interpretation that is accessible to the reader who was unfamiliar with poro-elastic theory before reading the present manuscript.

2.1.2 Loss Mechanism and Significance of Biot's Theory

When compared to linear-elastic or visco-elastic theory some results of Biot's theory might seem counter-intuitive. We can however understand the complex inertial and viscous effects of the fluid in terms of classic elastic theory by looking at the extreme ends of the dispersion curves when $\omega \rightarrow 0$ and $\omega \rightarrow \infty$. If we consider that the shear modulus μ_{fr} stays constant with frequency and we want to find an equivalent to Biot's wave speed in terms of the elastic shear wave speed expression $v_s = \sqrt{\frac{\mu}{\rho}}$, we immediately see that the density ρ needs to be replaced by an effective density ρ_{eff} ($\omega \rightarrow 0$) and ρ_{eff} ($\omega \rightarrow \infty$). At very low frequency, when $\omega \rightarrow 0$ Hz, the solid and the fluid phase are locked together through viscous coupling. This signifies that due to the viscous drag they move in phase without relative movement between the two. The wave speed is then defined

by the true shear modulus of the solid and an effective density made up of the densities of the solid and the fluid phase ($\rho_{eff} = \tilde{\rho} = \rho_s(1 - \phi) + \rho_{fl} * \phi$). In the case of biological tissues, those densities are approximately equal and the low-frequency limit is thus the velocity which we would have calculated using linear elasticity or visco-elasticity and the true shear modulus. At very high frequencies, when $\omega \rightarrow \infty$ Hz, which is conceptually similar to the case when the porous material is saturated by a superfluid, the shear wave velocity reaches its maximum value. Because of the lack of viscosity, the fluid is not dragged along by viscous coupling and rests on average¹. The fluid and solid displacements are thus out-of-phase and the shear wave experiences a different effective density: the solid frame density plus an apparent density due to the remaining added mass effect of the fluid. The added mass effect stems from the fact that the fluid is considered to be at rest on average, while there is relative movement between the accelerating solid and the fluid phase (see [38] I, 11). Ignoring the inertial coupling described in ¹, the expression for the effective density is then: $\rho_{eff} = (1 - \phi)\rho_s + \rho_a$. The higher the porosity of the medium, the lower the solid frame density and consequently due to $v_s = \sqrt{\frac{\mu}{\rho_{eff}}}$, the higher the shear wave speed in the high-frequency limit. Even for biological tissue, where fluid and solid densities are very similar, the shear wave speed at higher frequencies differs thus strongly from the elastic shear wave speed. While the idealized viscous-less case defines the high-frequency limit of the shear wave velocity and the phase-locked case defines the low frequency limit of the shear wave speed, it is obvious from the gap between the two values that a different physical mechanism is required to explain the evolution from low to high frequency. This is done through fluid viscosity, which introduces dissipation and defines the form of the dispersion curve in between the low and high frequency limits. Eqs 2, 3 account for the frequency dependence: $\tilde{\rho}$ is scaled by the factor q which is a frequency dependent function of the fluid viscosity. To stay within the effective density model, the term $q\tilde{\rho}$ could be interpreted as an effective density which is given through the frequency dependence of $q(\omega)$.

2.2 Visco-elastic Wave Propagation

In Section 3 we compare the poro-visco-elastic model to a simple visco-elastic model. The shear wave speed of the visco-elastic model is calculated using Voigt's model:

$$\mu = \mu_0 + i\omega\mu_1 \quad (4)$$

$$v_s = \sqrt{\frac{\mu}{\rho}}, \quad (5)$$

with μ_0 being solid elasticity and μ_1 being solid viscosity. In the present work we explicitly make the choice to compare Biot's model only to a simple visco-elastic model. Viscosity is still not

¹This is an oversimplification, in Biot's theory some rotation, which diminishes the added mass, is induced in the fluid by inertial coupling. The rotation is included in the calculation of the effective density by multiplying it with $(1 - \frac{\rho_{fl}^2}{(1-\phi)\rho_s + \rho_a})(\phi\rho_{fl} + \rho_a)$

widely inferred for in clinical practice [39] and most *in vivo* studies employ a Voigt model with limited success [7]. As a consequence, a model independent measure of the dispersion slope has been proposed as diagnostic value [7] and more complex visco-elastic models, such as Zener's model are being investigated [7, 40]. However, these include more springs and dampers and require complex inversions. One key difference between viscous and porous models is: the porous parameters in Biot's model are physical parameters that can be measured independently or determined from physical considerations [40], while the visco-elastic model is phenomenological. Hence, if we were to measure the porous and fluid parameters independently of shear wave elastography, the Voigt and Biot model would exhibit the same number of free parameters, the solid elasticity and viscosity. Note that [40] finds an equivalence of the Zener and Biot model for low frequency shear waves. However, this does not hold for deviations from Poiseuille flow and Zener's model does not explain the secondary longitudinal wave recently shown in a soft porous tissue [8].

2.3 Parameter Inversion

In order to fit the experimental data to the Biot and Voigt model we perform a constraint global minimization using the BasinHopping algorithm [41] of the Python library Scipy. The global method was chosen instead of a local method to avoid local minima in the optimization. In short, the BasinHopping algorithm performs a multitude of local minimizations through the L-BFGS-B (Broyden-Fletcher-Goldfarb-Shanno) algorithm, which is a quasi-Newton method [42]. For each minimization it uses varying starting values and keeps or rejects the result. In **Section 3** all minimizations are performed on the least squares [42] of the experimental and analytic shear wave speed of the investigated frequency range:

$$\epsilon = \sum_{i=1}^N \frac{[v_{exp}(\omega_i) - v_{sim}(\omega_i)]^2}{N}, \quad (6)$$

where N is the number of frequencies at which the shear wave speed was measured, v_{exp} is the experimental phase velocity, v_{sim} is the analytic phase velocity and ω_i is a experimentally measured frequency. **Eq. 2** is used to calculate the poro-visco-elastic shear wave speed v_{sim} . The shear wave speed v_{sim} of the visco-elastic model is calculated using Voigt's model from **Eq. 5**. The bounds for the different minimizations performed in **Section 3** are listed in **Table 3**.

2.4 Penetration Depth

The penetration depth is comparable to the skin depth in electromagnetics and relates the mathematical model outcome of any attenuation model to physical wave propagation (see [40] 2.3.2.2). For example, a model with an elasticity $\mu_0 \rightarrow 0$ and viscosity $\mu_1 \gg \mu_0$ will result in a calculated wave speed $v_s > 0$ but a penetration depth $\delta_{skin} \rightarrow 0$. Hence, the wave is a non-propagating one. We use the penetration depth as a physical constraint on the feasibility of a proposed model with:

$$\delta_{skin} = \frac{\omega}{2\pi v_s \alpha_s}, \quad (7)$$

where α_s is the shear wave attenuation, v_s is the shear wave speed and ω is the frequency for which the physicality of wave propagation is investigated. We classify a model as being physical if $\delta_{skin} > 0.5\lambda$. The theoretical penetration depth separating a diffusion from a propagating wave is lower (see [40] 2.3.2), but we judge time of flight and phase velocity measurements on less than half a wavelength as unreasonable in shear wave elastography because near-field effects are likely to dominate the wave-field [43, 44].

2.5 Shear Wave Propagation in a Foam

2.5.1 Experiment

Similar to [8] we saturate a rectangular Basotect® melamine resin foam and analyze the shear wave dispersion through the phase velocity measurement of a frequency sweep. Robust results are achieved through ultrafast shear wave imaging of plane shear waves inside the foam. **Figure 1** shows the experimental setup: The foam of dimensions $30 \times 18 \times 12$ cm is immersed in a water tank. A metal rod driven by a piston (ModalShop Inc. K2004E01) excites shear waves in the frequency range from 100 to 250 Hz. The chosen frequency range ensures sufficient wave propagation and avoids guided waves which exist at lower frequencies due to the finite sample size. The resulting wavefield is imaged in the $x-z$ plane using shear wave elastography. Ultrasonic plane waves are emitted orthogonal to the direction of shear wave propagation at 4,000 fps using a Verasonics Vantage™ scanner and a Philips L7-4 probe. Phase-based motion estimation [45] of the ultrasound reflection images results in retrieval of the z -component of the particle velocity throughout the imaging plane. Because the shear wave speed in the sample is two orders of magnitude lower than the ultrasonic wave speed, the particle displacement induced by the shear wave propagation is well resolved in time. The shear wave excitation is exemplary shown in **Figure 1A**) and the imaging principle in **Figure 1B**). Because the wave excitation source is extended in z , the resulting wave front is planar, which is visible in the insets in **Figure 1**. Hence, we sum the particle velocities along the z -axis in order to increase Signal-to-Noise Ratio. The resulting 2D space-time wavefield is then Fourier transformed using Matlab's™ FFT in order to measure the phase velocities of the frequency sweep. Because a plane wave evolves according to $e^{i(kx-\omega t)}$, the phase of a single frequency evolves linear in space. The phase velocity can thus be measured from the linear fit of the phase along the spatial dimension for each frequency. The phase is fitted using the Random Sample Consensus (RANSAC) algorithm [46, 47] adapted from [48]. The benefit of the RANSAC algorithm lies in defining a set of inlier points which are taken into account for slope estimation and a set of outlier points which are disregarded. We employ a coefficient of determination (R^2) larger than 0.98 and 70% inliers for each frequency. With $\phi_{meas} \in \text{Inlier}$, the phase velocity is thus measured as:

$$\phi_{meas}(\omega) = \beta x + C \quad (8)$$

$$v_{s\phi(\omega)} = \frac{\omega}{\beta}, \quad (9)$$

where ϕ is the phase, β is the linear slope of the phase, x is the coordinate of the spatial dimension, C is a Constant and ω is the

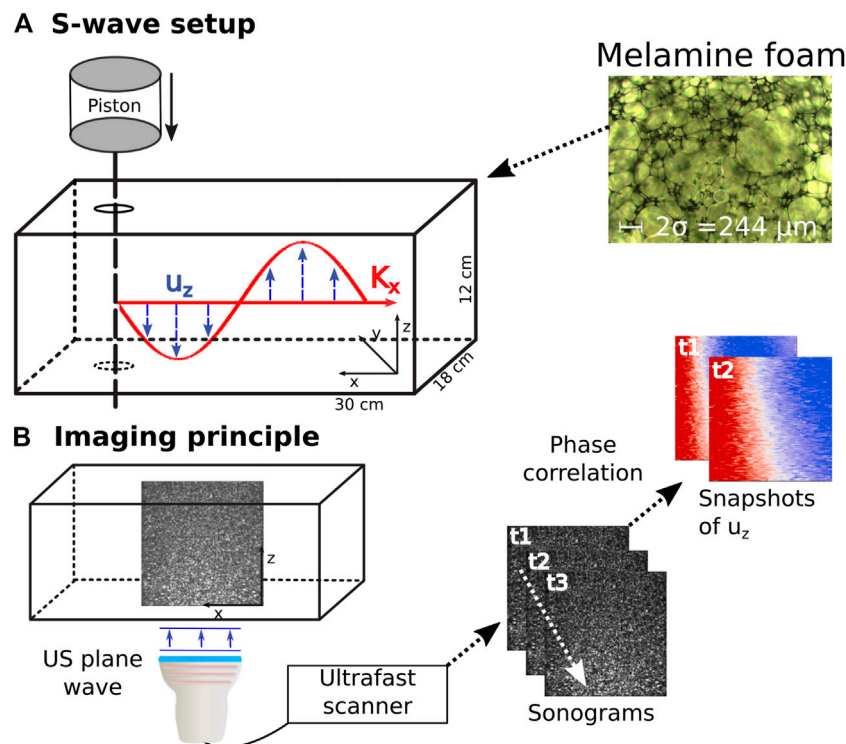


FIGURE 1 | Experimental setup. **(A)** Shear waves are excited in a water saturated melamine foam through a frequency sweep of a metal rod. The inset shows a microscopic image of the melamine foam used for the experiment. **(B)** Schematic sketch of the imaging principle in ultrasonic shear wave elastography. 2D snapshots of the plane wave ultrasonic reflection images are acquired at a high pulse repetition frequency. Particle movement of the shear wave induces changes in the sonograms and is retrieved through phase correlation. u_z is displacement along z , K_x is the wave vector in propagation direction. US is the abbreviation for ultrasound, t signifies timestep and σ is the viscous length.

frequency investigated. In contrast to our previous work reported in [8], we perform two experiments that differ only in the viscosity of the saturating fluid. For the high viscosity fluid we choose a 0.1% solution of Xanthan (E415) in water and measure the dispersion at 40°C, where 40°C ensures a good mixing of Xanthan with water. For the low viscosity fluid we measure the dispersion in water at 40°.

2.5.2 Poro-Visco-Elastic Dispersion Modeling in Melamine Foam

The Biot parameters of the foam were independently measured using the acoustic impedance tube method [49] and a Johnson-Champoux-Allard-Lafarge model [49–52] for [8]. For the different methods detailed in [49], the porosity ϕ was found between 96.7 and 99.7%, the tortuosity α between 1 and 1.02 and the permeability κ between 1.28×10^{-9} and $2.85 \times 10^{-9} \text{ m}^2$. The viscous length σ is between 11.24×10^{-5} and $13.02 \times 10^{-5} \text{ m}$ as indicated in the microscopic photo at the top of Figure 1, and the density is $8.8 \text{ kg m}^{-3} \pm 1 \text{ kg m}^{-3}$. The viscous length is typically employed in modeling air filled porous materials and not needed in the classical Biot model. The other quantities were fixed to $\phi = 99\%$, $\kappa = 1.276 \times 10^{-9} \text{ m}^2$ and $\alpha = 1.02$.

The elastic parameters are unknown and are likely to have been altered since the experiments performed by [8] on the same foam sample due to degassing, compression, immersion in water

and aging. To investigate if Biot's theory can explain the observed dispersion we perform a joint least squares inversion [53] on the unknown parameters μ_f (shear modulus of the foam frame) and η_f (fluid viscosity). To account for viscosity of the foam itself we introduce the complex shear modulus $\mu_{fr} = \mu_0 + i\omega\mu_1$, where the range for μ_1 is taken from the values given for melamine in [54]. This extended Biot theory, including solid relaxation mechanisms, has been described by Biot [31], and shown to be consistent with the Kramers-Kronig relations by Turgut [55]. We jointly invert using Eq. 6 and Python's Scipy package [56] as detailed in Section 2.3. Because we use the same foam sample at equal temperature in both experiments, μ_{fr} is constrained to be identical for both experiments. In contrast, the fluid viscosity is bounded to 0.1–100 mPas for the Xanthan experiment and fixed to 0.6 mPas (viscosity of water at 40°) for the foam in the water experiment.

2.6 Shear Wave Propagation in the Liver

2.6.1 Porous Parameter Values of the Liver

The liver can be roughly separated into hepatocytes, blood vessels, bile ducts and space of disse or perisinusoidal space. The latter is part of the interstitial space and contains extracellular fluid. The blood vessels follow a hierarchical tree-like structure with the central vein and portal arteriole at the top and the liver sinusoids at the bottom. The meso- and micro-circulation are exemplary

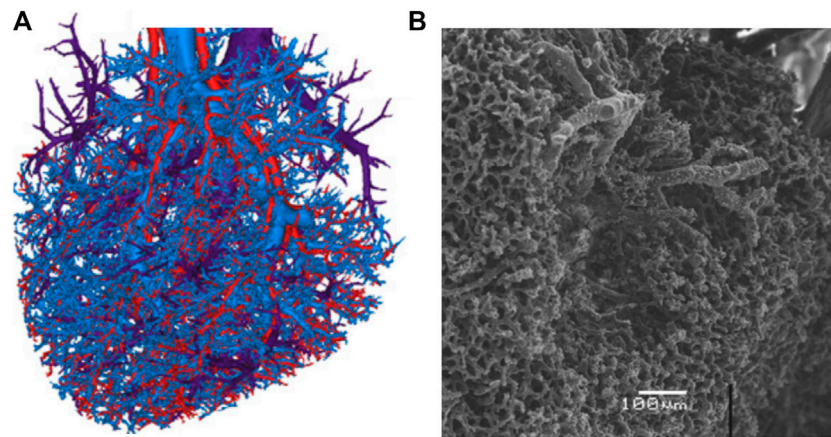


FIGURE 2 | Visualization of the liver vasculature as presented by [57]. The blood vessels, present at all scales, constitute the main source of porosity in our poro-visco-elastic liver model. **(A)**. Reconstruction of three mesovascular trees (hepatic artery, portal vein and hepatic venous system) from liver vascular corrosion casts. Reproduced with permission from Fig. 2 e) in [57]. **(B)**. Scanning electron microscopic image of the sinusoidal microcirculation. Reproduced with permission from Fig. 1 c) in [57].

show in **Figure 2**, which is reproduced with permission from [57], and are gained from a liver corrosion cast. The macro-, meso- and microscopic structures exhibit different scales of porosity and permeability. While the portal vein for example can be seen as a tubular conduct with a very high Darcy permeability (1×10^{-3} – $1 \times 10^{-2} \text{ m}^2$ [58]), the permeability of the sinusoidal space has been modeled with permeabilities as low as $1.56 \times 10^{-14} \text{ m}^2$ and the interstitial space with $3.12 \times 10^{-17} \text{ m}^2$ [13, 59]. Most studies reporting liver permeability values take a microstructure approach, investigating single liver lobules or the permeability across vessel walls. A more general approach was taken by [17], who modeled a 3D liver using a tissue permeability of 1.44×10^{-9} – $2.5 \times 10^{-9} \text{ m}^2$ and a vascular permeability of $5.0 \times 10^{-9} \text{ m}^2$. Here, tissue permeability is the permeability derived from the sinusoids. A permeability of $1.4 \times 10^{-9} \text{ m}^2$ for an avascular model was also found by [60] for a poro-visco-elastic liver model. These relatively high values are not surprising, considering that the blood flow into the liver sinusoids equals about 1.35 Lmin^{-1} at a pressure drop of 1.2 kPa [12]. As is the case of the permeability, care has to be taken when comparing literature values for porosity. Considering only sinusoidal porosity, [61] find $7.4 \pm 1.1\%$ while [13, 62] state values ranging from 14% for a healthy to 24.6% for a cirrhotic liver. According to [10], total liver porosity values range from 50 vol% to 70 mass% of total free fluid, including blood, water and bile.

These porosity and permeability values are higher than those used recently by [27] in a poro-hyper-visco-elastic model. They base their consideration on the values by [13], who modeled micro-circulatory or sinusoidal permeability based on hexagonal liver lobules. We believe however, that the sinusoidal permeability is not the relevant quantity for shear wave elastography. The region of interest for any liver elastography method is in the centimeter range and far exceeds the scale of a liver lobule (0.15^3 mm^3 in [13]). Therefore, one should take into account mesocirculation and potentially macrocirculation for proper modeling of the permeability and porosity in shear wave elastography.

2.6.2 Variability of Porous Parameter Values

For the average adult the normal blood volume in the liver equals about one third of its mass [63] or 450 ml. However, experiments in cats have shown that the liver can account for up to 400 ml of additional blood per kg of liver and rapidly expel half of the stored blood from the liver [63, 64]. Concordantly, [12] states that the human liver can occasionally store up to 0.5–1 L of additional blood in the hepatic vein and sinusoids. Using ultrasonic measurements [65] found mean diurnal variations of 17% in human liver volume. Recently [66], found that mice liver volume oscillates with the feeding and circadian cycle, gaining and loosing 34% each day. Furthermore, fibrosis and cirrhosis have been shown to change the capillarization and thus the porous microstructure of the liver [67–69]. For harmonic ultrasound elastography, [70] found that water uptake leads to a change in the hepatic shear wave velocity. They conclude that the shear wave speed is most likely related to a change in vascular load after water intake. These blood volume variations on short timescales for a single organism as well as pathologic changes due to liver disease influence the shear wave speed as predicted by poro-visco-elastic theory. They notably alter the porosity and permeability parameters in Biot's model. Furthermore, the higher the porosity, the more Biot's model becomes sensitive to the fluid viscosity. Our research revealed few studies reporting the tortuosity of the liver pore space. For rat liver sinusoids it was found to be 1.04–1.11 by [71].

The above cited studies give the range of available literature values for the porous parameters of the liver and will serve as input to the analytic modeling of the liver as a poro-visco-elastic solid in **Section 3.2**. **Table 1** summarizes the porous and elastic parameter values reported for the liver.

2.6.3 Permeability Calculation

The permeability of a porous sample with tubular pores of a single fixed radius r is given as [79]:

$$\kappa = \phi \frac{r^2}{8}. \quad (10)$$

TABLE 1 | Literature values for the poro-elastastic parameters of the liver. ϕ is porosity, κ is permeability, η is viscosity, α is tortuosity, E is Young's modulus and ν is Poisson's ratio. The different groups focus on different applications and use different approximations and assumptions, partly explaining the large variability of values. The most crucial point when referring to literature values with regards to elastography is if the microcirculation only or the meso- and macro-circulation are considered. The liver lobule model seems to focus on single lobules and micro-circulation in most of the literature we reviewed. For detailed description of measurements, approximations and error estimations, please see the provided references.

Source	Type	Type	ϕ [%]	κ [m ²]	η [Pa]	α []	E [Pa]	ν []
White et al. [17]	3D Virtual liver	Vascular space	29.8	$1.4\text{--}2.5 \times 10^{-9}$	3.5×10^{-3} []			
Rattanadecho and Keangin [72]	FEM model	Vascular space	60–70	$7.7 \times 10^{-11}\text{--}2.2 \times 10^{-10}$				
Debbaut et al. [13]	CT based 3D model	3D Sinusoidal	14.3	$7.0 \times 10^{-16}\text{--}1.1 \times 10^{-14}$				
Peeters et al. [62]	CT based 3D model	3D Sinusoidal	24.6	$1.1 \times 10^{-14}\text{--}7.7 \times 10^{-13}$				
Siggers et al. [59]	Lobule model	Interstitial space		3.1×10^{-17}	1.8×10^{-3}			
Bonfiglio et al. [73]	Lobule model	2D Sinusoidal	12	$7.0 \times 10^{-16}\text{--}3.3 \times 10^{-13}$	4.0×10^{-3}		1230 ^a	0.43–0.47 ^a
Komatsu et al. [71]	<i>In vivo</i> fluorescence microscopy	Sinusoidal (rat)				1.04–1.11		
Meyer [58]	Endoscopic Ultrasound	Portal Vein		$5.5 \times 10^{-5}\text{--}1.3 \times 10^{-3b}$				
Jang et al. [75]	Viscometer and Fibroscan	Healthy, Fatty, Cirrhotic human liver			$3.3 \times 10^{-3}\text{--}2.2 \times 10^{-2}$		2,669–76,497	
Rand et al. [76]	Viscometer	Human hematocrit 20–80°			$3.9 \times 10^{-3}\text{--}5.3 \times 10^{-2}$			
Kerdok [2, 10]	Porcine <i>ex vivo</i>	Perfusion test	50–70		$3.9 \times 10^{-3}\text{--}5.3 \times 10^{-2}$		40–116 ^d	
Moran et al. [60]	Porohyperelastoc Model	Parenchyma, avascular	20		1.4×10^{-9}		541.6	0.35
Lautt [63, 64]	Base value estimation ^e	Blood	20–35					
Greenway and Stark [77]	Base value estimation	Blood + Interstitial	37					
Chatelin et al. [78]	DMA	Porcine <i>In vitro</i>					325.7–1,323.7	
Chatelin et al. [78]	Fibroscan	Porcine <i>In vivo</i>					1,200–2,300	
Marchesseau et al. [9]	Rheometry	Porcine liver samples					300–900	
Wex et al. [3]	Rheometry	Porcine <i>Ex vivo</i> , time dependant					128–316 ^f	
Hall and Guyton [12]	Normal value	Blood volume	30 ^g					
Author's calculation	Based on corrosion cast by [57, 59] ^h	Macro- Meso- and Micro-circulation	0.35	2×10^{-9}				
Lautt [64]	Venous pressure change in cats	Additional blood storage	+400 ml					
Lautt [64]	Sympathetic nerve action	No liver function impairment	-50–60% of normal blood volume					
Hall and Guyton [12]	Backpressure in human liver	Additional blood storage	+0.5–1 L or up to 60% ⁱ					
Leung et al. [65]	Mean diurnal changes	Total human liver volume	±17% (Volume)					
Sinturel et al. [66]	Mice sections	Total liver volume	±34% (mass)					
Ipek-Ugay et al. [70]	Time harmonic elastography (human)	Fasting and water ingestion					±11% (of shear wave speed)	

^aBased on Chui et al. [74], who measured in pig livers. Note that the Poisson's ratio employed in our calculations is a property of the frame. It might this be slightly different from Chui's.

^bCalculated from flow speed and geometry in Meyer [58] using Darcy's law.

^cRepresents the extreme values of Hematocrit for human blood.

^dLinear elastic shear modulus for liver parenchyma.

^eThe authors state that large volume changes are observed (>20%).

^f20–27 and 1 h, respectively.

^gCalculated for a liver Volume of 1.5 L.

^hThe casting process might lead to shrinking and thus underestimation of porosity and permeability.

ⁱConsidering a liver mass of 1.5 kg

Following [80], a more realistic permeability can be calculated as:

$$\kappa = \int_0^{\infty} \frac{\phi}{8} a_{\mu}^2 f(a) da, \quad (11)$$

where a_{μ} is the mean radius of capillary tubes and $f(a)$ is the probability distribution density function of the tube radius. In **Section 4.3.1**, we use **Eq. 11** to calculate the permeability of the liver corrosion cast of [57].

2.6.4 Poro-Visco-Elastic Liver Model

In **Section 3.2**, we analytically model how the shear wave dispersion in the liver is influenced by the porosity and fluid phase in the poro-visco-elastic wave propagation model. The liver is regarded as a homogeneous solid saturated by a Newtonian fluid according to Biot's theory ([31, 55]). We compare these analytic curves of a hypothetical liver with some exemplary literature values of *in vivo* elastography measurements. The measurements are displayed in **Figure 4** and stem from **Figure 1** of [81]. The data was read off manually by taking seven and nine points from the experimental curves for the F1 and F3 curve, respectively. Finally, we compare the results to a best fit of the experimental results with Voigt's model. The poro-visco-elastic modeling is equivalent to the melamine foam modeling presented in **Section 2.5.2**. However, the foam parameters are now replaced by liver values from the literature.

Regarding blood as a Newtonian fluid is of course a simplification, albeit one that is commonly made. A more accurate description of blood is a non-Newtonian visco-elastic shear thinning fluid or sol where the viscosity depends on shear stress, oscillation frequency and Hematocrit [82]. Shear rate is a function of flow and vessel diameter, which vary widely throughout the liver [83]. We thus explicitly make the choice of reducing the model complexity by assuming an average Newtonian blood viscosity under systolic conditions as for example measured by [75]. The Biot parameters are inter-related, hence the sensitivity of the shear wave speed on one parameter depends on the choice of the others. This leads to a variety of possible dispersion curves within the range of literature values given in **Section 2.6.1**. Exploiting the parameter space is beyond the scope of this manuscript. Instead, in **Section 3.2** we present the dispersion curves for a low and a high porosity with changing viscosity. This shows the variety of dispersion curves which are covered by the porous model with fixed elastic and changing porous parameters.

To reduce complexity and because the Biot model is less sensitive towards tortuosity than to porosity ϕ , permeability κ , and fluid viscosity η_f we fix the tortuosity to 1.05 throughout our calculations, which is in the range reported in **Section 2.6.1**.

In **Section 3.2** we use the same modeling method to compare the poro-visco-elastic model to statistics of experimental shear wave dispersion data acquired by [81] and to Voigt's model. Equivalent to how [81] measure the experimental dispersion, the dispersion of the analytic model is evaluated using the slope of the linear fit between 73 and 250 Hz. With this range we account for the experimental conditions of the measurements of **Figure 5** [81]: The stated problems at low frequencies and the fact that the upper frequency for the fit of each point in **Figure 5** varied

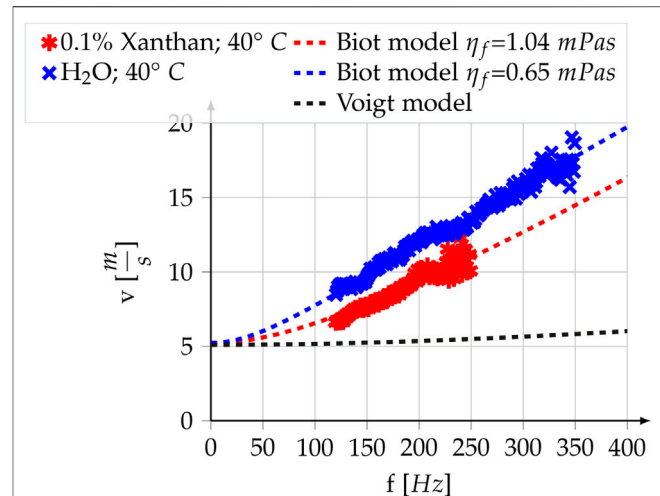


FIGURE 3 | Poro-visco-elastic model, visco-elastic model and experimental measurements for the melamine foam. In the two realizations of Biot's model all parameters are equal, except for the fluid viscosity. For all parameter values see **Table 2**. The Voigt model is displayed to highlight the differences with the poro-visco-elastic model. It is not a fit but uses the same shear modulus, solid viscosity and average density $\bar{\rho} = \rho_s(1 - \phi) + \rho_f\phi$ as the Biot model.

depending on the quality of the experimental data. The five parameter optimization of the Biot model exposes the ambiguity every multi-parameter inversion suffers. We do not claim to find the true values explaining the experimental data here, but merely want to evaluate if physical changes in porous parameters as reported in **Section 2.6.2** are able to explain the experimental trend observed by [81]. We thus explicitly compare with a two parameter, and thus less ambiguous, Voigt model, only. To facilitate the multi-parameter inversion for Biot's parameters we put further physical a priori constraints. The aim of the multi-parameter inversion here is to see if the observed statistical trend in the dispersion data can be explained by changes in the Biot model. We thus restrict the solid viscosity of the Biot model to not exceed that of the point with the lowest Dispersion, effectively fixing the Voigt viscosity for the entire observed data. Furthermore, we suppose increasing porosity and permeability for increasing dispersion, which is reflected in the optimization bounds. The latter results from **Eq. 11**: a higher porosity must be accompanied by a higher permeability if no geometry changes happen.

3 RESULTS

3.1 Experimental Shear Wave Dispersion in Melamine Foam

The red and blue crosses in **Figure 3** show the shear wave dispersion curves for high and low viscosity fluids. The dispersion for the foam in water (blue) differs significantly from the dispersion of the same foam in Xanthan solution (red). For example, the measured phase velocity in the foam

saturated by the Xanthan solution at 125 Hz is 2 m s^{-1} (>20%) lower than that of the foam saturated by water.

3.1.1 Poro-Visco-Elastic Dispersion Modeling in Melamine Foam

An inversion for the free parameters μ_0 and μ_1 of the Biot model, as described in Section 2.3, is performed on both experimental curves. The parameter bounds and values for the fixed parameters of the Biot model can be found in Section 2.5.2. The result is a μ_0 of 27.3 kPa, a μ_1 of 8.2 Pas for the foam and a fluid viscosity of 1.04 mPas for the Xanthan solution. The resulting analytic dispersion curves are shown as dotted lines in Figure 3 and expose a good fit with the experimental results. The only parameter changing between the two experiments is the viscosity of the fluid and hence solid elasticity and solid viscosity, the two free parameters of the Voigt model, stay constant throughout both experiments. As a consequence, we only model one dispersion curve for the Voigt model. It is displayed as the dashed black line and represents the dispersion resulting from a Voigt model, which uses the same elastic parameters as the Biot model. Due to the Voigt's model incapacity of modeling both curves, we did not perform a purely visco-elastic fit on the experimental data here.

3.2 Porous Dispersion in the Liver

Figure 4 shows the modeling results for a liver with different values of porosity and permeability and an identical set of visco-elastic parameters. The exact parameter values can be found in Table 2. The blue dashed curve represents a low porosity-low fluid viscosity liver, while the blue straight curve represents a high porosity-low fluid viscosity curve. The superimposed black curves are two exemplary shear wave dispersion curves in the liver acquired through ARFI/SWEI with an Siemens SONOLINE Antares scanner and a CH4-1 transducer (Siemens Healthcare, Ultrasound Business Unit, Mountain View, CA), taken from [81]. The dashed curve represents a liver classified with Fibrosis stage F1 and the straight curve represents a liver classified with Fibrosis stage F3. The least-squares best fit of the visco-elastic Voigt model and the experimental data in the frequency range of 100–400 Hz are plotted in red. Both, the poro-visco-elastic model results and the visco-elastic model results respect a penetration depth superior to 0.5λ (see Section 2.4). The drop-off of the experimentally measured wave speeds at low frequencies is attributed to a roll-off of the Fourier transform by Ref. [81]. They express confidence in their measurements for values >73 Hz. Within this confidence interval the measurements show an inflection at 100 and 150 Hz, which the Voigt model is incapable of reproducing without boosting μ_1 to large values that lead to a penetration depth $<0.5\lambda$. In contrast to that, the observed inflection is an intrinsic property of the Biot model with steeper slope at lower frequencies for smaller fluid viscosity.

The dotted blue curve represents the modeling result of using the same set of parameters as for the straight blue line except for the fluid viscosity η_f . Fluid viscosity is tripled for the dotted blue curve, leading to a significant decrease of the shear wave speed. The dash-dotted blue line was achieved likewise from the model of the dashed blue line. At 200 Hz, a commonly taken shear wave

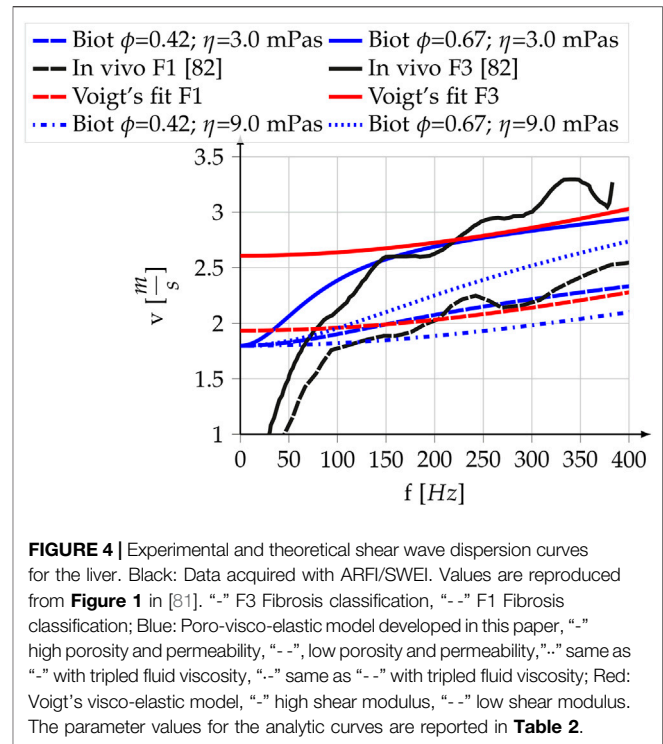


FIGURE 4 | Experimental and theoretical shear wave dispersion curves for the liver. Black: Data acquired with ARFI/SWEI. Values are reproduced from Figure 1 in [81]. “-” F3 Fibrosis classification, “--” F1 Fibrosis classification; Blue: Poro-visco-elastic model developed in this paper, “-” high porosity and permeability, “--”, low porosity and permeability, “...” same as “-” with tripled fluid viscosity, “-.-” same as “--” with tripled fluid viscosity; Red: Voigt's visco-elastic model, “-” high shear modulus, “--” low shear modulus. The parameter values for the analytic curves are reported in Table 2.

speed reference frequency, the difference between the low porosity-high fluid viscosity (dash-dotted) and the high porosity-low fluid viscosity (straight) model reaches 0.8 ms^{-1} or 42% of the lower value.

3.2.1 Experimental Support for the Poro-Visco-Elastic Liver Model

Reference [81] conducted a statistical analysis of *in vivo* measurements of shear wave dispersion slope at 200 Hz versus shear wave speed at 200 Hz for healthy and fibrotic human livers. In Figure 6 of [81], which is replotted in our Figure 5, one can observe a positive correlation ($R^2 = 0.3$) of the steepness of the dispersion slope with the measured speed at 200 Hz [81]. Eyeballing the trend we set three dispersion-speed target pairs for the inversion: $2.0 \text{ ms}^{-1} - 0.25 \frac{\text{ms}^{-1}}{100\text{Hz}}$, $2.5 \text{ ms}^{-1} - 0.45 \frac{\text{ms}^{-1}}{100\text{Hz}}$, $3.0 \text{ ms}^{-1} - 0.65 \frac{\text{ms}^{-1}}{100\text{Hz}}$. Using Eq. 6 and the Basinhopping algorithm [41] (see Section 2.3) the inversion for Voigt's model results in the points V1, V2, and V3 of Figure 5. They represent the least squares best fits of the Voigt model and the speed-dispersion pairs. Using the same speed - dispersion pairs, the least squares fit using the poro-visco-elastic model results in the points B1, B2 and B3. The exact parameter values for V1, V2, V3 and B1, B2, B3 can be found in Table 2 and the bounds for the inversion in Table 3.

4 DISCUSSION

4.1 Poro-Elastic Loss Mechanism of Melamine Foam

The experiment confirms the results by [8] that the shear wave dispersion in a highly porous soft phantom is governed by the

TABLE 2 | Modeling parameter values for the poro-elastic and visco-elastic models. The fluid density and the mean density are set to $1,050 \text{ kgm}^{-3}$, which is the reported value of arterial blood [100]. ϕ -porosity, κ -permeability, η_f -fluid viscosity, μ_0 -shear modulus, μ_1 -solid viscosity.

Fig	Data	Model	ϕ [-]	κ [m^2]	η_f [mPa s]	μ_0 [kPa]	μ_1 [Pa s]
Figure 3	"_ _"	Biot	0.99	1.28	0.6	27.3	8.2
Figure 3	"_ _"	Biot	0.99	1.28	1.04	27.3	8.2
Figure 3	"_ _"	Voigt	—	—	—	27.3	8.2
Figure 4	"_ _"	Biot	0.42	1.0	3	3.4	0.6
Figure 4	"_ _"	Biot	0.42	1.0	9	3.4	0.6
Figure 4	"_ _"	Biot	0.67	4.7	3	3.4	0.6
Figure 4	"_ _"	Biot	0.67	4.7	9	3.4	0.6
Figure 4	"_ _"	Voigt	—	—	—	3.9	1.2
Figure 4	"_ _"	Voigt	—	—	—	7.1	2.0
Figure 5	B1	Biot	0.5	2.5	3.9	2.5	0.6
Figure 5	B2	Biot	0.6	3.2	4.3	4.4	0.6
Figure 5	B3	Biot	0.7	3.7	3.9	5.9	0.5
Figure 5	V1	Voigt	—	—	—	3.6	1.6
Figure 5	V2	Voigt	—	—	—	6.2	2.8
Figure 5	V3	Voigt	—	—	—	9.4	4.3

$\times 10^{-9}$

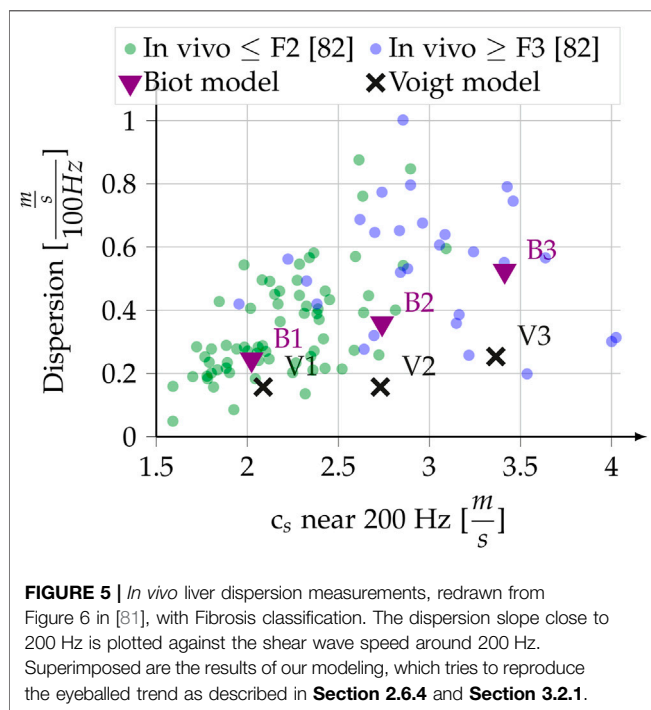


FIGURE 5 | *In vivo* liver dispersion measurements, redrawn from Figure 6 in [81], with Fibrosis classification. The dispersion slope close to 200 Hz is plotted against the shear wave speed around 200 Hz. Superimposed are the results of our modeling, which tries to reproduce the eyeballed trend as described in **Section 2.6.4** and **Section 3.2.1**.

viscosity of the fluid. Since the dispersion of the foam in Xanthan solution and in water differ significantly at the same temperature, viscous effects of the solid matrix cannot be the single reason for the wave dispersion. The experiment clearly shows that the difference in dispersion is due to the changed viscosity of the pore filling fluid. As a consequence, it is impossible for Voigt's model to reproduce the observed difference in the dispersion curves. Thus, modeling of shear wave propagation in soft porous materials by poro-elastic theory is justified. Biot's non-phenomenological theory of poro-elastic wave propagation takes the fluid viscosity into account by introducing a mechanism of fluid-solid interaction [28, 29].

It should be noted that the rheology of Xanthan solutions is non-Newtonian and as such Xanthan shares the shear dependency of the viscosity with blood. However, Biot's approximation of a Newtonian fluid with one constant apparent viscosity is sufficient for the small frequency range investigated herein, and in order to show that fluid viscosity is the single parameter which alters the shear wave dispersion. The good fit of the dispersion curves, which was retrieved by restricting the elastic and viscous parameters to lie within the range of the literature values for melamine foam, indicates that Biot's theory is well suited to explain the observed shear wave dispersion.

Our experiments show a fundamental difference between porous theory and other rheological models such as Voigt's model: the effect of a change in viscosity. A higher value of the fluid viscosity (Biot) leads to a smaller slope of the dispersion curve while a higher solid viscosity (Voigt) leads to a steeper slope of the dispersion curve. This can be understood in the following way: A higher fluid viscosity signifies stronger solid-fluid coupling. As a consequence, the shear wave encounters an increased effective density.

The experimental results were gained on a melamine sponge, which is an artificial, highly porous, soft solid. Melamine sponge has already been used to mimic ultrasonic scattering in biological porous tissue, namely the lung [84]. While the results of [8] and the experimental results presented herein show that Biot theory can explain shear wave dispersion in soft porous materials, its applicability on *in vivo* organs will be discussed in the following.

4.2 Porous Liver Modeling

The analytic shear wave dispersion curves in **Figure 4** are modeled without a change of the elastic parameters and cover the experimental curves for a F1 and F3 fibrosis stage liver. While the Voigt model requires a near two-fold increase of the elasticity and solid viscosity, the poro-visco-elastic model can reproduce both curves without a change in neither the elasticity nor the solid viscosity. Hence, the measurements above 73 Hz could in

TABLE 3 | Modeling parameter bounds for the poro-elastic and visco-elastic models. ϕ -porosity, κ -permeability, η_f -fluid viscosity, μ_0 -shear modulus, μ_1 -solid viscosity.

Fig	Data	Model	ϕ [-]	κ [m ²]	η_f [mPa s]	μ_0 [kPa]	μ_1 [Pa s]
Figure 3	"_ _"	Biot				10	1×10^{-4}
Figure 3	"_ _"	Biot			0.65	400	1×10^{-1}
Figure 4	"_ _"	Biot	0.2	1.0	2	6	1.6
Figure 4	"_ _"	Biot	0.5	3.0	5	9.5	2.5
Figure 4	"_ _"	Biot	0.5	3.0	2	6	1.6
Figure 4	"_ _"	Biot	0.7	5.0	5	9.5	2.5
Figure 4	"_ _"	Voigt	—	—	—	5	0.5
Figure 5	B1	Biot	0.35	1.5	1.5	2	0.1
Figure 5	B2	Biot	0.5	3.0	10	11	3
Figure 5	B3	Biot	0.6	3.9	4.3	2.5	0.1
Figure 5	B3	Biot	0.6	3.9	4.3	20	0.6
Figure 5	B3	Biot	0.6	3.7	3.9	4.4	0.1
Figure 5	B3	Biot	0.7	3.9	3.9	20	0.6
Figure 5	V1-3	Voigt	—	—	—	1	0.5
Figure 5	V1-3	Voigt	—	—	—	30	20

$\times 10^{-9}$

principle be explained without a change in solid visco-elasticity by using poro-visco-elastic theory. Since fibrosis classification by elastography relies on changes in shear modulus, this would indicate the possibility of false positives in fibrosis classification due to varying liver saturation. If the poro-visco-elastic model can be confirmed *in vivo*, shear wave elasticity imaging should thus take the porous parameters into account. Considering that perfusion and blood viscosity have been shown to be highly variable on short timescales (see **Section 2.6.2**), we think that these modeling results should be considered in future clinical research.

The fluid viscosity in the poro-visco-elastic model is 3.1 mPa s, which is below the commonly assumed whole blood viscosity of 3.5 mPa s but above the viscosities given for microcirculation and interstitial fluid in the liver by [59]. In a recent study [75], observed an anti-correlation of blood viscosity with liver stiffness as measured by transient elastography. The authors suggested pathologic changes as the source of this anti-correlation. The viscosity induced changes in shear wave speed of our porous modeling indicate the possibility of a different explanation. The correlation could be rooted in the inherent effect of the fluid viscosity on the shear wave speed, and as such be an artifact of the elasticity inversion.

4.2.1 Experimental Support for the Poro-Visco-Elastic Liver Model

Our hypothesis that the poro-visco-elastic model is more appropriate than Voigt's model to explain shear wave dispersion in the liver is supported by the analysis of the experimental results by [81] reported in **Figure 5**. In Voigt's model, an increase in the elasticity modulus will lead to an increase in measured speed at 200 Hz, but to a decrease of the dispersion slope. A much larger increase in solid viscosity (μ_1) than in elasticity (μ_0) could reproduce the correlation, but to our knowledge no such physical mechanism is known. On the contrary, the predominance of μ_0 for liver elastic behavior is

well documented [78]. Furthermore, such an increase in solid viscosity leads to very low penetration depths, effectively making the modeled wave a non-propagating one. Unlike Voigt's model, Biot's poro-visco-elastic model is able to reproduce a correlation of increased shear wave speed with increased dispersion slope for the reported *in vivo* measurements.

Ex vivo measurements do not reproduce this correlation of dispersion slope with shear wave speed [85]. This could be owed to the fact that *ex vivo* livers are partially drained. The drainage changes the porous properties, which adds to other factors, such as fluid pressure, that are inhibiting *ex vivo* experiments from properly reproducing the physics that govern shear wave dispersion *in vivo*.

While assuming the purely visco-elastic model, the porous and liquid properties of the liver might thus have largely been overlooked previously. Studies supporting this statement were conducted by [21–23, 86], who compared confined and unconfined *ex vivo* liver samples and found that the pressure on the fluid in the liver influences the shear wave speed. Reference [24] employed a microchannel flow model equivalent to Darcy's law and noted that it is superior to Kelvin-Voigt models, because it is based on tissue architecture instead of a priori information on springs and dampers. Both findings strongly support our deduction that porosity plays a crucial role in shear wave propagation in the liver. However, they do not discuss the main loss mechanism shown in **Section 3.1**: the viscosity of the fluid in the Biot model. While these works deduce elasticity changes from increased pressure on the tissue, we introduce the direct role of the fluid phase through porous physics into the calculation of the shear wave speed.

4.3 Future Research

4.3.1 Biot Parameter Measurements

Why should we introduce another multi-parameter rheological model? From a mathematical point of view, the introduction of additional model parameters improves model fit but also

increases model ambiguity. The critical reader might thus question the benefit of introducing another complex multi-parameter model. We agree of course, that conforming to Occam's razor [87], a simpler model is to be preferred over a complex model whenever sufficient experimental data for the model parameters is lacking. Biot's model however exhibits several traits that differentiate it from other complex rheological models such as Zener's model or a fractional Kelvin-Voigt model.

First, it is an entirely non-phenomenological model which is derived from first principles and the porous parameters can in principle all be measured independently. This is the main reason why it is widely applied in geophysical applications, e.g., in oil and gas exploration. Second, both the liver porosity and permeability depend heavily on blood vessel size and are not independent variables. The porosity, i.e., the space occupied by free fluids, can be estimated as the total volume of blood and extracellular fluid. Tubes are a good approximation for the average blood vessel shape and as a consequence permeability and porosity can be calculated from the knowledge of the distribution of vessel sizes with Eq. 11.

Reference [69] analyzed the 3D micro and mesoscopic structure of the hepatic blood vessel tree using high resolution Computer Tomography of a liver cast. As a final check for the Biot model we create a normally distributed probability function for each vessel generation based on their values of the mean and standard deviation of the pore size and length per vessel generation. For the interstitial fluid we assume a pore radius of $0.25\ \mu\text{m}$, a value derived by [59] for the space of disse, and a Volume percentage of 12% [77, 88]. Integrating over the permeabilities for each vessel generation we retrieve $\kappa \approx 2e^{-9}\text{m}^2$ and $\phi \approx 0.35$. These values are in the lower range of the literature values [17], which is in accordance with the assumption that the cast represents an un- or normally perfused liver. Reference [57] also evoke a possible shrinking of the blood vessel diameters due to the casting process.

We assume that a change in liver perfusion *in vivo* is reached through vaso-dilatation or vaso-contraction [63, 64, 89]. We can then calculate the necessary dilatational factor to reach the maximum porosity of 70% for the poro-visco-elastic model: For example, increasing the radius of the smallest 8 vessel generations by two and of the remaining generations by 1.2 as well as their lengths by 1.5 for the smallest 8 and by 1.2 for the remaining generations gives a porosity of 70% and a permeability of $5.6 \times 10^{-9}\text{m}^2$. This rough estimation is in accordance with the upper limits of the literature². Furthermore, the dilatational factors are not purely speculative, since [68] found a dilatational factor for the sinusoids of nearly two in cirrhotic rat livers.

As of now, these theoretical porous parameter values are estimated from the liver cast and the literature, but recent

imaging advances in ultrasound [90–92] and MRI imaging [93] have shown that porosity and blood vessel size estimation *in vivo* are becoming feasible. Another approach that might advance the imaging of porous properties with shear wave elastography was recently presented by [94]. They suggest to use Bayesian inference to deduce microstructure properties from effective wave parameters. Hence, poro-visco-elastic shear wave speed estimation could in the future replace the phenomenological springs and dampers of the currently applied rheological models with a physical loss mechanism that is determined by measurable porous parameters. From a physicist point of view, the added model complexity is then well justified by a reduction in the model ambiguity. If the poro-visco-elastic model will also result in improved disease classification compared to more complex visco-elastic models will have to be evaluated in the future.

4.3.2 Low-Frequency Dispersion

References [95, 96] show, that in the case of magnetic resonance shear wave elastography of the brain, a poro-elastic finite element model following [97–99] is more accurate than a visco-elastic model at very low frequencies. Physical reasoning for the observed low speeds at low frequencies requires much lower elastic moduli than are commonly inverted for in transient elastography. An elastography independent measurement of such low elastic moduli is given by [9, 78] for *in vitro* porcine liver. They find storage moduli as low as 300 Pa, using dynamic mechanical analysis (DMA). Similar values are reported for *ex vivo* bovine livers by [3] on fresh porcine liver grafts, using a rheometer and a fractional Kelvin-Voigt model, and by [60], who employed a poro-hyper-elastic model. Even lower elastic moduli are reported for in-vivo mimicking perfusion tests on ex-vivo liver samples. Reference [10] report linear elastic shear modulus values of less than 100 Pa for liver parenchyma.

These results in combination with poro-visco-elastic modeling indicate that the entire observable dispersion range, including the low frequencies, needs to be taken into account in order to accurately retrieve the elasticity of the liver. Future *in vivo* studies of the liver should thus focus on acquiring shear wave speeds over a large bandwidth. At very low frequencies, frequency dependent fluid viscosity and permeability should be taken into account due to the pronounced visco-elastic properties of blood.

5 CONCLUSION

Our results show, that for fluid saturated, soft porous materials, the porous parameters need to be taken into account to properly model shear wave dispersion. The shear moduli retrieved from simple visco-elastic models are prone to overestimations in the presence of porosity. Fluid viscosity, permeability, and porosity of the diseased as well as the healthy liver can vary inter- and intra-individual on short timescales. It is thus justified to assume that the wide range of observed shear wave dispersion curves in the *in vivo* liver is partly owed to changes in the porous properties of the

²The largest two vessel generations, e.g., the hepatic vein, are ignored, assuming that these are avoided by the practitioner in shear wave elastography. The radii of the biliary tree are also neglected, since its estimated volume percentage of 1.2% is relatively low.

liver. Our comparison of poro-visco-elastic and simple visco-elastic modeling shows, that porous parameter changes can easily be the reason for false positives in visco-elastic shear modulus inversion. In the here presented porous liver model porosity dominates at low frequencies, and solid viscosity is dominant at high frequencies. Furthermore, the stiffer the matrix of the liver, the more sensitive the shear wave dispersion becomes to the porous parameters in the Biot model.

Compared to more complex visco-elastic models, the poro-visco-elastic model does not require further phenomenological parameters. Instead it relies on directly measurable quantities. Furthermore, it gives a physical explanation for the observed shear wave dispersion in the liver. If our results are confirmed *in vivo*, they are likely to have implications for elasticity imaging of other porous organs, such as the spleen, the kidney and the brain.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

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AUTHOR CONTRIBUTIONS

JA and SC contributed equally to conception and design of the study. JA conducted the experiments, literature search and analytical modeling and SC participated therein. JA wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Actuators for MRE: New Perspectives With Flexible Electroactive Materials

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Since 1995, Magnetic Resonance Elastography (MRE) has been constantly developed as a non-invasive diagnostic tool for quantitative mapping of mechanical properties of biological tissues. Indeed, mechanical properties of tissues vary over five orders of magnitude (the shear stiffness is ranging from 10^2 Pa for fat to 10^7 Pa for bones). Additionally, these properties depend on the physiological state which explains the granted benefit of MRE for staging liver fibrosis and its potential in numerous medical and biological domains. In comparison to the other modalities used to perform such measurement, Magnetic Resonance (MR) techniques offer the advantages of acquiring 3D high spatial resolution images at high penetration depth. However, performing MRE tissue characterization requires low frequency shear waves propagating in the tissue. Inducing them is the role of a mechanical actuator specifically designed to operate under Magnetic Resonance Imaging (MRI) specific restrictions in terms of electromagnetic compatibility. Facing these restrictions, many different solutions have been proposed while keeping a common structure: a vibration generator, a coupling device transmitting the vibration and a piston responsible for the mechanical coupling of the actuator with the tissue. The following review details the MRI constraints and how they are shaping the existing actuators. An emphasis is put on piezoelectric solutions as they solve the main issues encountered with other actuator technologies. Finally, flexible electroactive materials are reviewed as they could open great perspectives to build new type of mechanical actuators with better adaptability, greater ease-of-use and more compactness of dedicated actuators for MRE of small soft samples and superficial organs such as skin, muscles or breast.

Keywords: magnetic resonance imaging, actuators, electroactive materials, magnetic resonance elastography, piezoelectricity

INTRODUCTION

For a long time, physicians have been using palpation to detect diseases revealed by qualitative changes of tissue stiffness. In parallel, the development of quantitative methods to assess mechanical properties of living tissues at different spatial scales and their link with physiopathological states of tissues have been constantly investigated [1–3]. Indeed, mechanical properties of tissues vary over five orders of magnitude (the shear stiffness is ranging from 10^2 Pa for fat to 10^7 Pa for bones). Additionally, these properties depend on the physiological state which explains the granted benefit of Magnetic Resonance Elastography (MRE) for staging liver fibrosis and its potential in numerous medical and biological domains [4]. For instance it has been shown that extracellular matrix, i. e. the

biochemical and mechanical micro-environment of tumors, is a key determinant in the metastatic process [5].

In the last decades, different imaging modalities such as ultrasound [6], magnetic resonance imaging (MRI) [1], or even optical coherence tomography [7] have been adapted to quantitatively map the mechanical properties of tissues. Today, one dimensional (1D) ultrasound elastography is used in clinical routine to non-invasively diagnose and classify patients with liver fibrosis [8]. Two-dimensional (2D) shear wave ultrasound imaging has demonstrated various benefits to diagnose for instance chronic liver diseases [9], or breast tumors [6]. Finally MRE has the potential of characterizing *in vivo* the mechanical properties of various tissues in 3D and has been approved by the Food and Drug Administration (FDA) for liver fibrosis grading [10]. Unlike ultrasound elastography, MRE is not limited by the presence of the skull which makes this technique also applicable to the brain [11, 12]. MRE can be used to quantify for instance neurodegenerative disease [13, 14], brain tumor malignancy [15, 16] or more recently, brain functional activity revealed by fast measurement of mechanical changes induced by neuronal activity [17].

Whatever the modality used to map the mechanical properties, shear waves have to propagate within tissue. To this end, different methods to generate mechanical waves, each with their pros and cons, have been developed which are based on external vibrating source, radiation pressure source or more recently natural source [18]. Among these methods, the external vibrating source has by far been the most used. The first reason is that it allows extracting mechanical parameters from the recorded shear wave field and not only the wavelength as done in the recently developed passive elastography [19]. The second reason is that the excitation must be compatible with the constraints and requirements of the imaging modality (space and time resolution, harmonic or transient recording etc...). In the case of MRI, the acquisition speed (maximum of 30 fps [20]) is much slower than it is with ultrasound (about 1,000 fps [21]). Therefore, MRI will have more capabilities to record low frequency signal generated by external driver than the transient wave generated by a radiation pressure source. On the contrary, ultrasound allow to record in real-time the propagating wave which does not imply any constraint on the type of excitation.

Overall, MRE relies on the use of an external mechanical actuator, which is challenging because of the constrained environment of the MRI system. Moreover, this actuator should be able, according to the dynamic approach of MRE (as opposed to the static approach), to generate low frequency (generally between 20 and 1,000 Hz) shear waves. Those actuators are often bulky, which burdens the use of MRI that is already a complex imaging modality to perform. Despite the high diagnosis performance of MRE [4], this technological lock prevents MRE to be democratized in clinic but also in preclinic experiment. In this challenging context, the question is: can technological progress allow designing an external mechanical actuator, as transparent as the shear wave generation is for users of ultrasound elastography? Among other vibration generators, piezoelectric ones offer interesting possibilities of integration, and

miniaturization. Therefore, we propose in this paper to review the current solutions for dynamic MRE actuation using piezoelectric actuators while underlining their pros and cons compared to the other methods. Finally, flexible electroactive materials will be reviewed as they could open great perspectives to build new type of mechanical actuators for MRE.

SHEAR WAVES AS AN “IN SITU” BIOMECHANICAL SENSOR—SPECIFICATIONS FOR A GOOD VIBE

Local Estimation of the Stress-Strain Relationship

In general, assessment of mechanical properties relies on the measurement of the strain induced by stressing a sample. Common non-tomographic rheological methods [22, 23] applied to soft biological tissues are coarse and integrative but the stress-strain relationship is almost directly obtained from the applied stress to the sample and its resulting strain which is derived from the displacement (linear or angular) of the actuator. Assuming that the sample is homogeneous and viscoelastic, and that the mechanical stimulation stays in the linear regime, the measurement sensitivity and accuracy relies only on the hardware and its calibration. On the other side, imaging methods require to measure the stress-strain relationship locally. For MRE this is done in two steps. First, an external actuator is used to generate waves within tissues that will locally probe the mechanical response of the tissue. Then imaging of local displacement is done by synchronizing the mechanical excitation with the MRI in order to encode the wave displacement into the phase of the complex MR signal. This allows derivation of both local strain from the displacement and local stress from the second temporal derivative of the displacement. The mechanical properties can then be identified by solving the equation of motion [1]. Therefore, the accuracy of the MRE measurements depends on the quality of the mechanical excitation source, the acquisition method of the displacement map and the reconstruction algorithm used to obtain the shear modulus.

Physical and Acquisition Constraints

The ability to generate low frequency shear waves of sufficiently high amplitude (about tens of micrometers in the tissue) is mainly constrained by potential loss effects occurring within biological tissues, due to attenuation (i.e., conversion into heat) or scattering (i.e., energy getting dispersed in different directions). This attenuation increases with frequency and with tissue stiffness which may result in a rapid decrease of wave amplitude with tissue depth. In practice, this attenuation can be partially compensated by increasing the excitation amplitude but can induce, close to the mechanical actuator, heavy phase wraps, difficult to eliminate. Therefore, generating high amplitude waves is especially difficult for large and deep organs. Finally, the presence of tissue interfaces might lead to wave absorption and mode conversion. Nonlinear effects induced by varying

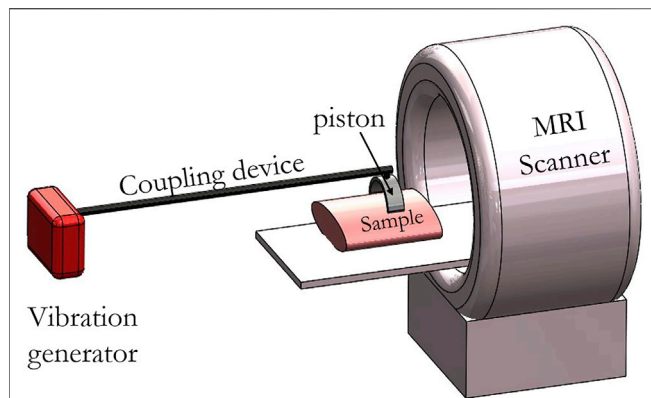


FIGURE 1 | Schematic representation of an MRE mechanical actuator positioned within the MRI machine. Generated waves are transmitted to the sample via the coupling device and a piston. Coupling devices and pistons are made of an MRI-compatible material such as plastic or PEEK for example which allows positioning inside the MRI scanner. Pistons can either fit the form of the sample, be rigid or be invasive in case of a needle. Coupling devices can be either flexible or rigid depending on the chosen material. Vibration generators are generally incompatible for usage close to an MRI scanner and have to be placed outside of the room.

preloading conditions (a beating heart, respiration etc) can influence the efficiency of high-amplitude mechanical wave transmission within biological tissues. These limitations can be overcome by a recent technique called reverberant shear wave elastography which uses multiple actuators to reinforce multi-directional waves. Unfortunately, this method is not yet available using MRI acquisition and may be limited to frequencies below 200 Hz [24].

From an acquisition point of view, the quality of MRE data can be estimated from phase to noise ratio which in a first approximation is tied to the signal to noise ratio (SNR) and the encoded phase shift [25]. Obviously the SNR will critically rely on the acquisition parameters but also on the spin transverse relaxation time (T_2^*) of the tissue that can be really low for example in liver with iron overload [26] or in lungs [27] and lead to very low SNR. Be that as it may, the encoding efficiency can be optimized by the acquisition sequence and the wave amplitude will mainly depend on physical properties and mechanical actuator settings as detailed in the following section.

Mechanical Actuator Requirements and its Characterization

As can be seen in **Figure 1**, a mechanical actuator for MRE is composed of a vibration generator which is associated with a coupling part that transmits as much as possible the mechanical excitation up to a piston which is positioned in contact with the surface of the sample. The coupling between the piston and the sample is the most difficult part of the MRE setup and has to be characterized in term of efficiency. When the vibration generator does not induce imaging artifacts, the size of the coupling device can be reduced which minimizes the associated losses. This can facilitate the integration of the mechanical actuator within the

magnet bore which leads to additional free space for instruments and a greater ease of use.

The Vibration Generator

Existing vibration generators are mainly based on electromechanical (acoustic actuators can be considered as electromechanical transducers with internal magnets) and piezoelectric actuators. Electromechanical actuators are able to deliver waves of high amplitude at low frequency (10–150 Hz), are also cheaper and easier to build and to control than the piezoelectric one. However their compatibility with MRI (due to the static field (B_0) distortions they induce) and related safety issues when positioned closed to the patient are problematic [28, 29]. One interesting alternative is to use a compressed air supply connected with an electromagnetic valve through a hose to control the vibration frequency and generate shear wave excitation. This approach is interesting to reduce the electrical power usually needed to generate high amplitude waves. Thereby the mechanical actuator is more MR-compatible and safe than an electromechanical one which requires higher power supply [30–32]. By comparison, piezoelectric actuators can be positioned closer to the patient without inducing image artifacts and they can work at higher frequency with high fidelity (up to 5,700 Hz) [33]. Nevertheless, this type of actuator lacks amplitude and requires amplification devices which are complex to develop. Finally, new transduction principles based on innovative materials such as optomechanical azobenzene liquid crystal open great perspectives with regards to MRI compatibility [34, 35].

Overall, the requirements for a powerful and efficient vibration generator are the following:

- A true single-frequency vibration.
- Ability to generate high amplitude displacement when loaded.
- A constant generated amplitude over a wide range of driving frequencies.
- MRI compatibility.

The Coupling Device

Due to the low MRI compatibility of most vibration generators, coupling devices are required to remotely transmit waves up to the piston. The drawback is that these coupling devices can degrade the performances of the vibration generator. The transmission lines are based on pneumatic pipes [36], rigid shaft [37–40], flexible shaft [41] or hydraulic pipes [42].

The pneumatic pipe is interesting in terms of MRI compatibility and safety as it allows easier positioning of the piston while satisfying space constraints. Nevertheless, the pneumatic transmission has low frequency accuracy (presence of harmonics) as it critically relies on the coupling with the resonant eigenmodes of the pipe. As demonstrated in [41], higher order harmonics could have an impact on the reconstruction accuracy depending on the number of cycles and the order of the harmonic. In addition to that, pneumatic pipes work mainly at low frequency (below 100 Hz), unless the extremity of the pipe is directly coupled to the sample [43, 44] or is used to actuate an unbalanced rotational mechanism [45, 46].

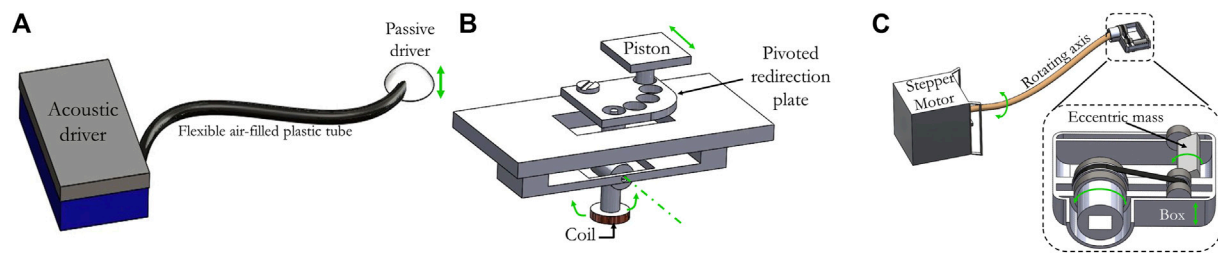


FIGURE 2 | 3D designs of three clinical actuators reported in the literature. **(A)** A pressure wave is generated by the acoustic active driver. Then the wave is transmitted through a plastic tube to a nonmetallic semi flexible membrane. To date, this is the only mechanical actuator which has been approved by the FDA and is commercially available. Depending on the application, the piston can be adapted [36]. **(B)** Displacement of the piston (green arrow) is achieved using an alternative current flowing in the coil which is placed in the static magnetic field B_0 of the MRI magnet. The coil is coupled rigidly via a revolute joint (revolution axis in green) to the frame. The pivoted redirection plate allows this actuator to provide motion of the piston in different directions unlike common electromechanical actuators [28]. **(C)** The stepper motor rotates the semi flexible PEEK rotating axis which is linked to the transducer through a locknut connection. Two PEEK rods can be found in the transducer. Those rods are glued to two PEEK timing pulleys and a timing belt. The second timing pulley is connected to a PTFE eccentric mass that induces a vertical vibration of the box. This later is positioned in contact with the sample to generate waves within the tissue. Motion direction of the different mobile parts is indicated by green arrows [41].

Recently a semi rigid coupling device has been developed using a flexible shaft made with PolyEther Ether Ketone polymer (PEEK) to transmit the mechanical wave generated by a stepper motor up to a gravitational transducer [41]. Thanks to the semi rigid coupling device, this actuator is easy to position and has a highly accurate on-resonance vibration frequency without harmonics.

The use of a hydraulic semi flexible hose allows, thanks to the incompressibility of water, better transmission than the pneumatic solution with up to a maximum of 2 mm amplitude for wave generation at the surface. This solution has been for example implemented for prostate MRE [42].

On **Figure 2**, 3D designs (Solidworks, Dassault Systèmes SE) of common clinical actuators are presented. The first actuator is an acoustic mechanical actuator with a flexible coupling device while the two others are electromechanical ones with a rigid and a semi-rigid coupling device.

The Piston

The front end of the coupling device, often called piston, requires dedicated shape and materials in order to optimize the efficient generation of shear waves within the biological tissues [47]. This piston can be actuated longitudinally which allows better penetration depth and design flexibility than with transversal actuation [47, 48]. Nevertheless, this latter generates more uniform shear waves parallel to the coupling surface. The shape of the piston is also crucial. For instance, for direct contact with soft tissues such as the breast, the efficiency of the longitudinal actuation can be enhanced by a 3D printed C-shaped breast holder with a non-planar surface to maximize the contact and potential shearing of the surface of the breast [49]. On the contrary, for indirect contact such as for the brain it is more the position of the piston relative to the skull that matters. The goal is to cause the head to experience a slight nodding motion of few microns (5–50 μm) [11, 12]. For small biological specimens it can be beneficial to generate shear waves by transversally shearing the surface of the sample to avoid bulk motion, in order to avoid the development of complex post processing tools [50]. In the case of small and fragile samples,

invasive solutions have often been preferred over external actuation setups. Indeed, the latter require dedicated miniaturized actuators with a sample holder to fit potentially complex 3D shapes. Therefore several research groups have based their MRE set-up on an oscillating needle inserted along the axis of the sample which is cylindrical in this case [51–53]. Needle based actuators have also been used in interventional MRE for MRI-guided procedures [54]. Nonetheless, this invasive solution is unsatisfactory, especially as the benefit of MRI comes from its non-invasiveness.

Characterization of the MRE Actuator

The mechanical actuator can be calibrated from the vibration generator to the front end in the presence or in the absence of a load. However, the amplitude of the waves and the homogeneity of their propagation can only be checked by MRI or investigated by simulations. These simulations are also mandatory to design the coupling device and in the case of piezoelectric actuator, to design an amplification structure as done for instance in [55]. In general, characterization of the mechanical actuators have been reported either in loaded [56–59] or unloaded conditions [56, 60] mainly using a laser Doppler vibrometer. This characterization method is beneficial to characterize the frequency response of the mechanical actuator in the real condition of use. This allows one to optimize the efficiency of the setup in terms of the frequency response in order, for instance, to maximize the motion amplitude at the working frequency. Changing parts for stiffer/softer ones, or working at the eigenmodes frequencies of the setup offer simple ways to achieve the awaited specifications [61].

MRI Compatibility

One another important constraint in the design of MRE actuators is the use of materials and active electronic parts which are MRI compatible, otherwise it may result in safety issues and deleterious image artifacts during MRI examination [62]. Overall, this MRI compatibility issue is particularly problematic in MRE experiments since materials can shorten the effective transverse relaxation time ($T2^*$) of tissues which is

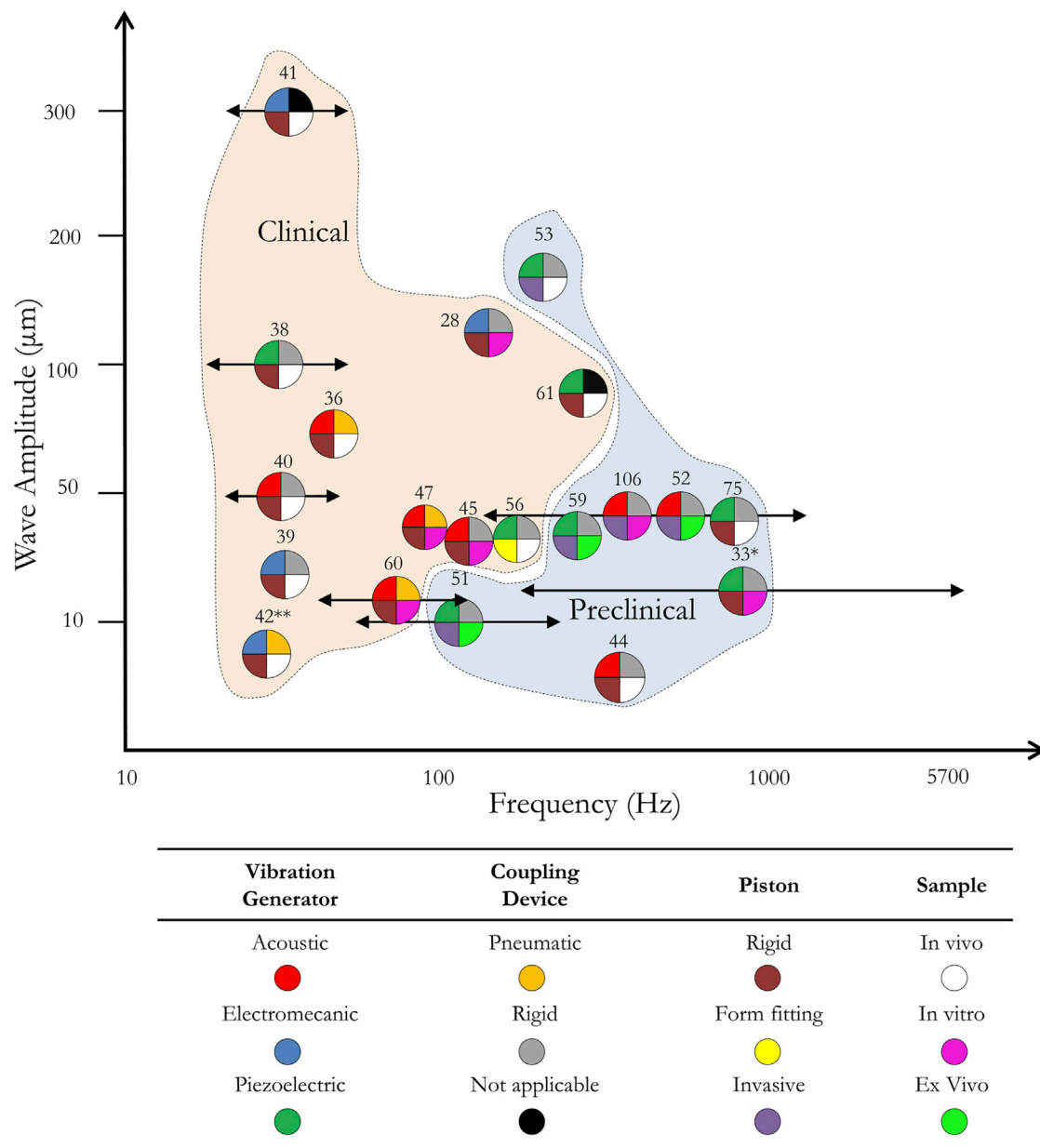


FIGURE 3 | Wave amplitude generated within the sample by mechanical actuators from the literature as a function of the frequency and the application. Each mechanical actuator is represented by a pie chart labelled by a bibliographical reference number and divided in four parts which corresponds to four actuator characteristics: the vibration generator (**top left corner**), the coupling device (**top right corner**), the piston (**bottom left corner**) and the type of experiment (**bottom right corner**). Each characteristic is divided in three colored categories given in the table. The X-axis shows the frequency at which the MRE experiment was run, and the Y-axis is the wave amplitude within the region of interest. Arrows are introduced for actuators which cover a wide range of frequencies. Clinical and preclinical zones provide information on the type of application the actuators were built for. This graph only shows actuators for which both the amplitude within the tissue and the frequency information was given. (*) here, the coupling device and the piston are combined in a single part. (**) here, the pneumatic coupling device has been replaced by a hydraulic system.

not compatible with the long echo time (TE) usually required for MRE sequences.

Regarding the MRI compatibility of materials, ferromagnetic and paramagnetic materials should be avoided near the scanner, as they would experience forces and torques caused by the high magnetic fields and gradients. It would result in serious safety

issues. Ferromagnetic materials must be distinguished from ferroelectric materials that have better MRI compatibility. That is why piezoelectric actuators that are mainly based on ferroelectric materials, can be positioned within the MRI scanner, closer to the sample. Nevertheless, non-magnetic and conductive materials especially metallic ones should be

positioned away from the sample as those materials can host eddy currents induced by the RF pulse and the switching magnetic field gradients. These currents may cause a significant increase of the material temperature and induce field heterogeneity which may lead to image artifacts and SNR degradation. This issue has been extensively studied and acquisition or hardware solutions have been developed to improve their MRI compatibility [63]. With regards to polymers, this type of material is highly MRI compatible even if some of them can lead to parasitic Nuclear Magnetic Resonance (NMR) signals, susceptibility artifacts or biocompatibility issues [64–66].

Another important source of safety issues and image artifacts are the active electronic parts. These have to be properly electromagnetically shielded and/or put away from the imaging region in order to avoid disturbance of the static magnetic field that would cause image artifacts. This source of artifact is particularly important for electromechanical and piezoelectric generators positioned close to the sample. A low-pass filter (cut-off frequency far below the Larmor frequency) is often necessary to limit electromagnetic coupling with RF and gradient MRI coils. Being one of the most common concerns in MRI, a standard test method has been established by the American Society for Testing and Materials (ASTM) to evaluate the MRI artifacts induced [67].

MRI Compatibility issues must be investigated only via an MRI examination. A multi gradient echo sequence with at least two echoes allows measurement of the static field map. More echoes could permit quantitative assessment of susceptibility maps [68]. Additional measurements can be necessary to quantify potential NMR signals from structures with short T2s as mentioned in [65]. Finally, degradation of the signal to noise ratio must be investigated by comparing SNR with and without the mechanical actuator.

Figure 3 graphically presents an overview of different actuators for MRE cited in the bibliography and their wave amplitude generated within the sample as a function of the working frequency. The three parts of the actuator defined previously as well as the type of sample on which the actuator has been tested are presented by a pie chart with four sections. Two areas of use of the actuator, corresponding to clinical and preclinical applications, can be identified. Overall actuators for clinical application (see **Figure 2**) are designed to work at lower frequency, i.e., below 100 Hz, than the ones for preclinical applications and can generate deep within tissues higher waves amplitude than the latter. Piezoelectric actuators offer the possibility to work at high frequencies and close to the sample which could be very beneficial to increase the mechanical coupling with small samples and integration capabilities.

As will be shown in the next sections, the use of piezoelectric actuators is justified in this case as it allows closer positioning of the vibration generator and design of a form-fitting piston without the need for a coupling device.

STATE-OF-THE-ART ON PIEZOELECTRIC ACTUATORS FOR MRE EXPERIMENTS

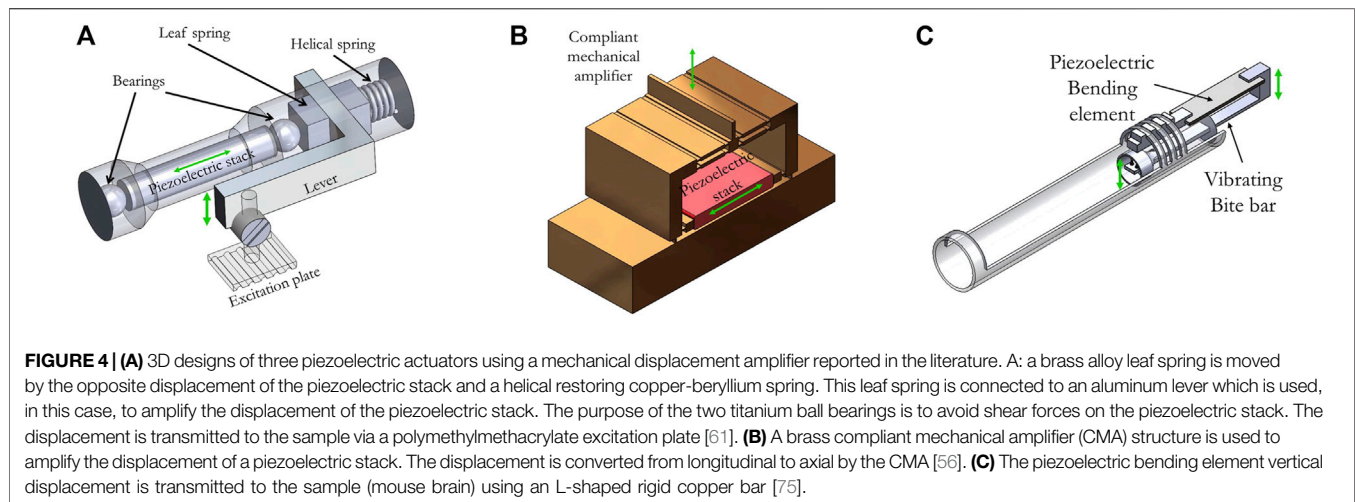
Piezoelectric actuators are becoming more attractive than pneumatic and electromechanical transducers because of

their fast response time, their independence concerning the orientation of the static magnetic field and the low image artifacts they tend to create. In the MRE context, they have three main drawbacks: generated heat from piezoceramic stacks, the complexity of their installation and their lack of displacement amplitude. The heat dissipated by the stacks has been managed by not putting them directly on top of the patient. With regards to the installation of such actuators, one concern is the high voltages needed to supply power to the piezoelectric actuators. The actuator itself is placed within the bore of the scanner close to the patient while the power is supplied via shielded coaxial cables that go through low pass filters connected to a power amplifier located outside the scanner room. This power amplifier is generally controlled via a computer that is also located outside the scanner room. Finally, adaptations have been made to overcome the amplitude limitations either by mechanically amplifying longitudinal piezoceramic actuators or by using contracting actuators with different coupling of the actuator to the tissue. In the following subsections, the different piezoelectric materials are presented as well as their integration in a set-up dedicated for MRE which requires in some cases, a mechanical amplification device.

Basic Principles of Piezoelectric Material and Actuators

Piezoceramic actuators are usually made from Lead Zirconate Titanate (PZT), Barium Titanate (BaTiO_3) or Zinc Oxide (ZnO). Due to its high electromechanical coupling factor (meaning the square root of the ratio of the electrical energy output to the mechanical energy), PZT is often chosen by the industry as the main material for the commercial actuators [69, 70]. The use of piezoelectric actuators is well documented in numerous fields of engineering [70, 71]. One important drawback of piezoelectric actuators is that displacement amplitude is decreasing with the working frequencies. In order to compensate for this, currently, two types of piezoelectric actuators arrangement have emerged:

- *Piezoceramic stacks*: with longitudinal piezoelectric actuators, an electric field is applied along the polarization vector that generates a material strain. This strain is too small for MRE applications but can be increased by stacking individual actuators. Actuators would be mechanically put in series with each other while being electrically connected in parallel. With such piezoelectric assemblies, nominal displacement generally reaches around 0.10–0.15% of the initial actuator length.
- *Piezoceramic bending elements*: They are constituted by contracting thin actuators attached to a deformable substrate. When activated, a bending moment vertical to the contraction is created. The advantage of this solution over the stack, is the larger displacement amplitudes for a smaller size but in return, the blocking force generated is much smaller which limits the application of this technology in the MRE context.



To date, developing a piezoelectric actuator for MRE has not been straight forward and has required combining a piezoelectric material with a mechanical amplifier or with a bending element.

Design and Integration of Piezoelectric Actuators for MRE

Mechanically Amplified Piezoelectric Actuators

First Xu et al. [72] then Ouyang et al. [73] proposed different compliant mechanical amplifier (CMA) topologies specially designed for piezoelectric displacement amplification. The lever arm and the double asymmetric 5 bars were the ones of interest when considering MRE applications. Lever arm-based structures are the easiest CMA to construct, therefore have been extensively used in industrial applications.

As reported in [61] and illustrated in **Figure 4A**, piezoelectric stacks have been used in combination with a lever arm to transmit its displacement to the patient. In this work, the stack was a cylinder, with a diameter of 18.5 mm and a length of 247 mm. The lever was built in a way that the axial displacement of the stack is converted into a sideways motion. Numerical simulations permitted to optimize the shape of the lever and the behavior of such actuators at the frequencies of interest. The chosen T lever provided a 5-fold amplification and the amplitude of the whole actuator reached 200 μm at frequencies up to 300 Hz. Finally, materials such as aluminum, copper, and titanium were chosen as building materials for their nonmagnetic and limited artifacts properties.

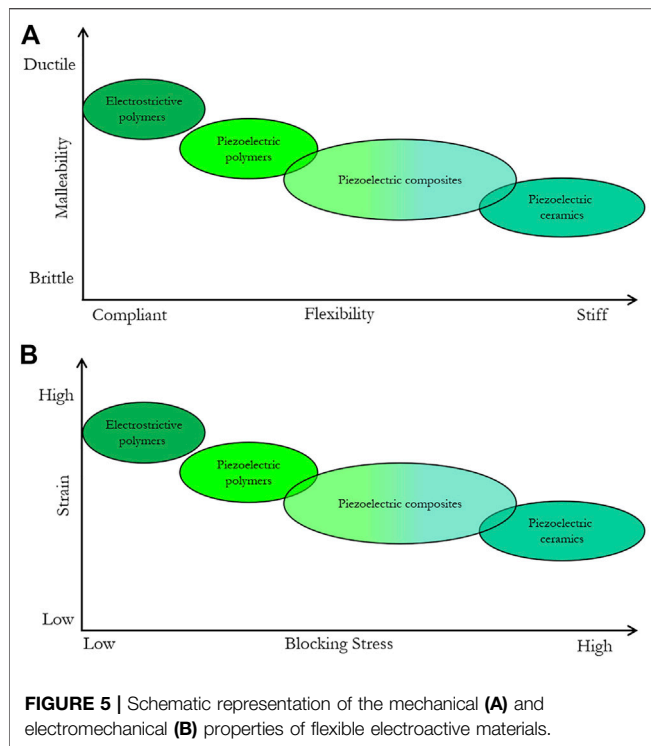
Tse et al. [74] built an actuator using the same principle but with an array of 10 bimorph ceramic piezoelectric disks and an L-shape lever that provided a 3-fold amplification. This actuator reached 336 μm amplitude at 400 Hz and 278 μm at 100 Hz.

As mentioned in [61, 72, 73], lever structures are convenient to build and provide significant amplitude amplification but their topology generally presents low natural frequencies, especially when the load increases over 3 g. This issue has been tackled in two different ways.

First, Arani et al. developed a symmetric 5-bar CMA brass structure [56] that can be seen on CAD modelling in **Figure 4B**.

Its advantages over the more common CMA topologies are the compactness, the absence of lateral displacement and the high natural frequency under various loads. In the laser vibrometer measurement, the CMA structure showed an amplification ratio from 10 (with a 325 g load) up to 70 (unloaded) at the respective natural frequencies ranging from 200 Hz up to 450 Hz. This paper put an emphasis on characterizing the actuator frequency response with regards to the loading. It resulted that while its natural frequency was dependent on the load in the low load regime (from no load to 240 g), its response stabilized at around 200 Hz and 240 g. The whole structure reached over 200 μm amplitude at 200 V. In the MRE experiments conducted on a gelatin phantom, the designed structure achieved a 7.6-fold amplification within the tissue mimicking phantom at a natural frequency of 200 Hz when loaded with an endorectal coil weighting 276 g. This work paved the way to reliable higher frequency MRE applications as the whole structure can be downsized, hence resulting in a higher natural frequency needed to perform MRE of small samples. Moreover, in case of high load, improving the amplification allows to reduce the input voltage which may result in a longer operating time before overheating of the stacks. This leads to additional degrees of freedom to perform the MRE acquisition.

Second, Meinhold et al. proposed a tunable resonant actuator using two mobile masses sliding on the actuator [55]. The amplified motion was perpendicular to the actuation of the ceramic. The design of this resonator was constrained by the size of the bore diameter of a small animal MRI scanner and the height of a commercially available piezoelectric stack. Two small linear piezoelectric actuators controlled the movement of the two masses between three locations: 1, 5 and 10 mm away from the center. The actuator was built in 655 silicon bronze and its dimension was determined in order to obtain a working frequency range of 680–1,117 Hz which corresponds to the high frequency range of MRE applications. The MRI compatibility of the resonator in the passive mode at a distance of 10 cm from a silicone rubber phantom was tested in a 7T scanner and showed no imaging artifacts. However, no tests have been conducted in the active mode. The proposed



actuator opens up the possibility of tuning, in the high frequency domain, an amplified actuator.

Non-amplified Piezoelectric Actuators

Using bending elements is another solution to increase the displacement amplitude of piezoelectric actuators. This relies on the bending of the tip of a bimorph piezoelectric to provide a displacement. Displacements are greater than the ones of regular longitudinal piezo ceramics. Alongside with Polyvinylidene fluoride (PVDF) films they find usage for example in low frequency energy harvester.

Chan et al. developed an actuator based on a piezoelectric bending element coupled with an acupuncture needle, produced a longitudinal motion of 200 μm without using a mechanical amplifier [53]. In this work, MRE experiments and comparison with an external driver positioned at the surface of the tissue were performed at 50, 100, 150 and 200 Hz. This work demonstrated that, compared to a surface actuator, this design allows to reduce the orientation-related error in wavelength estimation. However, the invasiveness of this actuator prevents its use for *in vivo* MRE.

Another group reported the development of an actuator for mouse brain MRE also based on a piezoelectric bending element connected to a vibrating bite bar [75]. The bite bar was made out of copper because of its rigidity and its nonmagnetic properties. The setup was mechanically characterized in loaded conditions using a laser vibrometer measurement to work at the resonance frequencies of the actuator. Taking advantage of the bending element, no mechanical amplification was needed to reach a displacement amplitude of 300 μm within a 1% agarose phantom and 30 μm within a mouse brain both at 877 Hz. A detailed view of this actuator can be seen in **Figure 4C**.

To our knowledge, bending elements have been more used in preclinical applications where the mechanical loading applied on the vibration generator is generally lower, avoiding the main drawback of this class of piezoelectric actuators: the low blocking stress which corresponds to the maximum stress generated by the actuator.

FROM PIEZOELECTRIC MATERIALS TO ELECTROACTIVE MATERIALS AND THEIR NON-MRE APPLICATIONS

Unlike electromechanical transducers, piezoelectric actuators do not suffer from any orientation limitation with regards to the static field. Nonetheless, bulk ceramic piezoelectric actuators are currently limited to a one-plane excitation and as mentioned earlier, to limited strain. One solution to this issue consists of using flexible electroactive materials to produce conformal actuators. These actuators can be made of the three following categories: piezoelectric composites, piezoelectric polymers and electrostrictive polymers. **Figure 5** presents different types of electroactive materials and their operating ranges in terms of strain vs stress and ductility vs stiffness. This graph shows that this type of new materials can allow a wide range of working domains for soft actuators from moderate stress/low displacement to low stress/high displacement. This field is rapidly evolving and, in this article, our aim is to explain their nature, the current applications and their potential for MRE. More information about basic principles and commercial application of electroactive materials can be found in [76].

Piezoelectric Composites

Piezoelectric composites have been known for a long time [77, 78]. These materials are made of a piezoelectric inorganic phase combined with a polymer matrix (in most cases not piezoelectric) [79, 80]. The goal is to combine the strong piezoelectric performances of the inorganic ceramics with the mechanical properties of polymers.

Flexible piezoelectric composites have been used as sensors for biomedical applications like the monitoring of biological low frequency signals like cardiac pulses [81–83]. Most of the studies deal with piezo sensors, but some flexible actuators have also been conceived. Dagdeviren et al [84] created a conformal array of actuators and sensors using a PZT composite for soft tissue characterization through mechanical actuation. Each actuator was made with a gold electrode and a platinum electrode deposited around a layer of nanoribbons of PZT before the whole stack was encapsulated in Polyimide. The array was designed to act simultaneously as an actuator inducing a mechanical excitation from 100 to 1,000 Hz in a tissue and a sensor able to detect small deformation at the tissue surface induced by the actuator part. In their work, the elasticity of various tissues was successfully determined. For *ex vivo* samples, both lung and heart were characterized. *In vivo*, the conformal modulus sensor was able to detect a difference in elastic modulus between a normal skin and a lesion. Arrays made of piezoelectric composite materials have also been used extensively for ultrasonic

transducers [85] and flexible transducers were designed using bulk PZT, polyimide and PDMS [86].

Piezoelectric Polymers

Ferroelectric polymers exhibit an intrinsic piezoelectric effect. There are various polymers exhibiting piezoelectricity, but the best known are mainly polymers based on PVDF and its copolymer of trifluoroethylene (PVDF-TrFE) [87], odd polyamides [88], and polymers of the polyurea [89]. However, the polymers of the PVDF (homopolymer or copolymer) have the highest piezoelectric properties and are therefore the most commonly used in applications. Using a cantilever structure based on a PVDF film, a displacement amplitude of 160 μm was reached [90] but without attempting to create vibrations. Furthermore, a bimorph actuator using two PVDF layers validated the use of such materials with a frequency range of 10 Hz–3 kHz reaching displacement amplitude of a few micrometers [91].

Electrostrictive Materials

During the last decade, much interest has been devoted to electrostrictive organic materials due to their large strain induced under electric fields, low weight, good mechanical properties, and easy processability. Electrostriction is a common phenomenon encountered in dielectric materials placed under the influence of an external electrical field that generates Maxwell stress which leads to material strain [92, 93]. Any dielectric material is electrostrictive by nature, but strain/stress effects are more pronounced in some of them. Such materials show promising potential in various applications where electromechanical conversion is required, such as low frequency energy harvesting or actuation in micropumps and artificial muscles. Despite lower generated stress than piezoelectric materials, electrostrictive polymers present high levels of electric field induced strain, more than 10-fold higher than piezoelectric fluorinated polymers at constant electric field. Among electrostrictive materials, we choose to underline two types of polymers that show, to our opinion, promising perspectives for the field of MRE actuation: Electrostrictive Polymers (EP) and Dielectric Elastomers (DE).

In addition to piezoelectricity, PVDF and its copolymers also exhibit electrostriction. However, plasticizers are needed to improve the electrostriction behavior and increase the strain that those materials can achieve [94, 95]. As an example of such EP, doped PVDF-TrFE films offer important relative strain (1–5%) under low electrical field. One example of a mechanical actuator based on EP was reported in [96] where a micropump diaphragm was developed. Quasi-constant displacement amplitude of 10–15 μm over a frequency range of 0.1–1000 Hz under high electrical field of 80 V/ μm was achieved. The frequency range investigated here matches the MRE one but the required electrical field could lead to image artefacts.

Interest in DE increased since Pelrine et al. reported on a commercially available acrylic elastomer able to reach a relative strain of 100% under applied voltage [97]. More theoretical details about those materials can be found in [98]. While their

strain can reach higher relative values than other electroactive materials, they usually have low stresses below 10 MPa compared to a hundred of MPa for classical bulk piezoceramic. The mechanical characterizations conducted in [97] of the acrylic elastomer VHB 4910 showed a rapid decrease of strain with frequency which limits its use to low frequency actuation (tens of hertz). However, further studies showed that other material like CF19-2186 silicones can be used at higher frequencies, but with lower achievable strains. In the context of MRI, Vogan et al. [99] used an acrylic DE to reconfigure imaging coils within the bore of a scanner which illustrates both the relatively high blocking stress of those materials (up to half the maximum stress of bulk piezoceramics [100]) and the MRI compatibility of the experimental setup with a DC voltage applied to the polymer [101].

PERSPECTIVES WITH FLEXIBLE ELECTROACTIVE MATERIALS FOR MRE MECHANICAL ACTUATOR

Pros and Cons About Current Mechanical Actuators for MRE

The quality of the MRE data and elasticity estimations are dictated by the strain-to-noise ratio as explained by [102–104]. However, a mechanical actuator that can generate high wave amplitudes within the region of interest of the investigated tissue is mandatory. To our knowledge, it is unclear exactly what minimum amplitude is necessary for reconstructing an elasticity map. However, none of the actuators shown in **Figure 3** have amplitude within the tissue below 7.5 μm . In addition, the mechanical actuator should operate at a well-suited operation frequency that offers the best compromise between spatial resolution and attenuation at a given depth. This mechanical actuator should also be able to run multi-frequency experiments in order to characterize the underlying microarchitecture of the tissue from the frequency dependence of the mechanical properties [38, 52, 105, 106].

As presented in this manuscript, electromagnetic compatibility restrictions near an MRI scanner require in most cases to place the vibration generator away from the center of the scanner. Therefore, a more or less flexible and long coupling device attached to a piston is needed to transmit the vibration to the patient/sample. As illustrated in **Figure 3**, actuators based on rigid coupling devices are the most frequently used in MRE experiments thanks to their high-fidelity at high frequencies (unlike actuators based on pneumatic coupling device). Nevertheless, the supplementary loading of the coupling device limits the choice of vibration generator that can be used. Larger loudspeaker, higher currents in the case of electromechanical actuators or mechanically amplified piezoceramic stacks must be used in order to create enough displacement under such loading conditions. Thus, the vibration generator is often oversized compare to the minimum requirement which is, as mentioned previously, only to generate few microns within biological tissues. This is particularly true in case of small soft samples (i.e. mainly

samples involved in preclinical studies) and in case of superficial organs such as skin [107], breast [49] or muscle MRE [108]. In case of adult brain MRE the question is much different as the generation of wave within the brain relies on both piston-skull and skull-brain coupling. Moreover, the use of a coupling device is often associated with complicated positioning and lack of integration which can limit experimental repeatability and reproducibility.

Regarding the piston, **Figure 3** shows that most of them are rigid despite the poor sample coupling that results from it. To our knowledge few groups have design rigid form fitting piston which makes sense to access soft tissue like breast [49], but which is more questionable for the head [40, 109]. Having a flexible form-fitting piston could ensure better sample coupling, in particular when the piston is in contact with a soft surface. To our knowledge only one group reported a flexible form-fitting piston dedicated to endorectal MRE, where the piston is in contact with a soft surface [56]. In this case, an inflatable flexible piston was designed in order to fit as much as possible to the colon wall. In this case, one major drawback is that the piston-sample coupling will critically rely on tissue stiffness which make measurements intra and inter patient dependent.

As seen on **Figure 3**, most of clinical and human *in vivo* applications of MRE are in the low range of frequencies (up to 100 Hz). Looking at the wave propagation equations it appears that reaching great depth with great amplitude is easier in this range than it would be at higher frequencies. Pneumatic devices have the drawbacks of inducing phase delay and non-harmonic mechanical excitation. However, these effects are minor in the low frequency range. Moreover, pneumatic devices are the easiest to handle among different coupling devices which explains their widespread use in that frequency range. In the preclinical environment (i.e., small samples in a small-bore preclinical system) where higher frequencies are required, piezoceramic and electromechanical are the go-to solutions as shown on **Figure 3** even though they require a more complex set up than pneumatic devices. In fact, attention must be paid for instance to the following fine-tuning operations: i/cable shielding for the high voltage supply in the case of a piezoelectric vibration generator; and ii/the orientation of the electromechanical actuator with regards to the static magnetic field.

Towards Integrated and Flexible Mechanical Actuators

There is room for improvement in both clinical and preclinical MRE applications. Smaller, easier to use and more adaptable actuators could generalize the use of MRE in the MRI community. In the path towards a more optimal mechanical actuator two objectives prevail:

- Reducing the number of parts that compose the actuator in order to reduce the load on the vibration generator. A lower load would allow for low stress soft actuators to be used. A reduced number of parts would also result in more compact

mechanical actuator which may simplify positioning of the MRE setup in the limited space of the MRI system.

- Using flexible form fitting pistons in order to achieve better sample coupling.

Flexible electroactive materials can be a key component of a new generation of mechanical actuators fulfilling the objectives previously exposed. As discussed above, these materials can require an electric field up to tens of V/ μ m in order to reach significant strain. Studying the MRI compatibility of working at such high electric fields is the first step towards reducing the number of parts for a better integration of the actuator. If the MRI compatibility of the electroactive material is validated, the piston would be the vibration generator itself without needing a coupling device. The mechanical actuator could be more compact and integrated to the MRI coil and positioned, just as a tiny patch for cardiac monitoring, directly in contact with the patient/sample. Their low blocking stress would no longer be a major issue and their compliance combined with a high strain would make them the perfect material candidate to play the role of a form-fitting mechanical actuator in contact with the patient/sample. As mentioned before, this actuation principle could work preferentially on small soft samples and in case of superficial organs. Nevertheless, to amplify the effect of such “elastography patch”, one could combine such actuators in arrays to increase the penetration depth without increasing too much the applied electrical field.

To our knowledge, only [110] succeeded in building a Dielectric Elastomer (DE) and tested its MRI-compatibility on a phantom inside a 3-T MRI scanner. In their work, they started by studying the effect of the high magnetic fields produced by the scanner on the mechanical properties of their actuator and saw no difference regarding whether the actuator was inside or outside the bore of the scanner. Moreover, they also analyzed the image artifacts that their actuator would create. No SNR decrease was observed while increasing the electric field through the elastomer. Both studies helped the authors conclude that DE actuators are fully MRI-compatible and could be used in various MRI-specific applications directly in contact with a biological tissue. However, translating this work into the context of MRE would require additional measurements such as frequency response of the material as well as its achievable motion amplitude in loaded and unloaded conditions. The mechanical capabilities of the actuator were evaluated using a classical Stress/E-field curve but wave amplitude/phase and elasticity studies were not run on a phantom. Additionally, the field-induced image artifact has been characterized up to 8 V/ μ m and it would be interesting to see if their results remain true at higher electrical fields. Nonetheless, this work is paving the way for a broader use of this type of material in MRE.

Available Commercial Flexible Materials Suited to Design MRE Actuators

From piezoelectric composites materials to dielectric elastomers a wide range of flexible materials have been developed and optimized which make them suitable for applications in

energy harvesting, sensing and actuation. The frequency range, the strain and the stress achievable with such materials are compatible with the requirements to build MRE mechanical actuators. Moreover, the relative high “technology readiness level” (5–7 [76]) of these mechanical actuators explains the recent development of commercial solutions that could be adapted to MRE experiments.

As a matter of fact, ready to use PVDF-based transducers are commercially available (Precision acoustics, TE Connectivity, PolyK, Durham Instruments etc). However, they tend to be dedicated to ultrasonic transducing. Nonetheless, thin films of PVDF are also available from the same suppliers in order to build one’s own actuators with specific sets of boundary conditions (clamping, thickness of the electrodes, stiffness of the electrodes, loading) to tailor the frequency behavior of the actuator.

Additionally, Sateco developed an actuator based on a multilayer stack of DE (silicone with carbon electrodes) with a wide operating frequency range from 0 to 2 kHz and a blocking force around 10N. The company has a starter kit with an all-in-one solution containing the actuator and all the necessary software and controllers.

After being actively involved in the field of DE [111, 112], Danfoss shut down its production of their DE solution called InLastor which could achieve an elevated blocking force of 16N, a relative strain of 2–3% and with rather low operating frequency (<100 Hz).

Another commercial solution sold by PI under the name of DuraAct is a flexible piezoelectric actuator based on a thin piezoceramic film. Custom versions with the necessary software and controllers can be ordered for specific applications such as structural health monitoring or precise actuation. The blocking force of the Power Patch model can reach 44N for a 10 $\mu\text{m/V}$ relative axial deformation while working from -20 V up to 120 V.

Smart Material commercialized an alternative to the DuraAct transducers with a piezocomposite solution called Macro Fiber Composite (MFC). The M8557P1 version reaches 150 μm of axial displacement with a blocking force of 900N and an operating range of -500 V up to 1500 V with a maximum working frequency of 10 kHz. As for the previous commercial solutions, Smart Materials also provide the drivers for their actuators.

To perform MRE experiment with one of these commercial transducers will probably require a slight adaptation of the setup

to be able to work in the constraint MRI environment. Particular attention must be paid to the MRI compatibility of the actuator that is often compromised by the presence of welds, electric cables and packaging parts. Fortunately, these are not always necessary for the proper operation of the actuator and can be removed with caution on a case-by-case basis.

In conclusion, recent progress made in the field of flexible electroactive materials could lead to a breakthrough in the field of actuators for MRE. These materials offer the possibility to design form-fitting actuators that are MR-compatible and can achieve relatively high displacement amplitudes with moderate blocking stresses. With few frequency limitations, they open a new path toward more adaptability, greater ease-of-use and more compactness of dedicated actuators for MRE of small soft samples and superficial organs such as skin, muscles or breast. These technological advances could be the way towards MRE experiment as easy to perform than ultrasound elastography but in 3D and with a better spatial resolution.

AUTHOR CONTRIBUTIONS

J-LG, S-AL conceived the manuscript. J-LG, J-FC, S-AL wrote the manuscript. TG, PL, VS, P-JC, MC, revised and edited the manuscript.

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In Hamstring Muscles of Patients With Knee Osteoarthritis an Increased Ultrasound Shear Modulus Indicates a Permanently Elevated Muscle Tonus

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Background: Some patients with knee osteoarthritis (KOA) show pain, stiffness and limited flexion and extension at the back of the knee, leading to dysfunction and affecting life. This may be related to changes in the biomechanical properties of skeletal muscles. Shear wave elastography (SWE) can detect these changes by measuring muscle shear modulus.

Aims: To investigate hamstring muscle shear modulus of healthy people and patients was studied using SWE method, and the correlation analysis between the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score of patients' subjective feeling and shear modulus of objective quantification was conducted.

Methods: The hamstring shear modulus was measured by SWE in 50 patients and 50 healthy individuals. Pearson correlation coefficient was used to evaluate the correlation between hamstring stiffness and shear modulus in patients.

Results: The hamstring shear modulus were significantly higher in the KOA group [the semimembranosus (SM) 15.23 ± 7.23 , the semitendinosus (ST) 15.94 ± 5.40 , the biceps femoris long tendinitis (BFL) 14.21 ± 6.55] than in the control group (the SM 10.95 ± 2.41 , the ST 11.25 ± 2.23 , the BFL 9.98 ± 2.81) ($p = 0.000$, $p = 0.000$, $p = 0.001$). The hamstring shear modulus in the KOA group was moderately positively correlated with pain, shear modulus, and physical function score.

Conclusion: Preliminary results show that the shear modulus of the hamstring of KOA patients is higher than that of healthy people, the WOMAC score and the shear modulus of patients are moderately correlated. These preliminary results show that ultrasonic shear wave elastography measurement of shear modulus may be enough to sensitive, can detect these effects, more targeted in order to assist the doctor's diagnosis and treatment.

Keywords: elasticity imaging techniques, hamstring, shear modulus, ultrasonography, osteoarthritis

INTRODUCTION

Knee osteoarthritis (KOA) is the most common form of arthritis, and the leading cause of disability and pain affecting middle-aged and elderly people worldwide (Reginster, 2002; Kurtais et al., 2011), caused by structural changes in joints resulting in pain, deterioration of function, and disability (Ding et al., 2007; Hunter, 2007). In clinical practice, we found that some patients with KOA presented with stiffness in the back of the knee, pain in traction, limited flexion and extension, and even abnormal gait. It has been hypothesized that patients with KOA present with excessive muscular co-contraction during gait which may help stiffen and stabilize the knee during gait through increased compressive forces and joint loading (Lewek et al., 2004; Mills et al., 2013; Brandon et al., 2014). Skeletal muscle undergoes structural changes with abnormal prolonged contraction of muscles which may alter its biomechanical properties. Histologically, changes in biomechanical properties is associated with changes in muscle composition including myosteatosis, myofibrosis (Faulkner et al., 2007) and dysfunction in extracellular elastic fibers (Kragstrup et al., 2011). Such changes may alter the biomechanical properties resulting in changes to muscle stiffness, limited movement, muscle pulling pain. Therefore, it is meaningful to evaluate the biomechanical properties of the muscle around the knee to further explore its biomechanical mechanism and provide standard guidance in rehabilitation plans.

Large number of studies have shown that the increase in muscle tension in patients is accompanied by changes in muscle stiffness. Guo et al. (2016) found that patients with neck and shoulder myofascial pain have increased trapezius muscle tone, and SWE can detect that the shear modulus of the trapezius muscle is higher than that of normal people. Mitsuhiro Masaki, Tomoki Aoyama and other studies found that medical staff with low back pain were accompanied by low back muscle stiffness (Masaki et al., 2017). SWE found that the multifidus muscle elastic modulus of the low back pain group was higher than that of the asymptomatic group. They believed that the back pain was related to the stiffness of the multifidus muscle. In the clinic, we found that some KOA patients have symptoms of stiffness on the back of the knee, traction pain, and limited mobility (Michael et al., 2010; Sharma, 2021). We believe that these symptoms are related to the stiffness of the hamstrings. At present, the muscle stiffness on the back of the knee has not been clearly clarified because it is often difficult to quantitatively assess the stiffness of individual muscles. However, it has become possible to use ultrasonic shear elasticity imaging to measure the shear elastic modulus. As early as 1991, the technology of ultrasound elastography was first proposed by Ophir et al. (1991). The basic principle is to display the different hardness of the tissue according to the degree of deformation of the tissue after being stressed, thereby reflecting the occurrence and development of the disease, and providing help for clinical diagnosis and treatment. Subsequently, in 1998, the shear wave elastic imaging technology proposed by Sarvazyan et al. (1998) and Catheline et al. (1999). The principle is that the sound source vibrates to generate sound waves. The tissue particles are caused to vibrate

laterally, thereby generating shear waves, and by tracking the propagation speed of the shear waves, the absolute value of the tissue elasticity-Young's modulus ($E = 3\rho c^2$, E : Young's modulus; c : shear wave propagation velocity; ρ : tissue density) is obtained, so as to quantitatively analyze and compare the elastic differences between the organizations, aid in clinical diagnosis.

Shear modulus of elasticity is used as an indicator of muscle stiffness. Previous studies have shown that SWE is an appropriate and reliable method for evaluating the shear modulus of muscle (Leong et al., 2013; MacDonald et al., 2016). The purpose of this study was to explore the difference in hamstring shear modulus between KOA patients and normal people, and the relationship between WOMAC subjective function score and hamstring shear modulus in KOA patients.

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score is widely used and has been validated mainly in the context of KOA since the 1990s (Bellamy et al., 1988; Davies et al., 1999; Escobar et al., 2002). It evaluates pain, function, and stiffness during everyday activities. Each score ranges from 0 to 10, with 10 being the worst outcome. But WOMAC functional scoring is based on the subjective feelings of the patient, objective quantitative analysis of muscle in patients with KOA is still lacking. Ultrasound shear wave elastography (SWE) is a new quantitative method used for assessing tissue shear modulus. It has been widely utilized in breast, liver and thyroid, it is also gradually applied to the research of skeletal muscle system (Sigrist et al., 2017). SWE can be used as a non-invasive diagnostic tool to evaluate the elastic modulus of soft tissues such as muscle ligaments in real time. It is simple to operate and has no radiation hazards. At the same time, it not only used to observe the overall shape of the soft tissue, but also to set interesting areas of the soft tissue to be inspected. Through the color depth, we can directly show the small elasticity of the target tissue. Moreover, through SWE, the elastic modulus of the soft tissue in the loosening state, active motion, passive motion state, and physical state and pathological state can be accurately evaluated.

In clinical practice, we found that some patients with KOA have symptoms of posterior knee stiffness, traction pain, and limited flexion and extension. We believe these symptoms are related to the shear modulus of the hamstring. Therefore our primary aim was to investigate to what extent hamstrings muscle shear modulus measured by SWE differed amongst KOA patients and healthy people of the same age. The secondary aim was to investigate the correlation between hamstring muscle shear modulus measured by SWE and WOMAC scores of subjective feelings in KOA patients.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Ethics Committee of Luoyang Orthopedic Hospital in Henan Province. Prior to the study, the steps of the experiment were fully explained to each subject. All subjects provided written informed consent before the

experiment, and all research procedures followed the principles of the Helsinki Declaration.

Subject

We recruited KOA patients aged 50–60 years, all of whom had symptoms of posterior knee stiffness, posterior knee traction pain, and limited flexion and extension. Among them, there were 22 males and 28 females (age 54.68 ± 3.25 years old), an average height of 163.46 cm and an average weight of 69.35 kg. Meanwhile, we recruited healthy subjects aged between 50 and 60, including 18 males and 32 females (age 53.85 ± 2.16 years old), their average height was 163.50 cm, and their average weight was 70.61 kg. At the same time, subjects were selected based on the following factors (Alfuraih et al., 2019): (1) no previous history of musculoskeletal or neurological disorders; (2) not currently taking or previously taken a corticosteroid treatment for the past 3 years with doses >5 mg/day; (3) not currently taking or previously taken a HMG-CoA reductase inhibitors (statins) for the past 3 years. The Luoyang Orthopedic Traumatological Hospital of Henan Province (Henan Provincial Orthopedic Hospital) approved this study, and each subject provided written informed consent to participate.

Ultrasound Imaging With Shear-Wave Elastography

Shear wave elastography was performed using the two-dimensional Aixplorer (Supersonic Imagine, Aix-en-Provence, France) system using the SuperLinear SL10-2 MHz probe. The scan was limited to the dominant side since limb dominance had no significant impact on muscle shear modulus (Alfuraih et al., 2018). Before scanning, all participants were placed in a prone position on the scanning bed and were asked to relax and be comfortable for 5 min. All participants were asked to refrain from any strenuous or sporting activities at least 1 day prior to the study to minimize possible confounding exercise effect. The transducer was oriented in the transverse plane over the region of interest on the measured muscle, and then the mode of SWE was activated to examine the shear wave modulus of the muscle or tendon. During the acquisition of the mode of SWE, the transducer was kept motionless for above 3 s (Zhou et al., 2019). Then the gray scale image was shown the appearance of the muscle under the longitudinal section. Image quality was closely monitored throughout measures. When the color in the ROI was uniform and several muscle fibers were continuously visible, the images were frozen, and then put the Q-box to obtain the shear wave modulus from the system and stored for off-line analysis (kPa) (Zhou et al., 2020). Three images were captured at each measurement site of tendon and muscle. The mean of the shear modulus from all three images were used for further analyses.

The hamstrings measurement position: (Alfuraih et al., 2017, 2019).

(1) Semimembranosus (SM): the junction of lower 1/3 and middle 1/3 of the mid-point of the popliteal transverse stripes from the mid-point of the gluteal transverse stripes. (2) Semitendinosus (ST): posterior medial side of the thigh, across the gluteal line to the middle of the popliteal line. (3)

Biceps femoris long tendinitis (BFL): posterolateral thigh parallel to semitendinosus.

From each elastography image, the spatial average of the shear modulus (kPa) in an 8-mm circular area was determined. The musculoskeletal mode was used to estimate the shear modulus of the upper trapezius muscle with temporal averaging (persistence), penetration mode, and 85% opacity. The range of the color scale was adjusted from 0 to 200 kPa. The values were averaged across three measurements in each condition. The probe was placed on top of the skin with a minimal load ensuring no external pressure could affect the measurements.

Western Ontario and McMaster Universities Osteoarthritis Index Knee Osteoarthritis Evaluation Scale

The WOMAC has demonstrated reliability, validity and responsiveness in patients with KOA (Bellamy et al., 1988). It is comprised of sub-scales (24 items), including pain (5 items), stiffness (2 items), and physical function (17 items). This scale, which evaluated osteoarthritis-related disability in the hip and/or knee osteoarthritis, is also very sensitive to the changes in health states. As scores increase in all dimensions, the intensity and severity of the complaint also increase (Bellamy, 2002).

Data Analysis

Data analysis was performed using the SPSS 22.0 program. The cases distribution was evaluated by Kolmogorov–Smirnov test, and according to the results of this test, there was normal distribution. For that reason, we have used parametric tests for statistical evaluations to compare means. Pearson correlation analysis was used to evaluate the correlation between the hamstring shear modulus and WOMAC score. *p* values of less than 0.05 were defined as statistically significant.

RESULTS

Demographic Data

Demographic information including age, gender, and BMI, for all subjects are shown in **Table 1**. Forty-six patients were included in the KOA group. Forty-six healthy subjects served as control group. These two groups were similar regarding demographic findings (**Table 1**).

TABLE 1 | Patients' demographic findings.

		Observation (N = 50)	Control (N = 50)	<i>p</i> value
Age (years)		56.87 $\pm$ 8.41	56.15 $\pm$ 8.94	0.693
Gender	Male	22 (44%)	18 (36%)	0.414
	Female	28 (56%)	32 (64%)	
BMI (kg/m ²)		24.60 $\pm$ 3.67	23.6 $\pm$ 3.43	0.872

BMI, body mass index. Data are expressed as frequency (percentage) or mean $\pm$ standard deviation.

TABLE 2 | Intra- and interoperator reliabilities for assessing the three hamstrings stiffness.

	SM			SL			BFL		
	Mean $\pm$ SD	SEM	MDC	Mean $\pm$ SD	SEM	MDC	Mean $\pm$ SD	SEM	MDC
Operator A in test 1	12.91 $\pm$ 2.78	0.62	1.72	12.83 $\pm$ 2.76	0.62	1.72	11.27 $\pm$ 3.21	0.62	2.0
Operator A in test 2	12.37 $\pm$ 2.82	0.63	1.75	12.45 $\pm$ 2.23	0.50	1.39	11.56 $\pm$ 3.84	0.86	2.38
Operator B	12.32 $\pm$ 2.42	0.54	1.50	12.85 $\pm$ 2.64	0.59	1.64	11.51 $\pm$ 3.38	0.76	2.11
ICC ^a (95% CI)	0.84 (0.43–0.94)			0.85 (0.45–0.94)			0.94 (0.73–0.98)		
ICC ^b (95% CI)	0.97 (0.86–0.99)			0.98 (0.88–0.99)			0.98 (0.89–0.99)		

ICC, intraclass correlation coefficient; CI, confidence interval; SEM (kPa), standard error of measurement of kPa; MDC (kPa), minimal detectable change; SD (kPa), standard deviation of kPa; kPa, kilo Pascal.

^aIntraoperator reliability.

^bInteroperator reliability.

Intra- and Interoperator Reliabilities

The related statistical parameters for intra- and interoperator reliabilities for assessing the hamstrings shear modulus are summarized in **Table 2**. The mean shear modulus values of the SM, SL, and BFL were 12.91 kPa, 12.83 kPa, and 11.27 kPa for operator A in test 1; 12.37 kPa, 12.45 kPa, and 11.56 kPa for operator A in test 2; 12.32 kPa, 12.85 kPa, and 11.51 kPa for operator B. The intraclass correlation coefficient (ICC) values of intraoperator reliability were good in SM [ICC = 0.85; 95% Confidence Interval (CI) = 0.45–0.94; standard error measurement (SEM) < 0.63 kPa; and MDC < 1.75 kPa], in SL (ICC = 0.84; 95% CI = 0.43–0.94; SEM < 0.62 kPa; and MDC < 1.72 kPa) and excellent in BFL (ICC = 0.94; 95% CI = 0.73–0.98 SEM < 0.86 kPa; and MDC < 2.38 kPa). The ICC values of interoperator reliability were excellent in SM (ICC = 0.97; 95% CI = 0.86–0.99; SEM < 0.63 kPa; and MDC < 1.75 kPa), in SL (ICC = 0.98; 95% CI = 0.88–0.99; SEM < 0.62 kPa; and MDC < 1.72 kPa) and in BFL (ICC = 0.98; 95% CI = 0.89–0.99; SEM < 0.86 kPa and MDC < 2.38 kPa).

Differences of the Hamstring Shear Modulus Between the Knee Osteoarthritis and Control

The comparison of the hamstring shear modulus between the two groups is demonstrated in **Table 3**. The hamstring shear modulus were significantly higher in the KOA group than in the control group ($p = 0.000$, $p = 0.000$, $p = 0.001$).

Correlation Between the Hamstring Shear Modulus and Western Ontario and McMaster Universities Osteoarthritis Index Score in the Knee Osteoarthritis Group

The correlation between the hamstring shear modulus and WOMAC score in the KOA group is demonstrated in **Table 4**. The hamstring shear modulus in the KOA group was moderately positively correlated with pain, stiffness, and physical function score.

TABLE 3 | Shear modulus (kPa) of the three hamstring in two groups.

	SM	ST	BFL
Observation ($N = 50$)	15.23 $\pm$ 7.23	15.94 $\pm$ 5.40	14.21 $\pm$ 6.55
Control ($N = 50$)	10.95 $\pm$ 2.41	11.25 $\pm$ 2.23	9.98 $\pm$ 2.81
p value	0.000	0.000	0.001

TABLE 4 | Correlation between the three hamstring shear modulus and WOMAC score in the observation group.

		SM	ST	BFL
Pain	r	0.477	0.519	0.631
	P	0.001	0.000	0.000
Stiffness	r	0.437	0.461	0.539
	P	0.002	0.001	0.000
Physical function	r	0.605	0.543	0.605
	P	0.000	0.000	0.000

DISCUSSION

In the present study, we found that SWE is a feasible tool for evaluating hamstring muscle shear modulus in patients with knee osteoarthritis, with good intra- and interoperator reliabilities. This study is the first to compare the hamstring muscle shear modulus of patients with knee osteoarthritis with that of healthy volunteers. The results show that the hamstring muscle shear modulus is significantly different between healthy volunteers and patients with knee osteoarthritis. The hamstring muscle shear modulus of patients has a negative correlation with the functional score.

Cortez et al. (2015) reported that the medial gastrosoleus and tibialis muscles had fair to good levels of intraobserver (ICC, 0.42–0.70) and interobserver (ICC, 0.69–0.73) reliability. Taş et al. (2017) reported that intraobserver reliability (ICC, 0.91–0.92) and interday reliability (ICC, 0.81–0.83) were excellent, and interobserver reliability (ICC, 0.71) was good of SWE in the patellar tendon, and the intraobserver reliability (ICC, 0.93–0.94), interday reliability (ICC, 0.81–0.91), and interobserver reliability (ICC, 0.95) were perfect in the rectus femoris. Koppenhaver et al. (2018) reported that overall reliability estimates were fair to excellent with ICCs ranging from 0.44

to 0.92 in low back musculature, and reliability was higher in the lumbar multifidus muscles than the erector spinae muscles, slightly higher during contraction than during rest, and substantially improved by using the mean of three measurements.

In the present study, intraoperator reliability were good for assessing the SM (ICC = 0.85) and the SL (ICC = 0.84), intraoperator reliability was excellent for assessing the BFL (ICC = 0.94), but the interoperator reliability were excellent for assessing the SM (ICC = 0.97), the SL (ICC = 0.98), and the BFL (ICC = 0.98) corresponding to the results of SEM and MDC. The findings from this study have indicated that the SWE is a credible instrument for evaluating the hamstrings shear modulus.

According to the new findings of the preliminary study, the shear modulus of SM, ST, and BFL in patients with KOA with pain of posterior knee traction and limited flexion and extension significantly increased compared with normal people, and the higher the shear modulus of the muscles in the hamstring, the higher the WOMAC (pain, stiffness, physical function) score of patients' subjective feelings. The increase in the shear modulus of the patient's hamstring muscle reflects the change in muscle biomechanical properties, that is, the deterioration of the

elasticity of the muscle tissue, which in turn affects the functional activities of the knee joint of the patient, which explains the correlation between the two. These results suggest that shear wave elastography can quantitatively detect changes in hamstring shear modulus in KOA patients, in the form of shear modulus, which is closely related to patients' functional activities. These preliminary findings provide a proof-of-concept that would be beneficial to support this technique as it would assess the shear modulus of the diseased muscles of the patient and validate the WOMAC score. Many researchers have found that the technique can assess the effectiveness of interventions such as dry needles and manual interventions in muscles.

In the past (Hoang et al., 2009), two-dimensional ultrasound has been used to obtain rich morphological parameters, such as muscle thickness, cross-sectional area, muscle fiber length, feather horn, etc. To some extent (Cartwright et al., 2013), ultrasonic technology has been used to evaluate neuromuscular, tendon, ligament and joint lesions and guide rehabilitation treatment, but two-dimensional ultrasound cannot provide bone images. Mechanical properties of muscle system and anisotropy of muscle tissue also increase the limitations of two-dimensional

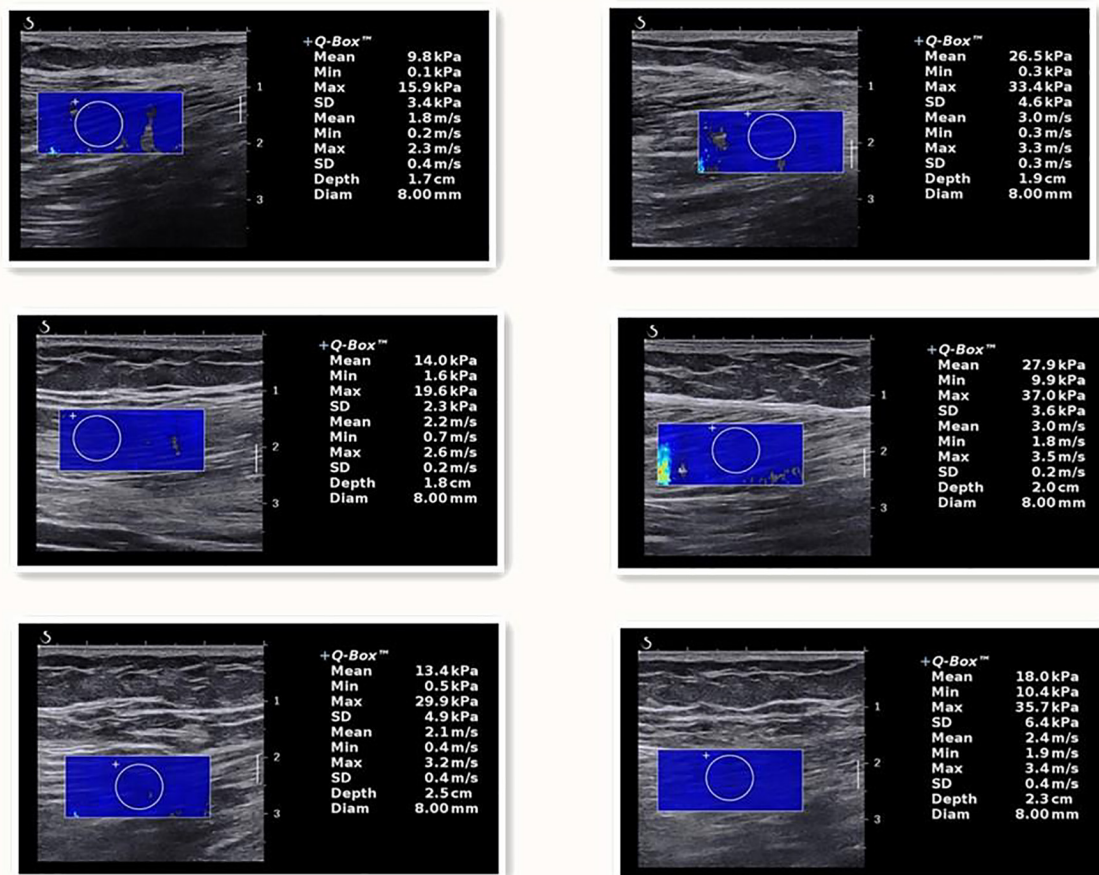
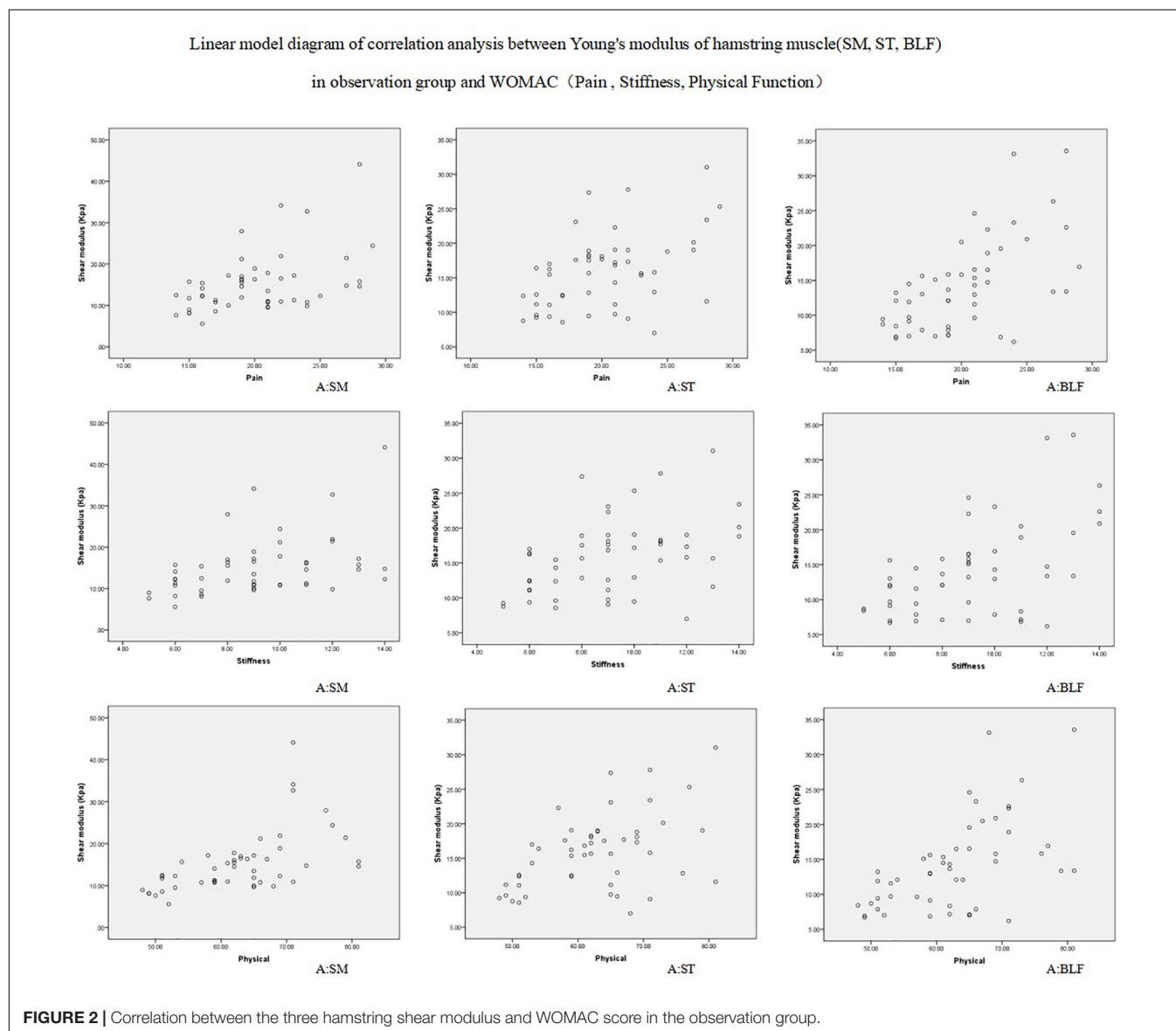


FIGURE 1 | The one on the left is listed as a healthy person, and the SM, ST, and BFL are listed from top to bottom. The one on the right is a patient, and the SM, ST, and BFL are listed from top to bottom.

ultrasound application (Botar et al., 2012). In recent years, French Aixplorer ShearWave real-time SWE ultrasonic diagnostic instrument was produced. Based on the automatic generation and analysis of real-time Shear Wave, young's modulus value, as an important biomechanical parameter, was obtained through the quantitative analysis system, so as to realize the full quantification of ultrasonic elastography and promote the extensive clinical research of SWE.

Rodrigues and Rodrigues Junior (2000) proposed that the elastic fiber system in the muscle extracellular matrix gradually loses its resistance with age, promoting the loss of normal muscle shear modulus. According to Alfuraih et al. (2019) research on the muscles of the lower limbs, the shear modulus of resting muscles gradually decreases throughout the adult life, especially in the elderly (>75 years old). Obviously, these are studies of healthy people. Maher et al. (2013) study

showed that the shear modulus of the trapezius muscle under the action of myofascial trigger points (MTrPs) was sensitive enough to detect the change of the dry needle treatment and the influence of posture on the shear modulus. The cause (Hubbard and Berkoff, 1993; Simons, 1996) may be abnormal activity in the endplate of neuronal movement at the end of muscle fibers at the MTrPs. Continuous muscle segment contracture leads to increased local metabolic demands, pressure on capillary circulation, and increased metabolic by-products, resulting in local muscle shear modulus. However, the etiology and pathophysiology associated with KOA are currently being studied but remain unclear. This study starts from the mechanical properties of muscle and discusses the relationship between muscle and function. To our knowledge, this is the first study to combine subjective functional scores with objective quantitative indicators.



Our findings show that tissue shear modulus can be visualized in real time, while the difference between the sick and the healthy are readily observed and displayed via a color-coded map (**Figure 1**). These findings may shed more light on the mechanisms by which patients experience pain, stiffness, and limited functioning. This study demonstrated the feasibility of SWE techniques for assessing muscle stiffness in KOA patients. But the muscle assessed in this study was a superficial muscle that is easy to palpate. The same cannot be said for deeper muscles such as those in the lumbar region or deep abdominal area. Consequently, an inability to palpate and reproduce the classic referral patterns is difficult to accomplish for the clinician. Shear wave elastography would provide such a means for an objective evaluation of tissues of different types and depths, and a means to determine the efficacy and longevity of therapeutic interventions. Currently (Kwon et al., 2012; Berko et al., 2014), there are still no relevant reports on the normal young's modulus of muscle tissue and the reference range of abnormal values, which will become the future research direction and play a greater role in the diagnosis of muscle injury, monitoring the development of diseases and evaluating the curative effect of diseases.

CONCLUSION

Preliminary results show that the hardness of KOA patients hamstring were higher than in healthy people, show that muscle hardness change is a major feature of KOA patients, the patients' subjective aspect WOMAC score has a moderate correlation, the greater the hardness of muscle, feel stiff and dysfunction in patients with more serious (**Figure 2**), these preliminary results show that ultrasonic shear wave elastography measurement of shear modulus may be enough to sensitive, can detect these effects, more targeted in order to assist the doctor's diagnosis and treatment.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by the Ethics Committee of Luoyang Orthopedic Traumatological Hospital of Henan Province. Prior to the study, the steps of the experiment were fully explained to each subject. All subjects provided written informed consent before the experiment, and all research procedures followed the principles of the Helsinki Declaration.

AUTHOR CONTRIBUTIONS

FL and Z-YW designed the study and drafted the manuscript. J-YG, Y-XG, and FL helped to conceive the study. Z-JZ, S-HS, and Y-RF helped to perform the statistical analysis. LZ, Y-BW, Y-FZ, Y-MF, and M-MF participated in the data collection. All authors reviewed and approved the final manuscript.

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Cardiac Strain Imaging Using Multi-Perspective Ultrafast Ultrasound

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The heart is a complex organ with a high level of deformation occurring in different directions. Ultrasound imaging has proven to be a valuable tool to quantify these deformations. However, strain is not always measured accurately in vital parts of the heart when only using a single probe, even at a high frame rate, because of the anisotropic spatial resolution and contrast. Therefore, a multi-perspective ultrafast ultrasound (US) strain imaging method was developed, aiming at investigating improvements in strain while operating two probes at different relative angles. In an *ex-vivo* experiment of a beating porcine heart, parasternal short axis views of the left ventricle (LV) were acquired by two phased array probes with different relative angles (30° – 75°) at a frame rate of 170 frames per second (FPS). A fully automatic registration algorithm was developed to register the image datasets for all cases. Next, radio frequency (RF) based strain imaging was performed. Axial displacements were compounded based on the unit axial vectors of the dual probes to improve motion tracking and strain estimation. After performing multi-perspective strain imaging, compounded radial and circumferential strain both improve compared to single probe strain imaging. While increasing the inter-probe angle from 30° to 75° , the mean tracking error (ME), mean drift error (MDE) and strain variability (SV) decreased, and signal-to-noise ratio (SNRe) increased for both strain components. For the largest angle (75°), large reductions in ME (-42%), MDE (-50%) and SV (-48%) were observed. The SNRe increased by 253 and 39% for radial and circumferential strain, respectively, and strain curves revealed less noise for each region. In summary, a multi-perspective ultrafast US strain imaging method was introduced to improve cardiac strain estimation in an *ex-vivo* beating porcine heart setup.

Keywords: ultrasound imaging, multi-perspective ultrasound, ultrafast imaging, cardiac strain imaging, image registration, strain fusion

INTRODUCTION

Speckle tracking echocardiography (STE) has emerged as a quantitative and comprehensive method to accurately estimate LV myocardial function and contractility [1, 2]. STE allows the measurement of myocardial deformation by tracking speckles on B-mode or RF data, in 2-D and 3-D, in the long-axis, short-axis, and apical views of the hearts [3]. Numerous studies have shown that STE can provide valuable information for early detection of local LV dysfunction and thus significant insights into the pathophysiology of ischemic heart disease [4–6]. STE algorithms generally use a block-

matching approach to track the speckles in a sequence of B-mode or RF image data [7]. STE is superior to Tissue Doppler imaging, particularly regarding noise and angle dependency [8]. Moreover, STE provides information on strain in more than one direction. By tracking each speckle in the region of interest during one cardiac cycle frame to frame, strain in different directions can be estimated. However, the accuracy of speckle tracking is dependent on image quality and frame rate, and performs considerably worse in the non-axial directions.

Currently, the optimal frame rate of speckle tracking is between 50 and 80 FPS for clinical applicability to measure myocardial strain [9]. However, to obtain such a frame rate using conventional US machines, image resolution and scan-line density are inevitably reduced. High image quality is beneficial to measure strain accurately, but at the cost of low temporal resolution, resulting in under-sampling, decorrelation, and anisotropic speckle patterns in the images. In some cases, such as the evaluation of coronary artery disease and stress echoes (where the heart rate increases), a higher frame rate over 80 FPS is required [10]. For this reason, using conventional US imaging machines to acquire the entire LV myocardium and quantify the LV myocardial deformation locally at such a high frame rate is not feasible.

Recently, ultrafast US imaging is playing an important role in new advances in echocardiography since its high frame rates enable capturing rapid cardiac motion, leading to an abundance in novel diagnostic or prognostic information [11–13]. Both focused and unfocused US transmission technologies have been developed. Multi-line acquisition (MLA) reduces the number of scan lines and transmits beams with a wide opening angle together with parallel receive beamforming. As a result the frame rate can be increased [14]. Multi-line transmit (MLT) is an alternative that applies receive beamforming in parallel and transmits multiple focused beams into different directions simultaneously. The frame rate can be improved accordingly [15]. Moreover, MLT can easily be combined with MLA techniques to gain an 8-fold increase in frame rate. However, the main disadvantage of these technologies is the degradation of lateral resolution and artifacts [16]. Plane wave or diverging wave imaging methods have also been proposed to achieve a high frame rate using the full aperture of the transducer [17]. Unfocused acquisition schemes allow more lines to be reconstructed in parallel while keeping good energy penetration. However, the spatial resolution decreases vastly due to the width of the broad beam increasing with depth. As a solution, coherent spatial compounding of steered spherical waves was introduced to improve spatial resolution and SNR [18]. However, coherent spatial compounding is only beneficial in a limited, overlapping region-of-interest in deeper lying structures. In cardiac imaging, limited by the anatomy of the ribs, using a phased array probe only allows for steering with small angles due to its intrinsic small footprint.

Therefore, using a single probe to perform STE and measure cardiac strain is limited, even at a high frame rate, due to the limited resolution in certain areas of the heart. Because of the complexity and dynamicity of the heart, inhomogeneous and high-level strain occur in all directions. When the strain direction is parallel to the direction of the US beam (axial direction), it can be measured accurately, benefiting from the good contrast and

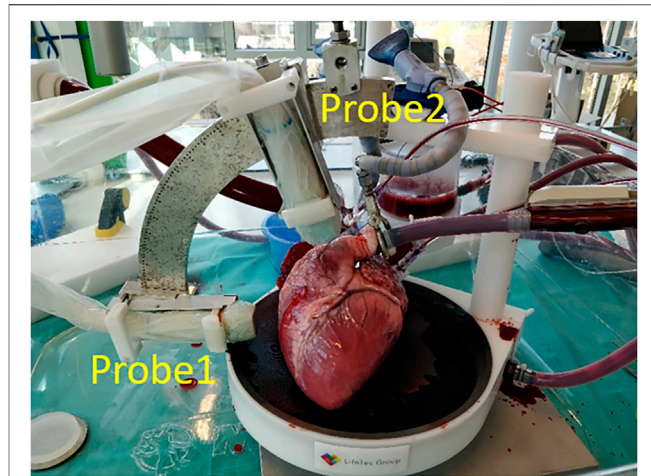


FIGURE 1 | Experimental setup of the beating porcine heart.

resolution in this direction. However, suffering from the low image contrast and poor resolution in the lateral direction, it is difficult to measure lateral strain precisely.

Multi-view and multi-probe imaging have been introduced for cardiac applications, showing promising results in improving lateral resolution and contrast [19–24]. However, its major shortcoming is the use of separate US image acquisitions, obtained manually at different time points, which can result in misalignment of the images due to changes in hemodynamic variables such as heart rate or ventricular pressure. Moreover, compounding the strain data acquired from different time points using multi-view imaging is non-trivial and will unavoidably lead to inaccuracies, given the rapid motion and contraction of the heart, as well as possible variability in heart rate from cycle to cycle.

Hence, a different approach based on multi-perspective US imaging that can perform ultrafast interleaved imaging and compound strain data has the potential to greatly improve performance. The major advantage is, that the US data acquired by the two probes are highly correlated, which makes image registration and strain compounding feasible. In addition, no separate recording and temporal registration are needed. Improvements of this method in image quality and vascular strain imaging have been demonstrated in a mock circulation setup using a porcine aorta [25–27]. However, for cardiac imaging, considering the limited imaging windows, asymmetrical and inhomogeneous deformation pattern, and high strain levels in different regions of LV, a different imaging setup and strain compounding method are required. Furthermore, this approach for cardiac imaging needs to be based on phased array probes and requires testing and validation under realistic conditions, before translation into the clinic.

This study introduces a multi-perspective ultrafast cardiac strain imaging method to improve 2-D cardiac strain measurements. The advantages and performance of multi-perspective STE will be demonstrated experimentally using a sophisticated beating porcine heart setup [28], and evaluated for

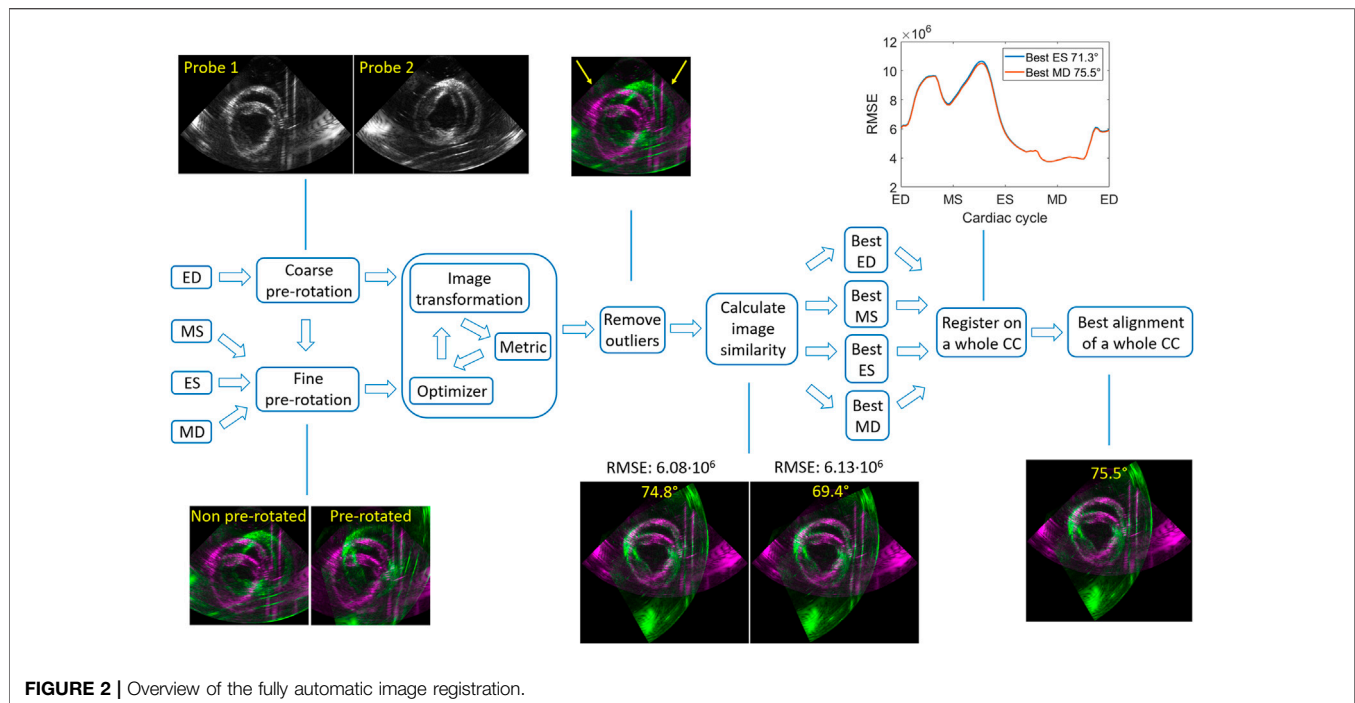


FIGURE 2 | Overview of the fully automatic image registration.

different relative probe positioning, i.e., increasing relative angle between the two probes. Moreover, a novel compounding method for interleaved multi-perspective US based strain imaging is introduced (unit axial vector based strain imaging) and compared to a strain fusion approach.

METHODS

Experimental Setup and Data Acquisition

In the *ex-vivo* experimental setup, a porcine heart was paced at a rate between 90 and 120 beats per min (bpm) to assure a steady heart rhythm through the whole experiment (see **Figure 1**). The heart was entirely submerged in a saline solution at 38°C in order to perform US imaging. More details about the setup of the beating porcine heart can be found in [28, 29], where a similar design was used.

US Data were acquired using a 256-channel Vantage open research system (Verasonics, Redmond, United States) equipped with two phased array probes (type: P4-2v, center frequency: 2.95 MHz, bandwidth: 1.64–4.26 MHz).

The two probes were attached to a mini-arch that ensures that they were exactly aligned in the same imaging plane, as can be seen in **Figure 1**. The arch allows to adjust the relative angle between the probes, as well as the probe positions in the radial direction to adjust the distances with respect to the heart. Probe 1 (P1) was positioned to acquire the parasternal short axis view, while the second probe (P2) was rotated every 15° from 30° to 75° with respect to the first probe on the mini-arch. All US data were acquired at a frame rate of 170 FPS, which was more than 3 times higher than conventional cardiac US imaging (<50 FPS). The two probes acquired US data in an interleaved scanning scheme, where they took turns to transmit and receive diverging waves

under 11 steering angles between -12° and 12°, i.e., the angle of the virtual sources with respect to the center of the aperture. US data were compounded in receive mode to improve image quality at the cost of a lower frame rate.

The frame rate Fr of the multi-perspective ultrafast imaging system is calculated as follows:

$$Fr = \frac{1}{(2N_a - 1)t_{BA} + t_{BF}} \quad (1)$$

where $N_a = 11$ is the number of steering angles, $t_{BA} = 260$ us is the waiting time between different steering angles, $t_{BF} = 400$ us is the waiting time to the next acquisition, needed for transfer of the acquired data to the host computer.

During the acquisition, all the channel data (raw US data) of each probe were sampled at four times the effective center frequency. All 320 frames of the acquired raw US data were reconstructed and stored as in-phase quadrature (IQ) data before envelope detection in MATLAB R2019a (The MathWorks, Natick, MA, United States) for further offline processing.

Image Registration and Fusion

Temporal registration was not required since the US images from the two separate probes were obtained in an interleaved scanning sequence. A fully automatic spatial registration method was developed to align the images (after envelope detection) of all different relative angles (30°–75°) acquired by the multi-perspective ultrafast imaging system.

Four frames in the cardiac cycle of the 75° dataset (ED: end of diastole, MS: mid of systole, ES: end of systole, MD: mid of diastole) were selected to demonstrate the algorithm (see **Figure 2**). First, the ED was used to perform a coarse pre-rotation, where the image obtained with probe 2 (P2) was

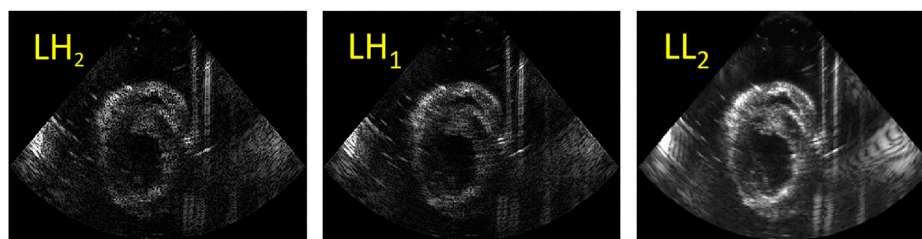


FIGURE 3 | An example of DWT decomposition using 2 layers: the low horizontal and vertical high subbands (LH_2 , LH_1) (left two images); the low frequency subband LL_2 (right).

TABLE 1 | Parameters of speckle tracking.

Iteration	1 (coarse)	2 (intermediate)	3 (fine)
Axial kernel	251	175	101
Lateral kernel	11	1	1
Axial maximum displacement	40	25	10
Lateral maximum displacement	3	0	0
Axial skip factor	40	13	13
Axial filter (axial $\times$ lateral)	21×9	15×7	15×7
Lateral filter (axial $\times$ lateral)	21×9	—	—

*Number means pixel, axial pixel size: 0.056 mm, lateral pixel size: 0.76°.

rotated every 10° from 10° to 90° with respect to the image acquired with probe 1 (P1). Next, these ten pre-rotated P2 images were registered to P1 by an image intensity based optimization algorithm [30], yielding a global rotation angle.

The iterative process of the optimization algorithm started with generating the initial transformation matrix based on the initial condition of the two images. Next, the metric compared the transformed moving image (P2) to the fixed image (P1) and create a metric value. Finally, the optimizer checked the stop condition based on the metric value and adjusted the transformation matrix for the next iteration. The process terminated when the metric approached a point of diminishing returns or when the number of maximum iterations was reached. The optimizer was based on a one-plus-one evolutionary algorithm (growth factor: 1.05, epsilon: 1.5, initial radius: 0.0063, maximum iterations: 100) and the metric used was the mutual information of the two images [31, 32].

Next, outliers were removed by calculating the overlapping percentage of the field-of-views (the sector/fan shapes) for the registered ten image sets. When the overlapping percentage of the two field-of-views was larger than 90%, it was considered to be an outlier (Figure 2). The score of the best alignment for ED was based on calculating the root mean square error (RMSE) of the gray values, where the lowest RMSE determined the best alignment of the ten registration results.

Three more frames (MS, ES and MD) in a whole cardiac cycle were chosen to repeat the previous steps applied on ED. To decrease the computation time, a fine pre-rotation was performed instead of the coarse pre-rotation, using the best three pre-rotation angles derived from the lowest RMSE values in ED. Similarly, the best alignments for these three frames were obtained (as Figure 2 shows).

Finally, all these four best alignments (ED, MS, ES, MD) were registered on a whole cardiac cycle, where the lowest mean of RMSE determined the best alignment of the whole cardiac cycle.

A fusion algorithm based on the discrete wavelet transform (DWT) was adapted to fuse the US images obtained from the two probes with different relative angles [33, 34]. The Haar wavelet is commonly used in image processing due to its fast and orthogonal properties [35–37]. It was selected to decompose the images into 2 layers with 7 subbands (see Figure 3). The fusion rules applied on DWT were defined as follows: for low frequency subband LL_2 which encompasses the structures of the left ventricular wall, the maximum values of the two images were taken. High frequency subbands (LH , HL and HH) show small structures, e.g., the noise and speckles, in the US images. Hence, for these subbands, averaging was performed. Finally, after fusion, all the subbands were reconstructed into one fused image.

Speckle Tracking

The speckle tracking algorithm encompasses a 2-D block matching method that calculates the normalized cross-correlation as an index of similarity between frames to determine displacements in both axial and lateral directions of the US sector grid during a full cardiac cycle [38]. First, US envelope data were used to obtain coarse displacements in both directions. After median filtering, these coarse displacements were used as input for the next iteration step (intermediate) to refine the displacements, where a smaller kernel size was applied on RF data instead. Next, in the third iteration (fine), the fine displacements are estimated. The parameters used for speckle tracking are shown in Table 1. RF data were chosen to perform speckle tracking since the resolution and precision are significantly better than B-mode speckle tracking [39]. Speckle tracking was performed separately on the RF datasets of the dual probes.

The myocardium was segmented in the fused B-mode image at the end-of-diastole frame (ED) by manually selecting the inner (endocardium-myocardium) and outer (epicardium-myocardium) walls. Considering the non-conventional nature of the data, automatic segmentation algorithms were not considered. Based on the result of automatic registration, the segmented mesh of the fused image was converted to meshes for the images obtained by the single probes. The segmented mesh consisted of 11 by 90 samples in the radial and circumferential directions, respectively. The mesh was tracked over the cardiac cycle using the displacements estimated by the speckle tracking algorithm to estimate myocardial motion [38].

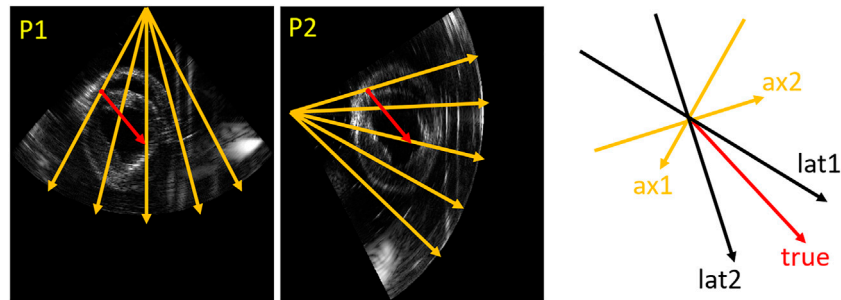


FIGURE 4 | Vector representation. An example (relative angle 75°) in the tracking mesh is shown (red point), where orange (ax1, ax2), black (lat1 and lat2) and red (true) arrows represent axial, lateral and true directions, respectively.

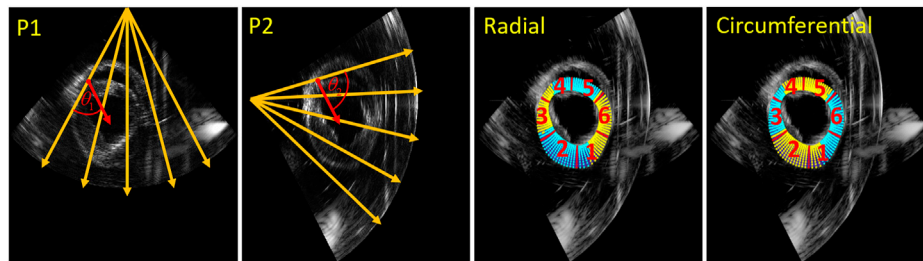


FIGURE 5 | Strain fusion: an example on how to select the optimal radial strain from the two probes for in this case a relative angle of 75° (left two images). The orange arrows indicate the US beam directions, the red arrows indicate the radial strain direction of the red point for the two probes respectively. θ_1 and θ_2 are the angles between US beam and the radial strain direction of the red point respectively. Masks for radial and circumferential strain fusion (right two images), where cyan means strain selected from P1 and yellow indicates strain chosen from P2. Red numbers show the segmented myocardial regions (11 by 90 samples) for regional strain analysis.

Displacement Compounding and Strain Estimation

To compound the displacements, benefitting fully from the multi-perspective imaging data, two different methods were considered. The first compounding method used in this study to improve strain estimation is unit axial vector compounding (VC). The fine axial displacements were compounded based on the unit axial vectors of the dual probes to improve the motion tracking of the myocardium for all relative angle datasets. Lateral displacements were discarded, given the relatively poor accuracy compared to axial displacements. Hence, 2-D kernels were only used to correct for lateral motion to improve axial displacement estimates. The axial displacements of P2 were transformed and interpolated into the polar coordinates of P1, allowing for axial displacement compounding.

As **Figure 4** shows, the true direction of the red point in the tracking mesh is decomposed into the axial and lateral directions. Therefore, the estimated true displacement can be represented using unit axial vectors and axial displacements of the dual probes:

$$\begin{bmatrix} m_x & m_y \\ n_x & n_y \end{bmatrix} \cdot \begin{bmatrix} U_x \\ U_y \end{bmatrix} = \begin{bmatrix} U_{ax1} \\ U_{ax2} \end{bmatrix} \quad (2)$$

where $\vec{m}_{ax} = (m_x, m_y)$ and $\vec{n}_{ax} = (n_x, n_y)$ are the unit axial vectors of the two probes. U_x and U_y are the estimated true

displacements in Cartesian coordinates. U_{ax1} and U_{ax2} are the axial displacements measured by the two probes.

Since **Eq. 2** consists of a system of two linear equations with two unknowns, a unique solution of the true displacement U_x and U_y can be estimated. Horizontal and vertical strain were calculated with respect to the initial configuration (Lagrangian frame of reference) by taking the spatial derivative of the derived displacement fields (U_x and U_y) in the horizontal (x) and vertical (y) directions using a 2-D least-squares strain estimator (2DLSQE) with a fixed size of 5 by 9 pixels [40]. The resulting horizontal and vertical strain were converted to radial and circumferential strain using the following equations [41]:

$$\begin{aligned} \epsilon_{rad} &= \epsilon_{xx} \cos^2 \beta + \epsilon_{yy} \sin^2 \beta + 2\epsilon_{xy} \sin \beta \cos \beta \\ \epsilon_{cir} &= \epsilon_{xx} \sin^2 \beta + \epsilon_{yy} \cos^2 \beta - 2\epsilon_{xy} \sin \beta \cos \beta \end{aligned} \quad (3)$$

where β is the angle between the ultrasound beam and the radial strain. ϵ_{xx} , ϵ_{yy} and ϵ_{xy} represent horizontal, vertical and shear strain in Cartesian coordinates respectively. ϵ_{rad} and ϵ_{cir} are the resulting radial and circumferential strain.

Strain Fusion

The second method to improve strain estimation is to fuse the strain instead of compounding the displacements, so-called strain fusion (SF). Derived radial and circumferential strain of the two

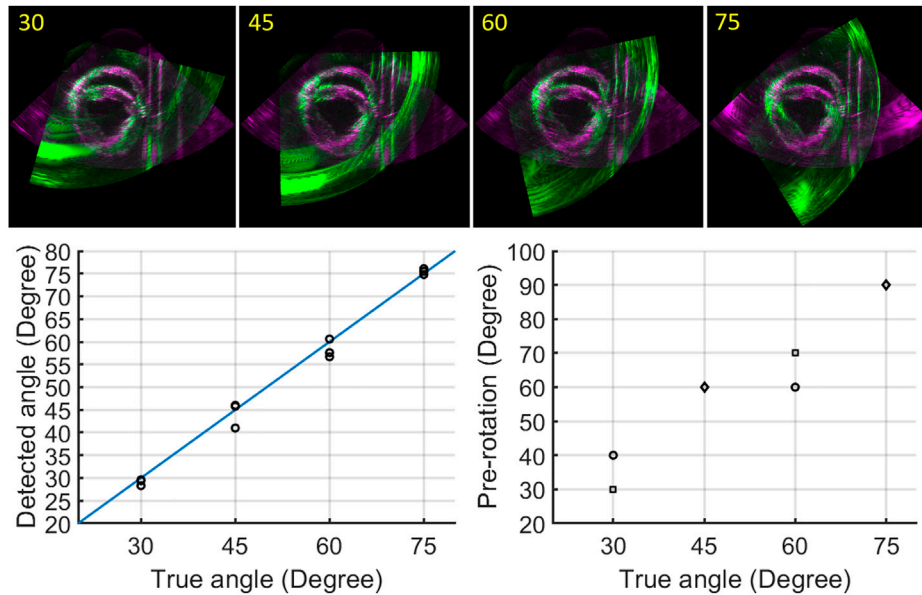


FIGURE 6 | Registration results: registered image data for angles ranging from 30° to 75° (top row); true angle versus detected angles, black circles indicate three different cardiac cycles (bottom left); true angle versus pre-rotation angles (circle: one cardiac cycle, square: two cardiac cycles, diamond: three cardiac cycles) (bottom right).

probes for all relative angle datasets were fused based on automatically generated strain fusion masks. In the first step, for each point in the segmentation mesh (11 by 90 samples in total), the angle between strain and US beam direction was calculated for both probes. Next, all the calculated angles in the mesh of the two probes were compared, where the smaller angles were selected to create the masks for strain fusion. Finally, as **Figure 5** shows, the masks for radial and circumferential strain fusion were created, where cyan shows strain selected from probe 1 (P1) and yellow indicates strain chosen from probe 2 (P2). Furthermore, the mesh was segmented into six regions for regional strain analysis [42].

Analysis of Results

To quantify the accuracy of the registration results, the rotation error was calculated with respect to the ground truth derived from the mini-arch.

To compare the motion tracking performance between single-perspective (SP) and multi-perspective using VC, the mean error (ME) of the entire tracking mesh was estimated between the last (ED) and first frame (ED) of a tracking cycle:

$$ME = \frac{1}{n} \sum_{i=1}^n \sqrt{(x_i - x'_i)^2 + (y_i - y'_i)^2} \quad (4)$$

where n is the number of pixels in the tracking mesh, (x_i, y_i) and (x'_i, y'_i) are the positions of a pixel in the tracking mesh at the first and last frame, respectively.

The elastographic signal-to-noise ratio (SNRe) at the end-systolic radial and circumferential strain were calculated to analyze the strain estimation for SP, and multi-perspective VC and SF:

$$SNRe^{rad} = \frac{\mu_{rad}}{\sigma_{rad}} \quad (5)$$

$$SNRe^{cir} = \frac{\mu_{cir}}{\sigma_{cir}}$$

where μ_{rad} and σ_{rad} are the mean and standard deviation of the end-systolic radial strain, and μ_{cir} and σ_{cir} are the mean and standard deviation of the end-systolic circumferential strain.

The mean drift error (MDE) and strain variability (SV) of radial and circumferential strain were calculated for the last frame, since MDE and SV should theoretically be zero at the end of a tracking cycle:

$$MDE = \frac{\sum_{i=1}^n |e_i|}{n} \quad (6)$$

$$SV = \sqrt{\frac{\sum_{i=1}^n (e_i - \mu_e)^2}{n}}$$

where e_i is the strain value of a pixel in the tracking mesh at the last frame and μ_e is the mean of strain values for all the pixels at the last frame.

RESULTS

Registration

The fully automatic registration method was tested for all different angle datasets (30°–75°) for three cardiac cycles, as **Figure 6** shows. In all cases, the best alignment was found when the pre-rotation angle applied was $< \pm 15^\circ$ with respect to the true angle. Overall,

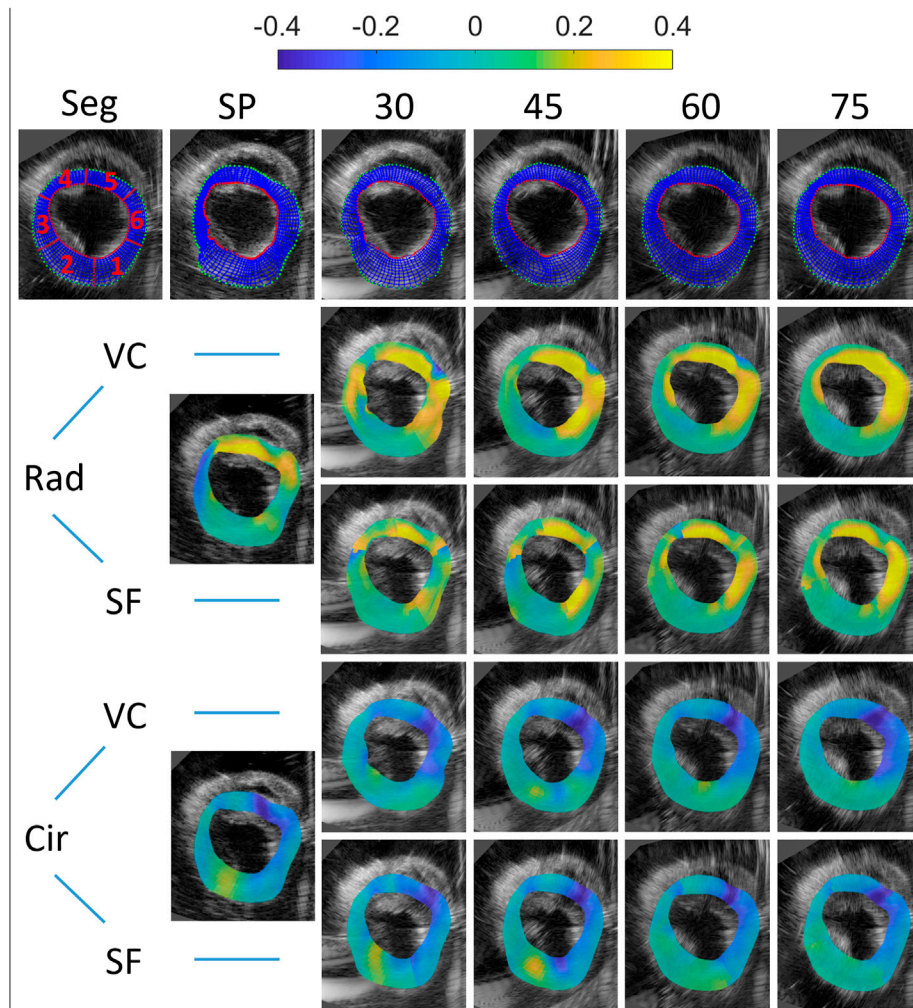


FIGURE 7 | Motion tracking and end-systolic strain for all multi-perspective datasets (relative angles 30°–75°). Top row: the initial segmentation mesh (first frame), and final tracked meshes at end-diastole are shown (SP and 30°–75°). Both radial and circumferential end-systolic strain images are shown for single-perspective imaging (SP), and multi-perspective strain imaging using unit axial vectors based compounding (VC) and strain fusion (SF), respectively.

final registration result revealed a mean rotation error of 1.4° with respect to the ground truth (angle set on the arch).

Motion Tracking

Figure 7 shows the performance of motion tracking at the end of a cardiac cycle. More quantitative results of the mean error for motion tracking are demonstrated in **Table 2**. The myocardium contours of SP show that the myocardium coordinates are more uniformly distributed in the upper wall regions (R4, R5). This coincides with motion tracking performing better in the axial direction where the ultrasound beams propagate. While increasing the imaging depth, the lateral resolution decreases accordingly, leading to a reduced tracking accuracy (R2) with an error drift of 1.9 mm. In the side wall regions (R6, R3), where the wall motion is mainly occurring in the lateral direction, the error drift increases by 0.3 and 0.9 mm, respectively.

After unit axial vector based compounding, with the largest angle 75° (as **Figure 7** top right shows), the tracking mesh (at the

last frame) shows the most similar shape as the segmentation mesh shows (see **Figure 7** top left). The mean error reduces from 1.4 to 0.82 mm in total, with the largest reduction found at side wall region (R3, reduction of 1.37 mm) and lower wall (R2, reduction of 1.36 mm). This can be appreciated by the reduction of lateral drift compared to single probe imaging.

Strain Estimation

End-systolic strain are shown for single perspective, unit axial vector based compounding and strain fusion in **Figure 7**. MDE, SV and SNRe were calculated for both radial and circumferential strain using these three methods, as can be seen in **Table 2**.

For radial strain, an average radial strain of 0.08 ± 0.05 was measured in single perspective strain imaging. The largest positive strain values are found at the regions of the myocardium where radial strain is estimated from mostly the axial displacements (R4, R5), and the SNRe reaches up to 7.6. In

TABLE 2 | Quantitative results of motion tracking and strain estimation for myocardium (R1–R6).

Measure	Method	Total	R1	R2	R3	R4	R5	R6
ME (mm)	SP	1.40	1.10	1.90	2.20	1.10	0.94	1.30
	VC	0.82	0.63	0.54	0.83	1.10	1.10	0.68
RMDE	SP	0.15	0.07	0.06	0.25	0.17	0.08	0.23
	VC	0.08	0.04	0.03	0.07	0.12	0.15	0.09
	SF	0.07	0.05	0.04	0.07	0.09	0.08	0.09
CMDE	SP	0.09	0.14	0.18	0.04	0.07	0.09	0.05
	VC	0.04	0.03	0.03	0.04	0.03	0.07	0.05
	SF	0.05	0.07	0.04	0.06	0.04	0.07	0.05
RSV	SP	0.19	0.07	0.10	0.16	0.20	0.10	0.11
	VC	0.10	0.02	0.03	0.10	0.15	0.14	0.10
	SF	0.09	0.05	0.02	0.09	0.11	0.10	0.09
CSV	SP	0.12	0.09	0.21	0.05	0.07	0.11	0.06
	VC	0.06	0.03	0.04	0.05	0.04	0.09	0.05
	SF	0.07	0.04	0.05	0.07	0.04	0.08	0.06
SNRe ^{rad}	SP	3.0	4.6	0.7	−9.3	7.6	5.2	9.0
	VC	10.6	11.0	2.9	7.7	4.2	18.9	18.9
	SF	6.7	4.8	7.2	5.2	4.0	5.5	13.5
SNRe ^{cir}	SP	6.1	7.3	−3.3	2.1	7.0	10.5	12.4
	VC	8.5	3.4	−1.5	1.7	9.4	16.8	21.0
	SF	5.7	2.5	−0.9	1.5	4.9	12.5	13.8

*ME, mean error; RMDE, mean drift error of radial strain; CMDE, mean drift error of circumferential strain; RSV, radial strain variability; CSV, circumferential strain variability; SNRe^{rad}, radial elastographic signal-to-noise ratio; SNRe^{cir}, circumferential elastographic signal-to-noise ratio; SP, single perspective; VC, unit axial vectors based compounding; SF, strain fusion. The results of both VC and SF are shown for the largest angle of 75°.

the lateral regions of the myocardium (R3, in the middle of R1 and R6), where the deformations are perpendicular to the direction of US wave, negative strain occur with a low SNRe of −9.3, caused by the inaccurate lateral displacement estimates used. After VC (largest angle 75°), the overall radial strain reaches up to 0.20 ± 0.07 and the SNRe increases from 3.0 to 10.6. The largest strain values (0.35) are found in R5 and R6 with the highest SNRe of 18.9 in all regions. The largest improvement of SNRe (increases from −9.3 to 7.7) is found at R3. A similar trend is shown in the SNRe after SF (largest angle 75°), despite only a small improvement (over SP) with respect to VC, in terms of the maximum strain value and SNRe. Compared to single perspective strain imaging, the overall radial strain measured is 0.17 ± 0.05 and the SNRe increases from 3.0 to 6.7 after SF. The largest improvement of SNRe (increases from −9.3 to 5.2) is also found at R3, where single perspective strain imaging performed worst, caused by the inaccurate lateral displacements.

For circumferential strain, an average circumferential strain of -0.09 ± 0.03 was estimated in single perspective strain imaging. The largest negative strain values are found at R5 and R6, and strain precision is higher where the strain direction are equal to the US beam propagation (R6, R3), with the largest SNRe found to equal 12.4 (R6). In the lateral region with a large depth (R2), where the strain estimation mostly relied on the lateral displacements with the lowest resolution, a positive strain

value of 0.06 and the lowest SNRe of −3.3 are found. After VC (largest angle 75°), the overall circumferential strain reaches up to -0.12 ± 0.03 and SNRe increases from 6.1 to 8.5. The largest strain value increases from 0.27 to 0.30 in R5 and the highest SNRe of 21.0 is found in R6. In the lateral regions (R2, R5), a large increase of SNRe (increase of 6.3) is shown in R5, while only a small increase of SNRe (increase of 1.8) in R2. After SF (largest angle 75°), improvements of SNRe are shown in R2, R5 (lateral regions for single perspective) and R6, despite a small decrease of overall SNR (decrease of 0.4).

The mean drift error (MDE) and strain variability (SV) of radial and circumferential strain were calculated for the last frame, as can be depicted from Table 2 and Figure 8. Overall, no significant differences were found between VC and SF for MDE and SV. After VC (largest angle 75°), the mean drift error decreases 0.07 and 0.05 for radial and circumferential strain, respectively. The largest improvements for radial strain are found in R3 and R6, where strain directions are perpendicular to the US beam directions (for single perspective). Similarly, extensive improvements for circumferential strain are found in R1 and R2. Strain variability reduces by 0.08 for both strain components on average. For radial strain, a large reduction of 0.06 is shown in R3 as expected. A similar trend is shown for circumferential strain with a decrease of 0.17 in R2.

DISCUSSION

This study explored the benefits and feasibility of using multi-perspective ultrafast US to improve cardiac strain imaging. More specifically, the improvements in myocardial strain were investigated when positioning the dual probes at different relative angles and using different strain compounding methods, in an *ex-vivo* beating porcine heart setup. A fully automatic registration algorithm was developed to accurately align the two image datasets acquired from the dual probes for all different angle cases. A novel axial displacement compounding method based on unit axial vectors was proposed to improve the cardiac strain estimation for both strain components, and was compared to a strain fusion algorithm and conventional single probe strain imaging to quantitatively compare the improvements of strain estimation. Furthermore, a quantitative comparison of inter-probe angle for multi-perspective ultrafast US imaging was demonstrated, in terms of improvements in motion tracking and strain estimation.

The mini-arch was designed for experimental verification purposes only, not for *in vivo* use. In future *in vivo* studies, it would be difficult to rotate the second probe on the mini-arch as we did in this *ex-vivo* study, given the imaging windows are limited and ribs are present. Ultimately, the technique could be used *in vivo* by mounting two probes onto the subject's thorax, allowing multi-perspective imaging in a freehand fashion. With this in mind, an automatic image registration algorithm was developed.

To cope with the large rotation, anisotropic resolution, and regional contrast differences from the two different perspectives in this application, a good initial condition needs to be provided before the optimization algorithm operated. This was done by an automatic pre-rotation step, where the moving image (P2) was pre-

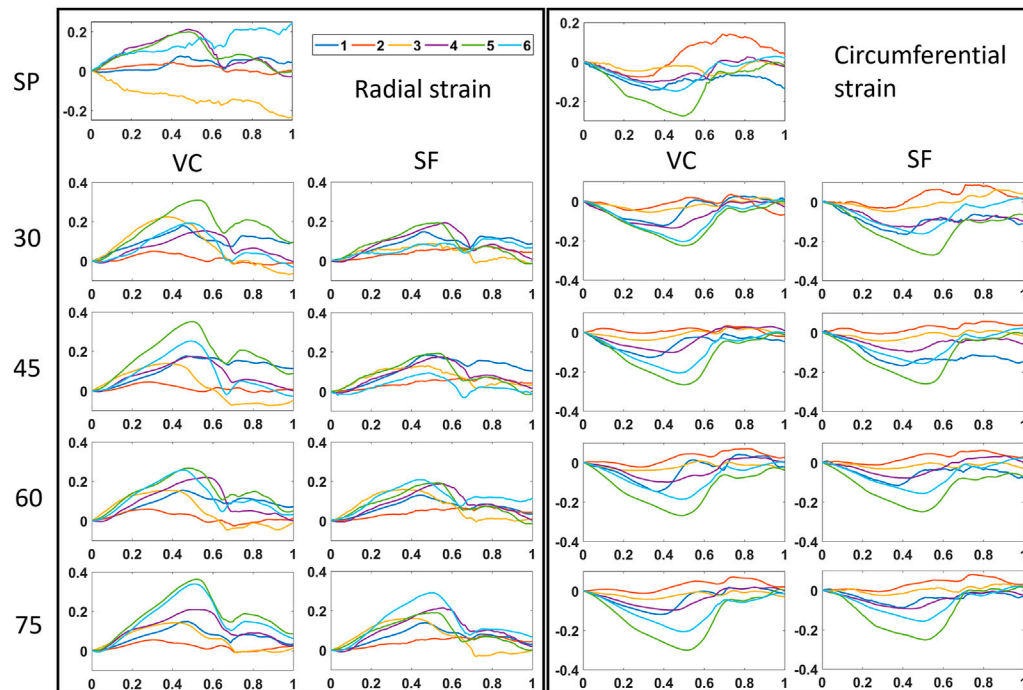


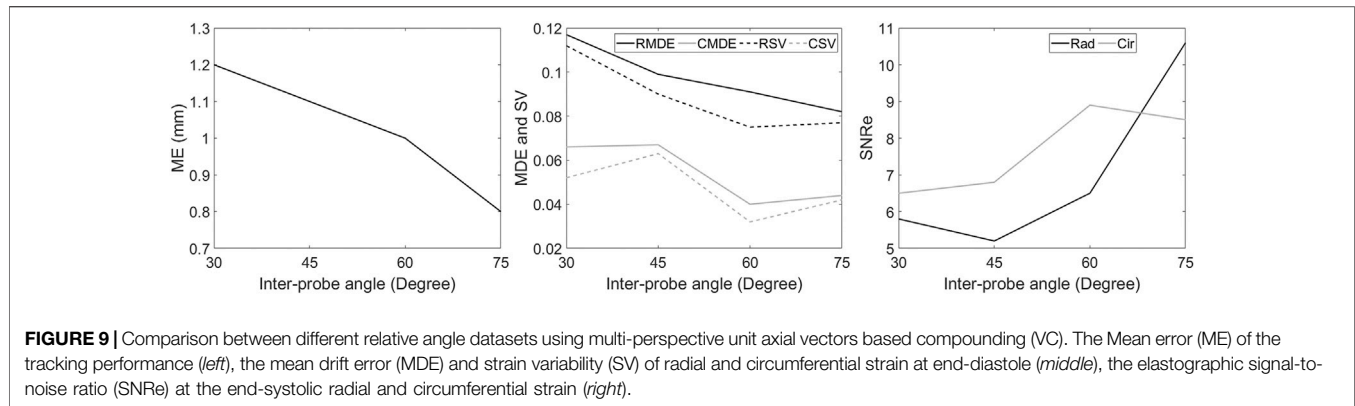
FIGURE 8 | Time strain curves for all multi-perspective datasets (30°–75°). Both radial and circumferential strain curves are shown for single-perspective imaging (SP), and multi-perspective, unit axial vectors based compounding (VC) and strain fusion (SF), respectively.

rotated in a step of 10° from 10° to 90° with respect to the fixed image (P1). The registration algorithm performed well but results do indicate that an initial estimate of the inter-probe rotation angle ($\pm 15^\circ$ with respect to the true angle, see **Figure 6**) is required. To decrease the computation time, this pre-rotation angle could be estimated in clinical practice by a simple mechanical sensor mounted to the probe.

Since no temporal registration was required in this study, the two image sets of a whole cardiac cycle can be registered by applying the registration result of any frame during a whole cardiac cycle. Four frames were selected for registration to ensure the accuracy and efficiency of the registration process. More importantly, the algorithm we developed determined the best registration automatically by calculating the RMSE of the two images in a whole cardiac cycle without using the ground truth from the arch. The results of the fully automatic registration algorithm revealed a rotation error of 1.4° in all cases, indicating good agreement for different datasets (different cardiac cycles and angles). The largest rotation bias (4.0°) with respect to the ground truth was found when the inter-probe angle was 45°, mainly caused by the inaccurate selection among the best four alignments for a whole cardiac cycle. This can be explained by the extensive artifacts, which were present in the image acquired by P2 near the boundaries of the overlapping regions (green image of 45° in **Figure 6**). These artifacts were created by the supporting devices for the beating porcine heart and the strong reflection from the bottom of the tank. In this case, the lowest RMSE (7.35×10^{-6}) of the two images failed to represent the best alignment, since the registration result with more of these artifacts appearing inside the overlapping regions will obtain a lower

score for RMSE. In the datasets of other angles, less artifacts were present around the boundaries of the overlapping regions. Therefore, the scores of RMSE for these cases were less sensitive to the small shift of artifacts occurring in the best four alignments, leading to more accurate registration results. It goes without saying that the presence of these artifacts affects the accuracy of the registration. *In vivo*, these artifacts will not appear, although there near field clutter will be present caused by neighboring structures such as the ribs. A possible solution to make the selection of the best alignment more accurate would be only calculating the RMSE for the region of interest, i.e., the LV. However, this requires a dedicated automatic segmentation algorithm to precisely segment out the LV, which will unavoidably and extensively increase the computation time.

For motion tracking, one may notice that the maximum lateral displacement for the second and third iterations of speckle tracking were set to zero (see **Table 1**), which means only in the first step, lateral displacement estimates contributed to motion tracking and strain estimation. By doing so, numerous accumulated lateral drift errors caused by the poor lateral resolution and high number of frames from the last two iteration steps were reduced, although the tracking became insufficient and inaccurate in the lateral direction (as **Figure 7** shows). After compounding the axial displacements of the two probes (largest angle 75°), the performance of motion tracking improved vastly compared to single probe imaging, the error drift reduced 40% on average, with the largest reduction found at the side wall regions (R3, R6). The tracking mesh showed a more similar shape as the segmentation mesh showed.



A quantitative comparison between different relative angle datasets using VC is shown in **Figure 9**. Overall, after VC, all inter-probe angles show improvements in terms of motion tracking, SNRe, MDE, SV, strain magnitudes and strain curves over SP (also see **Figures 7, 8** and **Table 2**). While increasing the inter-probe angle from 30° to 75°, ME, MDE and SV decrease and SNRe increases for both strain components, with the largest difference found for angles $\geq 60^\circ$. For the largest angle (75°), ME (-42%), MDE (-50%) and SV (-48%) reduce significantly, SNRe increases by 253 and 39% for radial and circumferential strain, respectively, and strain curves reveal less noise for each region. The investigation of inter-probe angle shows that motion tracking and strain estimation improve when the angle increases. For future *in vivo* measurements, one should aim for a large angle to benefit the most, ideally 90°. Due to the presence of ribs and limited imaging windows, this remains challenging and still needs to be validated. However, in some cases, when only a small inter-probe angle might be feasible (30°–60°), motion tracking and strain estimation can still be improved when using VC compounding (see **Figures 7–9**).

This study introduced a unit axial vector based displacement compounding method to improve motion tracking and strain estimation. The advantages of VC over SF have been quantitatively illustrated in terms of SNRe, MDE, SV, strain magnitudes and strain curves for both strain components. This can be explained by the fact that VC only uses axial displacements to derive strain while SF includes a certain contribution of lateral displacements. It is generally known that due to a lack of phase information and a lower resolution in the lateral direction, strain derived from the lateral displacements are less accurate than derived from the axial displacements [43, 44]. Especially, in the intersections of strain fusion masks (see **Figure 5**, intersections of cyan and yellow), the angles between strain direction and US beam direction for both probes are larger than 45°, which means more than 50% lateral displacements are used to derive strain in these regions. Although SF shows improvement over SP in all aspects, the fact of using lateral displacements to derive strain makes it less accurate than VC which only uses axial displacements to derive strain [41, 45, 46].

The method of unit axial vectors based displacement compounding we proposed is straightforward and solves the true displacements with a unique solution using two axial displacement datasets acquired by the dual probes. It certainly has merit for *in vivo*, high quality strain imaging. Conventional single probe beam

steering, as shown in, e.g. carotid imaging, are not sufficient at these depths with phased array transducers. Of course, the use of two probes will make an *in vivo* examination more complex, although probe mounting (possibly in a more dedicated arch with more flexibility that ensures common image planes) or passive robotic devices could assist in the exam. Ultimately, for *in vivo* application, the methods introduced in this paper should be extended to 3-D, which will make precise probe alignment less of a bottleneck.

In vivo interleaved multi-perspective US strain imaging, however, will still require extensive US safety testing and ethical approval, as well as a step to handheld or partially mounted dual probe imaging in 3-D as explained earlier. Once this is achieved, multi-perspective US strain imaging could play an indispensable role in the diagnosis and management of virtually any disease that affects the myocardium. Future studies will focus on performing multi-perspective ultrafast US myocardial strain imaging *in vivo*. The improved motion tracking and myocardial strain estimation could provide a more sensitive means for detecting regional myocardial dysfunction, and improve the reliability of strain estimation for the heart in general, thereby boosting the use and diagnostic value of cardiac strain imaging in general.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Dutch Ministry of Agriculture, Nature and Food Quality, and Food and Consumer Product Safety Authority.

AUTHOR CONTRIBUTIONS

PL has setup experiments, performed data acquisition, developed algorithms, analyzed data, created the manuscript. J-WM has played a significant role in the development of the unit vector axial

projection method. H-MS has collaborated on US acquisition methods, data analysis and manuscript writing. RL is responsible for the study design, helped in algorithm development, data analysis and interpretation, manuscript writing, and obtaining study funding.

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Quantitative Compression Elastography With an Uncalibrated Stress Sensor

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Tissue stiffness is a key biomechanical property that can be exploited for diagnostic and therapeutic purposes. Tissue stiffness is typically measured quantitatively *via* shear wave elastography or qualitatively through compressive strain elastography. This work focuses on merging the two by implementing an uncalibrated stress sensor to allow for the calculation of Young's modulus during compression elastography. Our results show that quantitative compression elastography is able to measure Young's modulus values in gelatin and tissue samples that agree well with uniaxial compression testing.

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1 INTRODUCTION

In modern medicine, diagnostic and treatment decisions are becoming increasingly influenced by measurements of biomechanical properties of tissues. This is especially true in the fields of oncology [1], dermatology [2], and hepatology [3]. Because disease states necessarily involve the physical change of the affected tissue, biomechanical properties are often a good indicator of both disease presence and extent.

A key biomechanical property that is most often measured in clinical use is tissue stiffness. Changes in tissue structure on the microscale result in changes in tissue stiffness on the macroscale. For example, tumors often vary in stiffness from the surrounding tissue. Subsequently, tumor stiffness can be used to detect tumors [4], aid in staging them [5], determine their metastatic potential [6], and aid in the evaluation of treatment [7]. Similarly, a liver disease resulting in fibrosis or steatosis can be diagnosed *via* liver stiffness measurement [3, 8]. Treatment of many diseases in addition to the aforementioned diseases involving soft tissues, can benefit from the analysis of tissue stiffness for diagnostic and therapeutic planning and evaluation.

The field of elastography is generally focused on the measurement of tissue stiffness *via* various imaging modalities. For superficial or excised tissues, this can be accomplished with noninvasive optical techniques such as optical coherence elastography (OCE) [9–11], which can provide high-resolution elastograms. However, due to light attenuation in tissue, optical-based elastography methods cannot be used in tissues that are deeper than a few mm beneath the surface unless an invasive probe is utilized [12]. Magnetic resonance elastography (MRE) does not have this limitation and is used to make whole-organ and whole-body elasticity maps but suffers from relatively poor resolution, typically in the mm range [13]. Additionally, it is expensive and requires a large amount of space. A third option is ultrasound elastography [14], which is portable, comparatively inexpensive, and has been practiced the longest and studied the most of the three techniques. It can offer both deep tissue penetration and high resolution, though there is a tradeoff between these two factors.

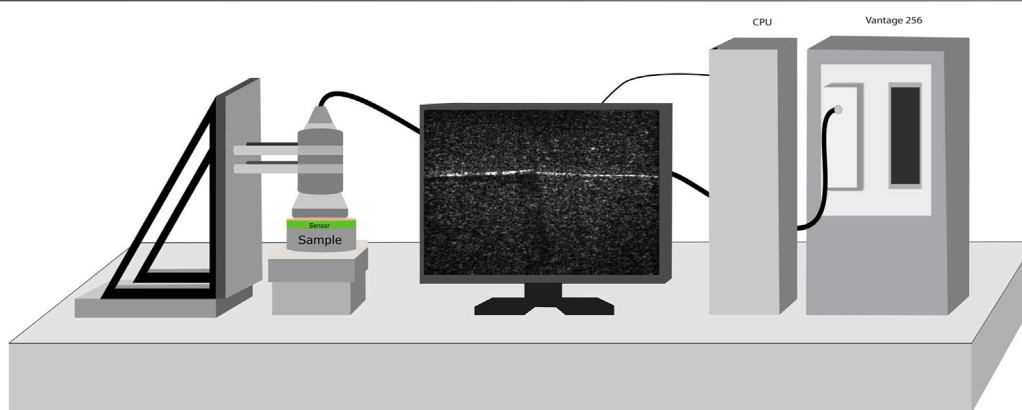


FIGURE 1 | The experimental setup, consisting of a custom probe mount, Verasonics Vantage 256 system, and a z-axis stage. The transducer is held firmly in place as the z-axis stage is incrementally raised to compress the sample, during which shear wave and compression data is obtained.

Ultrahigh-frequency ultrasound transducers with frequencies in the hundreds of MHz are capable of approaching optical imaging resolution both axially and laterally at the cost of penetration depth; however, these are still at an early stage and are currently manufactured in laboratories for use in experimental setups [15]. Elastography is typically performed at lower frequencies (< 5 MHz), resulting in resolutions of hundreds of microns up to mm scale [15].

The two main branches of ultrasound elastography are static/quasi-static compressive strain elastography [16, 17] and shear wave elastography [18, 19]. In strain elastography, a series of images are taken as the tissue is slowly compressed, which results in a measure of how the strain distribution changes with compression. This method typically results in semi-quantitative measurements and shows the ratio between the strains in different regions. Because of the manual compression, there can be high operator error and low repeatability [20], which is compounded by the nonlinear biomechanical properties of tissues [21]. Shear wave elastography, on the other hand, involves inducing a mechanical wave inside the tissue and measuring its speed. This allows for a quantitative measurement based upon the shear wave speed, i.e., shear modulus or Young's modulus. These mechanical parameters are absolute measurements of tissue elasticity and are more valuable because they allow for comparisons between different time points during the treatment of a single patient, between different patients, and between different diseases. Strain-based compressive elastography can also obtain absolute Young's modulus in tissues but requires the use of a precisely calibrated compliant sensor for measurements of surface stress, which has been utilized widely in optical coherence micro-elastography [22, 23]. However, use of a stress sensor in ultrasound elastography is not as prominent as in optical methods.

In this work, we combine strain and shear wave elastography using a stress sensor placed on the sample to obtain two-dimensional quantitative stiffness maps in a new technique called quantitative compression elastography (QCE). Unlike

quantitative micro-elastography, our method does not require a calibrated sensor with precisely known mechanical characteristics. Instead, shear waves are generated within the sensor, which can be converted to quantitative elastic modulus values. Using these values and the strains measured within the sensor and the tissue of interest, we measure quantitative tissue stiffness values. We also compare these values to uniaxial compression testing (UT) for validation. To our knowledge, this is the first translation of strain imaging in ultrasound elastography to the quantitative mapping of biomechanical properties through the use of an uncalibrated stress sensor.

2 MATERIALS AND METHODS

2.1 Experimental Setup

The experimental setup is shown in **Figure 1**. It consists of a Vantage 256 (Verasonics, Kirkland, WA, United States) ultrasound system with an L11-5 V transducer. The imaging and push frequencies were 7.8 MHz and the pulse duration was 128 μ s. The transducer was placed into a custom holder to ensure stability and prevent movement. Each sample was placed on a linear z-axis stage which served to produce the compression. Ultrasound gel was degassed using a vacuum chamber and a thin layer was placed between the ultrasound transducer and the sample. The transducer was placed above the surface of the sample and guided into place by raising the z-axis stage.

2.2 Phantom Preparation

Gelatin phantoms were created by mixing gelatin (gel strength 300, type A. Sigma-Aldrich Corp, MO, United States) with distilled water and pouring it into a cylindrical mold with a diameter of approximately 8 cm. Silica powder was added to all phantoms and sensors to promote acoustic scattering and to make the boundaries easy to visualize on ultrasound imaging. Prior to measurement, all phantoms were removed from their molds *via* a custom lift mechanism to avoid tearing the phantom. This lift mechanism consists of a circular piece of thin acrylic cut



FIGURE 2 | Photograph of the steak sample. The left side was cooked and the right side was uncooked. The boundary can be clearly seen in the middle. The scale bar is approximately 1 cm in each direction.

with a laser cutter to match the diameter of the cylindrical mold. Two thin strips of metal were bent at a 90-degree angle and glued to the bottom of the acrylic to allow for the whole acrylic disk and the solidified phantom to be lifted as a single unit. When possible, the sensor was created by pouring the sensor gelatin mixture on top of the sample to avoid acoustic impedance mismatch that could create artifacts in the presence of an air gap or the insertion of bubbles. For each concentration, a separate small cylindrical phantom with a diameter of approximately 3.5 cm was also cast for validation *via* uniaxial mechanical compression testing.

2.2.1 Homogeneous Phantoms

Homogeneous phantoms with concentrations of approximately 10% gelatin (w/w) and 16% gelatin (w/w) were created. Silica powder at a concentration of approximately 0.8% (w/w) was added for scattering. They were poured into the aforementioned cylindrical mold allowed to solidify. A sensor layer of 9% gelatin (w/w) containing 0.5% silica powder (w/w) with a thickness of approximately 4 mm was poured on top and allowed to solidify.

2.2.2 Heterogeneous Phantoms

A half and half phantom was created to assess the capability of the technique to assess spatial variations in stiffness. Half of the phantom consisted of 16% gelatin (w/w) with 0.8% silica powder (w/w), and the other half was 10% gelatin (w/w) with 0.6% silica powder (w/w). The silica powder was varied to allow for visualization of the boundary between the halves. The gelatin was poured into the aforementioned cylindrical mold, allowed to solidify. A sensor layer of 9% gelatin (w/w) with 0.4% silica powder (w/w) with a thickness of approximately 4 mm was poured on top and allowed to solidify.

2.3 Tissue Preparation

Beef steak was acquired fresh from a local grocery store and cut into a small strip of approximately 5 cm by 10 cm. Half of the sample was cooked on a hot plate at 150°C for ~1–2 min per side to change the local biomechanical properties of the region. Both sides of the cooked region were heated to ensure biomechanical changes were consistent through the entire depth, and the tissue was allowed to cool to room temperature before measurements.

An image of the tissue is shown in **Figure 2**, which clearly shows the delineation between the cooked and uncooked regions. When placed under the transducer, a copper wire was aligned with the visible cooked boundary for visualization on the ultrasound B-mode image before a 16% gelatin (w/w) sensor with a thickness of approximately 5 mm was placed on top. Prior to the acquisition, the wire was carefully removed from beneath the sensor.

2.4 Uniaxial Compression Testing

Phantoms of each concentration underwent uniaxial mechanical compression testing (Model 5,943, Instron Corp, Norwood, MA, United States) to measure Young's modulus by the "gold standard" for comparison. The height and radius of the cylindrical phantoms were measured with digital calipers, and the sample was coated with water to prevent friction between the compression testing frame plates. After coming into contact with the phantom, the test began and continued until either 20% strain or 50 N of force was reached. Compression was performed at 0.25 mm/s. The phantom was removed, and rewet after each measurement, and a total of five measurements were performed on each sample.

The raw mechanical testing data was loaded into MATLAB (Mathworks, Inc., Natick, MA, United States), and stress and strain were calculated using the measured dimensions of the phantom. A polynomial best fit line to the stress-strain curve was obtained, and the derivative was calculated. This derivative equation corresponded to the tangent Young's modulus as a function of strain and was used for validation of the ultrasound compression elastography method.

2.5 Data Acquisition

Custom acquisition software was written in MATLAB to control the Vantage 256 system in conjunction with the z-stage. For each compression position, 120 plane-wave ultrasound images were taken and saved as IQ data. Following this, a shear wave was generated approximately 0.5 mm below the sensor layer, and an additional 120 plane-wave ultrasound images were taken to monitor the wave as it passed through the sensor and sample. An image was taken every 100 μ s. The shear wave was generated by using 32 elements of the transducer, and the apodization and delays were calculated *via* software provided by the ultrasound manufacturer. This ensured that the wavefronts from each transducer element arrived at the shear wave excitation focal point at the appropriate time to generate a shear wave. The z-stage was raised by 100 μ m, and the process was repeated until the desired number of compressions was reached.

2.6 Data Processing

A flow chart of the processing steps is shown in **Figure 3**. The shear wave data sets were processed as follows. First, the particle velocity was obtained in each of the 120 frames using the vector method [24] with a kernel size of 20 pixels, which corresponded to 1.35 μ m. The particle velocity, i.e., the temporal derivative of the displacement in the axial direction, for each pixel was summed in time to obtain displacement in each frame. Then, the displacement images were smoothed using a Savitzky-Golay

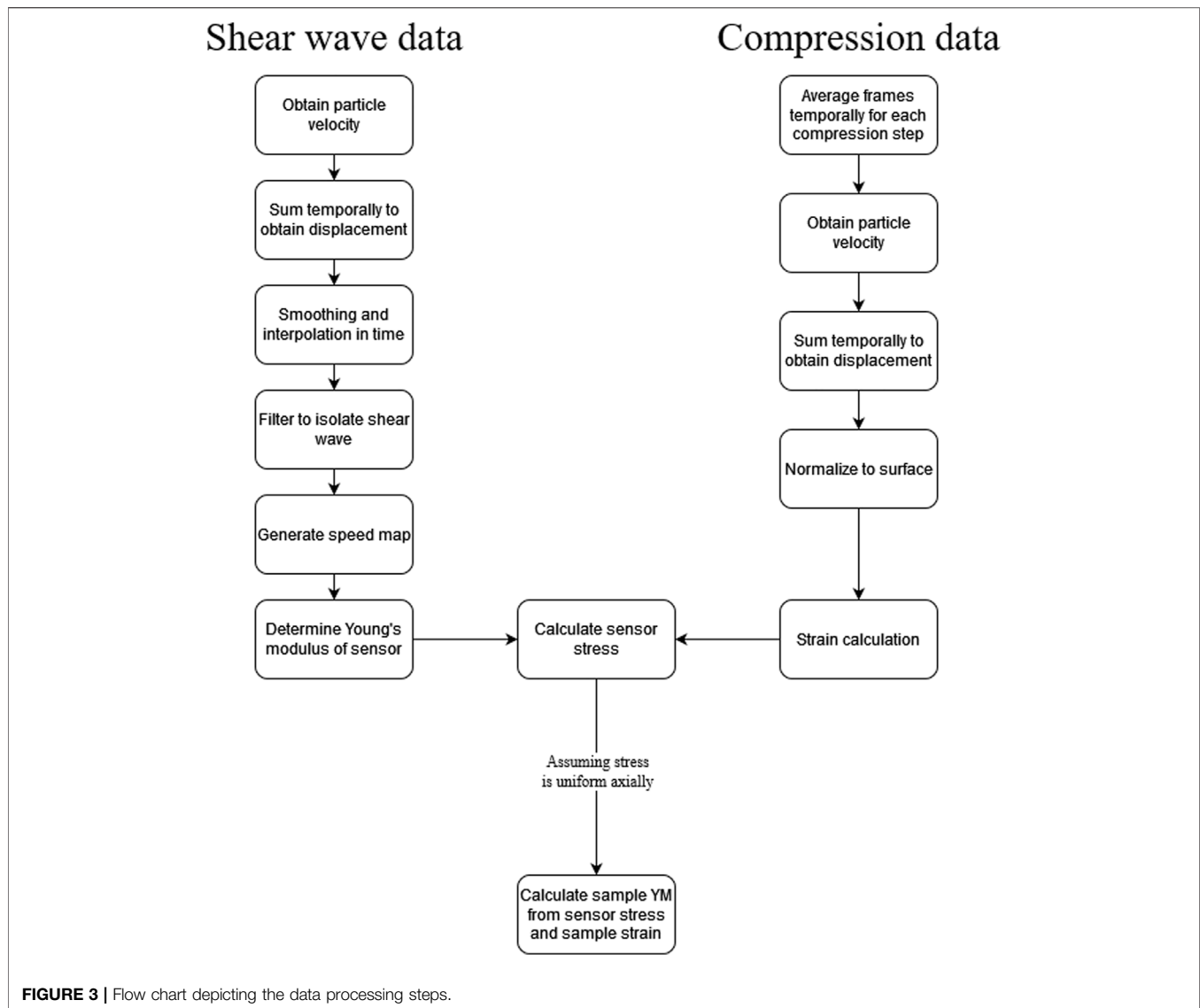


FIGURE 3 | Flow chart depicting the data processing steps.

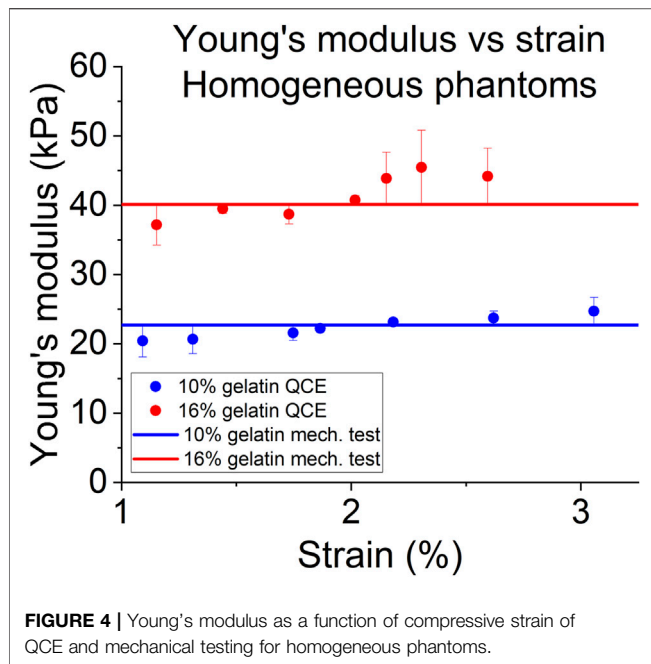
filter with polynomial order of one and a frame length of 21. The data were interpolated in time by a factor of 5. The Fourier transform of the displacement data in time was taken at the level of the sensor in the path of wave propagation to determine the frequencies corresponding to the shear wave. The displacement data stack was then bandpass filtered to isolate the frequencies corresponding to the shear wave. A cross correlation-based method was utilized to obtain the temporal lags of the wave propagation in the sensor. A shear wave speed map was generated from the wave propagation time lags and spatial coordinates using a sliding window with a kernel size of approximately 1.69 mm.

The compression data was processed as follows. The IQ data for each compression data set was loaded, and the 120 frames were averaged in time to remove noise for each compression step. The result was a stack containing complex B-scan images of the sensor and sample. The complex images were converted to intensity grayscale images, which were then segmented using

MATLAB's volumeSegmenter tool. The vector method was used on the complex image stack to generate particle velocity maps, which were then cumulatively summed in time to generate displacement maps. Following this, each displacement image was normalized such that the displacement at the interface between the sensor and the transducer was zero. The strain was calculated from the displacement frame by using the least-squares regression fit of the axial displacement with a window size of approximately 0.225 mm [25].

The Young's modulus of the sensor was calculated from the speed of the shear wave. The area of the excitation pulse was removed to avoid near-field effects, which overestimates the velocity of the waves generated in or near the shear wave excitation focal point. The wave speed map of the sensor was converted to Young's modulus, E , via the following relationship [10]:

$$E = 3\rho c_s^2 \quad (1)$$



where ρ was the mass density of the sensor material and c_s was the shear wave speed. The mass density was assumed to be $1,000 \text{ kg/m}^3$. The speed in the sensor was averaged to obtain Young's modulus.

The stress in the sensor was then calculated *via* the following relationship:

$$\sigma = E\epsilon \quad (2)$$

where σ was the stress in the sensor and ϵ was the strain in the sensor. Assuming that the stress was uniform axially between the two rigid boundaries of the setup, i.e., the transducer and lower plate, Young's modulus was computed for each pixel in the strain map of the sample by

$$E_{\text{sample}} = \frac{\sigma_{\text{sensor}}}{\epsilon_{\text{sample}}} \quad (3)$$

The resulting images were then smoothed *via* cubic spline interpolation. The average of the segmented area was then taken after outliers were removed to obtain the final average Young's modulus value for the sample or region of interest.

3 RESULTS

3.1 Gelatin Phantoms

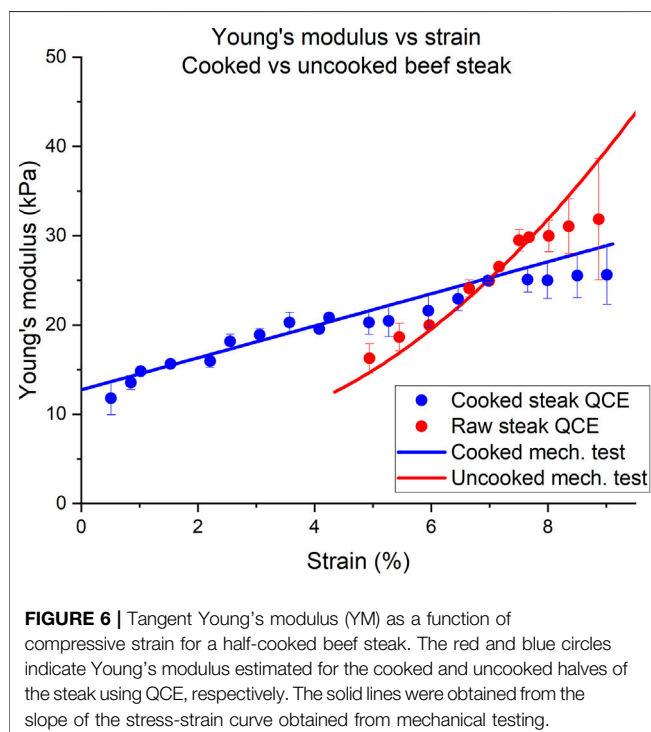
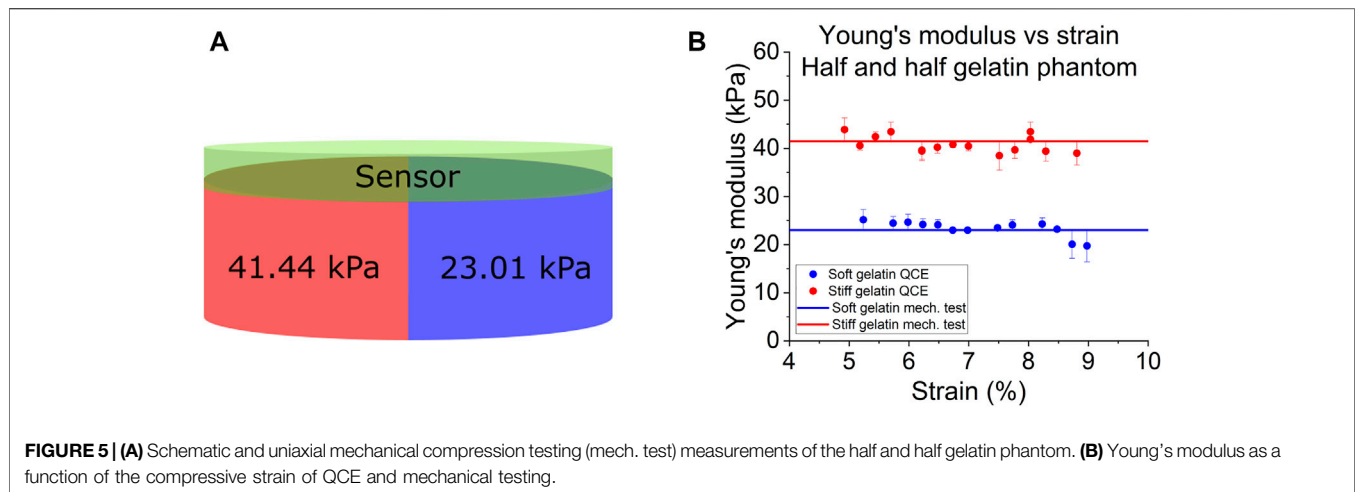
The results from the homogeneous phantoms are shown in **Figure 4** and in **Table 1**. For the 10% phantom, the average value was $22.36 \pm 1.59 \text{ kPa}$, and the average for the 16% phantom was $41.39 \pm 3.15 \text{ kPa}$. The slope of the stress-strain curve from uniaxial testing resulted in Young's modulus of 22.73 kPa for the 10% phantom and 40.14 kPa for the 16% phantom. The average percent errors for the 10% phantom and 16% phantom were 5.91% and 6.68%, respectively. The results from the half and half gelatin phantom are shown in **Figure 5**. For the softer side, the average value of the measurements using the ultrasound compression method was $23.33 \pm 1.66 \text{ kPa}$. The slope of the stress-strain curve from uniaxial testing resulted in Young's modulus of 23.01 kPa . Overall, there was an average percent error of 5.60% by QCE as compared to mechanical testing. For the stiffer side, the average elasticity was $40.83 \pm 1.75 \text{ kPa}$ as measured by compression elastography. The slope of the stress-strain curve from mechanical testing was 41.44 kPa . Overall, there was an average percent error of 3.98% by QCE as compared to mechanical testing.

3.2 Tissue Sample

The measured elasticity values for the tissue sample can be seen in **Figure 6** and in **Table 2**. The elasticity of the uncooked steak increased from 16.25 kPa to 31.84 kPa over a compressive range of 4.94%–8.87%, as estimated by QCE.

TABLE 1 | Values of stress and strain for the soft and stiff sides of the phantom experiment.

Phantoms				
Step	Soft side strain (%)	Soft side stress (kPa)	Stiff side strain (%)	Stiff side stress (kPa)
1	5.24	131.71	4.92	215.95
2	5.74	140.15	5.18	210.03
3	5.99	147.55	5.44	230.83
4	6.23	150.78	5.70	247.51
5	6.48	156.22	6.22	245.19
6	6.73	154.88	6.22	246.14
7	6.98	160.47	6.48	260.37
8	7.48	175.69	6.74	274.66
9	7.73	186.05	6.99	282.89
10	8.23	199.84	7.51	288.93
11	8.48	196.50	7.77	308.21
12	8.73	175.12	8.03	336.29
13	8.98	176.99	8.03	348.75
14	—	—	8.29	326.50
15	—	—	8.81	343.42



Mechanical testing measured Young's modulus of the uncooked steak as 14.61–38.66 kPa over the same region. Overall, there was an average percent error of 6.06% by QCE as compared to mechanical testing. The elasticity of the cooked steak increased from 11.79 to 25.62 kPa over a compressive range of 0.5%–9.01%, as estimated by QCE. Mechanical testing measured Young's modulus of the cooked steak as 13.64 to 28.95 over the same region. Overall, there was an average percent error of 5.59% by QCE as compared to mechanical testing.

For the strain ranges shown, the cooked half of the steak had a much more linear slope with increasing compression than the

uncooked half of the steak. The solid blue line is the derivative used to approximate the stress-strain curve of the uniaxial testing data. The raw tissue did not have a linear trend of tangent Young's modulus beyond ~7%. The mechanical testing uniaxial data is plotted in **Figure 6** as the solid red and blue lines for the uncooked and cooked steak, respectively.

4 DISCUSSION

Our results show good agreement between Young's modulus measured with QCE and Young's modulus measured by uniaxial testing. These results validate QCE as a simple, multimodal, accessible, and relatively low-tech method to obtain quantitative information on tissue stiffness.

Gelatin was chosen because, for small strains, Young's modulus is approximately constant as a function of strain [26, 27]. Some studies have shown an approximately linear stress-strain curve up to approximately 20% strain [28]. We found that this remained a good approximation in the strain region we considered, which was below 10% strain. When measuring the homogeneous phantoms, the errors remained below 7% when compared to uniaxial compression testing. When estimating the elasticity of each side of the heterogeneous phantom using QCE, the errors remained below 6% as compared to uniaxial mechanical compression testing, indicating that QCE is capable of a high degree of accuracy for tissue stiffness assessment.

Hooke's law is valid only in the case of materials with linear-elastic behavior; i.e., in situations where the stress-strain curve is linear. Here, we assume that we are within the linear-elastic region, that the strain increases slowly, and that there is no permanent modification to the material.

The tangent Young's modulus values measured in the tissue sample (steak) *via* QCE were in good agreement with mechanical testing as well. There was a significant preload on the raw side prior to elastography measurements being taken. This is because the tissue shrank during cooking, and thus, the initial strain on the thicker uncooked side was greater as

TABLE 2 | Values of stress and strain for the cooked and uncooked sides of the steak experiment.

Steak				
Step	Cooked strain (%)	Cooked stress (kPa)	Raw strain (%)	Raw stress (kPa)
1	0.50	6.87	4.93	72.04
2	0.89	12.81	5.50	94.05
3	1.05	15.35	6.00	117.36
4	1.51	23.25	6.66	154.10
5	2.19	36.58	6.99	175.51
6	2.58	44.85	7.16	186.97
7	3.04	55.27	7.48	211.46
8	3.58	68.67	7.66	225.12
9	4.04	80.70	7.98	253.04
10	4.27	87.02	8.31	283.55
11	4.97	107.42	8.89	343.59
12	5.27	117.03	—	—
13	5.97	139.81	—	—
14	6.43	155.82	—	—
15	6.97	175.80	—	—
16	7.65	202.35	—	—
17	7.97	215.24	—	—
18	8.50	237.77	—	—

compared to the thinner, cooked side. Though the raw and cooked tissues were under different loads during the experiment, accurate results were still obtained without the use of a calibrated stress sensor. As in the gelatin phantom, the error remained low at ~6%, proving further that QCE is able to accurately measure tissue stiffness.

The tissue did not exhibit linear elasticity, which is as expected [21, 29, 30]. While most tissues show strain hardening, this behavior varies by tissue type [31, 32]. This is characterized by a large initial compression with low levels of stress followed by strain hardening, where the stress increases in a nonlinear fashion with increasing strain [31, 33]. The raw tissue exhibited this to a greater degree than the cooked tissue, but QCE can accurately measure tissue stiffness regardless of whether it is in the linear or nonlinear regime since the stress measurements are made for a given strain.

Typically, to perform quantitative strain elastography, a compliant sensor with precisely known properties is needed [22, 23]. This involves uniaxial testing beforehand to measure these properties and precise measurements of the pre-stress before imaging. Uniaxial testing machines are expensive and are unlikely to be found in most hospitals or clinical settings, unlike ultrasound imaging systems. Additionally, if the sensor breaks or needs to be changed, calibration must again be performed on the sensor, which is particularly noteworthy in cases where sterility is necessary. One major novelty of the presented method is that it eliminates the need for the calibration step. The sensor need only be linear in the strain region that will be utilized in the sensor and sufficiently scattering to visualize shear wave propagation. If the sensor enters the nonlinear region, the elasticity is no longer accurately estimated by Eqs 4–1, which means that Young's modulus value will not be correct. An alternative form of the relationship in Eq. 3 shows the problem more clearly:

$$E_{\text{sample}} = \frac{\sigma_{\text{sensor}}}{\epsilon_{\text{sample}}} = E_{\text{sensor}} \frac{\epsilon_{\text{sensor}}}{\epsilon_{\text{sample}}} \quad (4)$$

If E_{sensor} is nonlinear with compression, then this nonlinearity will be transferred into E_{sample} and give an incorrect measure of elasticity despite having the proper strain ratio.

An additional constraint on the sensor is that it must not be too soft or stiff. If the sensor is too soft, then during compression, most of the deformation will go into the sensor instead of being distributed between the sensor and sample, and there will be no measurable strain in the sample. If the sensor is too stiff, it will not deform during compression, and there will be no measurable strain and, thus, stress. In both cases, the method will likely fail due to the lack of a measurable strain. The strain must be accurately measured in both the sensor and the sample for this method to be successful. In the clinic, acoustic coupling gel is often used, but acoustic coupling gel pads are also commercially available, which may serve as stress sensors. One such gel pad is Aquaflex, which has been studied previously [34] and is widely available. However, their mechanical properties are not often known, and thus, this technique would be useful in such use cases assuming the stiffness of the gel pad is not too low or high. One difficulty in using commercial gel pads is that they are designed to be acoustically transparent, which would limit visualization of the shear wave propagation and, therefore, the estimation of shear wave speed. A commercially manufactured gel pad with a low concentration of acoustic scatterers would be preferable for this application.

Clinical ultrasound imaging machines typically do not allow the transducer to act as a shear wave source, which means that this method cannot be used in those settings. Though it is most convenient when the transducer can act as both a shear wave generator and an imaging modality, this method could be performed with an additional wave source, such as a mechanical actuator utilized in MRE [35]. The main concern

is that wave propagation with a sufficient signal-to-noise ratio [36] must be observed in the sensor.

Shear wave propagation in the sample is not necessary when using this method. This means that the method can work for any deformable tissue even in the presence of high dispersion or attenuation, provided that strain in the tissue can be accurately measured. If the underlying structure is not deformed enough, then strain cannot be determined, and therefore the value of E_{sample} is unreliable. In this case, it is unlikely that either strain or shear wave elastography would be possible, e.g., in bone. However, having shear wave propagation in the sample is advantageous, as it allows for additional analysis and comparison, such as for multimodal elastography [37, 38], which has shown improvements in disease detection as compared to ultrasound imaging alone.

Two key processing steps have the potential to change the accuracy of QCE. First is the estimation of the shear wave speed in the sensor. If the speed in the sensor is poorly estimated due to poor wave propagation, poor scattering, or other factors limiting wave propagation or its detection, then the accuracy of this method will decrease. The algorithm with which speed is estimated also determines accuracy to some degree. If using windowed cross-correlation, for example, the choice of window size will influence the accuracy [39]. The second key step is the estimation of strain within both the sensor and the sample. The algorithm used here will determine the key factors that must be considered. Like the estimation of speed, the window across which the strain is estimated will also influence the accuracy [40, 41].

Studies in optical coherence elastography have shown that quantitative strain elastography has better contrast and mechanical spatial resolution than wave-based elastography methods [42, 43]. These methods rely upon high SNR because they depend upon being able to track small movements either in *via* phase-sensitive methods or *via* speckle tracking [40]. Additionally, the use of a sensor decouples the measurement from the optical (in the case of OCE) or acoustic properties of the tissue, meaning that stiffness can be estimated in samples with poor imaging visibility because the underlying mechanical properties can be measured by their effects on the sensor. This

may also mean the ability to detect the effects of structures that lay beyond the imaging depth [22].

There are several limitations to this technique. First, this technique is only capable of capturing a 2D representation of tissue stiffness. These experiments were performed in a very controlled manner by using the custom holder. Ideally, this technique would be performed clinically by manually controlling the transducer on patients where discrete 100 μm steps would not be possible or practical and where significant out-of-plane motion is expected. It may be possible to overcome this problem with transducers that provide 3D information or by incorporating a fixed transducer or sample holder. Additionally, the effect of compression step size on decorrelation between image frames was not considered. This method relies on the correlation between successive stepped frames, and too much movement within the frame will degrade this correlation. Future work will focus on applying QCE to various tissue types, investigating the relationship between window size and accuracy, assessing the repeatability of QCE across different tissue samples, examining the effect of the rate of loading on measurements, and evaluating the technique on highly anisotropic and highly viscoelastic tissues.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JR created the setup, designed and manufactured the custom holder, performed the experiments and analysis, and wrote the paper, MS and AN provided expertise throughout and helped guide processing methodology. MS and AN also helped with strain estimation algorithm implementation. SA and KL provided technical guidance, supervised data analysis, and initiated the research. JR, MS, SA, and KL came up with the concept. All authors contributed to manuscript revision, read, and approved the submitted version.

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Magnetic resonance elastography of malignant tumors

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Cancer biomechanical properties, including high stiffness, solid stress, and interstitial pressure, as well as altered micro-architecture, are drivers of tumorigenesis, invasiveness and resistance to treatment. Magnetic resonance elastography is an emergent non-invasive imaging method to assess the tumor mechanical properties in a spatially resolved fashion. Several MRE acquisition and reconstruction methods have been developed to assess tumors and surrounding tissues. It is increasingly recognized that the visco-elastic properties assessed with MRE are useful for characterizing malignant tumors and evaluating treatment response in various organs. Generally, malignant tumors, except brain tumors, have high stiffness and high visco-elastic ratio or fluidity. MRE transducers, acquisition sequences and reconstruction algorithms are continuously improved to increase depth penetration and spatial resolution, and limit artifacts at spatial discontinuities. Moreover, assessment of compression stiffening might provide new biomarkers of the altered physical traits of cancer. Increasing research and clinical validation will improve the efficacy of MRE for cancer characterization.

KEYWORDS

magnetic resonance elastography, cancer imaging, biomechanical properties, malignant tumor, biomarker

1 Introduction

Malignant tumors have four physical hallmarks which are high stiffness, solid stress, and interstitial fluid pressure, as well as altered micro-architecture. These biomechanical properties facilitate tumor progression and limit treatment efficacy by hindering drug delivery [1]. Magnetic resonance elastography (MRE) is increasingly used to assess tumor visco-elasticity [2–5]. This assessment may be useful for characterization of cancer severity and evaluation of treatment response [6–8].

2 MRE for tumor assessment

For reconstructing the tissue mechanical properties, MRE can be decomposed in three steps [9, 10]. First, an actuator is placed close to the tumor to generate mechanical vibrations. Second, a phase contrast MRI acquisition sequence, centred

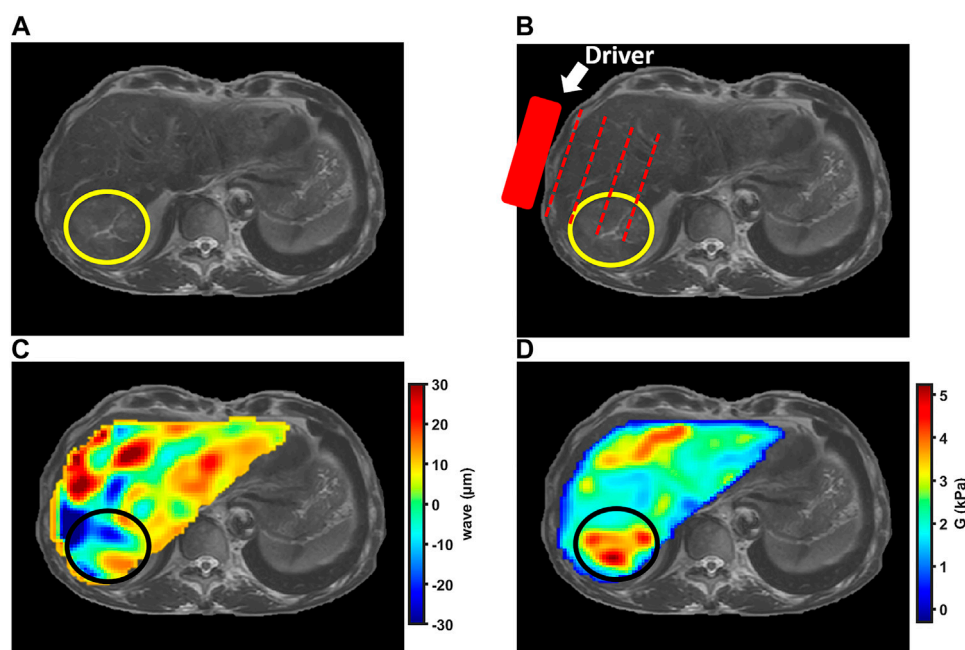


FIGURE 1

MRE examination in patient with hepatocellular carcinoma indicated by the circle. **(A)** Anatomic T2-weighted MR image of liver showing slightly hyperintense hepatocellular carcinoma in right liver lobe. **(B)** Anatomic MR image illustrating external driver position. **(C)** Shear wave color map showing propagating waves within the liver and tumor. **(D)** Shear modulus color map showing liver and hepatocellular carcinoma stiffness.

on the tumor, is used to encode the motion. Third, a reconstruction algorithm is used to assess the mechanical properties from shear wave propagation (Figure 1).

2.1 Actuators

Several types of actuators have been developed to propagate shear waves in tissues and tumors. The shape, size and power of the actuators are adapted to the explored organ. Moreover, drivers have been specifically developed to assess small animals in which tumors can be induced or implanted for preclinical studies.

2.1.1 Clinical studies

In early MRE studies, electromagnetic actuators have been used [11]. The drivers are composed of a coil connected to a piston. Using the B₀ of the MR scanner, an alternating current in the coil generates mechanical vibrations of the piston. This kind of actuator has been used to detect tumors located in breasts [11], to assess the mechanical properties of liver tumors [2] and to compare MRE results with histologic features in prostate cancer [12]. However, using this system, near field artefacts may occur with echo planar imaging [13].

Alternatively, pneumatic drivers which use air pressure waves to obtain mechanical vibrations, have been proposed. A speaker system generates sinusoidal displacements in a passive driver placed against the explored organ. System simplicity and easy configuration of frequency and wave amplitude are advantages of pneumatic drivers. Furthermore, the geometry of the membrane can be optimized for the explored organ, such as a pillow-like driver for propagating shear waves inside the brain. Pneumatic drivers have been used to explore various tumors in the brain [14, 15] and the liver [16]. They are easily positioned but they work mainly at a frequency below 100 Hz and have low frequency accuracy because of the relation with the resonant eigenmodes of the pneumatic pipes [17]. A specific transducer has been designed for tomoelastography [18]. It is composed of air driven pressure pads, one or two pads being placed on the anterior side and two pads on the posterior side [19, 20]. The multiple excitation sources allow for exploring retroperitoneal located organs such as the pancreas [20].

A mechanical actuator using a rotational eccentric mass to generate shear waves has also been developed. It is designed to easily be placed close to different organs such as the brain [21]. One disadvantage of the system is the coupling between frequency and amplitude of the shear waves. Hence, the actuator may be less tolerated at high frequency by sensitive patients.

2.1.2 Preclinical studies

In preclinical high field (7T) MRE scanners, external actuation is induced with piezoelectric transducers, electromechanical uniaxial shakers or loudspeakers. The last two systems need to be placed outside the bore. Shear waves are propagated to the tumors with a rigid carbon rod. Small animal studies have shown the ability to assess tumors implanted subcutaneously [22] or orthotopically in brain [23, 24], breast, pancreas [5] and colon [6]. Prostate tumors have also been examined ex-vivo [25]. Pepin et al. [26] used an electromechanical driver connected to a silver needle inserted in mice Hodgkin's lymphoma.

2.2 Motion encoding

In MRE, oscillating magnetic field gradients, also called motion-encoding gradients (MEGs), are used to encode the harmonic displacement in phase contrast images. The MEGs can have various shapes. Rectangular and trapezoidal MEGs have generally higher encoding efficiency than sinusoidal gradients. MEGs are typically inserted at each side of the refocusing pulse. One or more directions can be encoded, and for complete wave field characterization, encoding of the three spatial directions is needed.

The MEGs are inserted in several types of imaging sequences, including gradient-echo, spin-echo or echo-planar imaging. Echo-planar imaging reduces the acquisition time [27]. However, this kind of readout is associated with distortion artifacts which may have an impact on the displacement estimation. Moreover, echo-planar imaging is characterized by long echo times caused by the long echo train. At long echo times, the image signal may be drastically decreased, especially in tissues with short T2, such as the liver. In clinical practice, gradient-echo and echo-planar imaging are mainly used [28, 29].

MRE provides an image of the harmonic wave displacement at a particular time point of the oscillation. Since the motion is synchronized with the pulse sequence, several time points of the displacement are imaged by delaying the MEG. Typically, four to eight-time steps are acquired along a period of the oscillating displacement. Fourier transformation is applied to extract the fundamental harmonic component of the displacement field.

2.3 Mechanical properties assessment

Several reconstruction methods have been developed to assess the mechanical properties with MRE. The methods vary according to unwrapping algorithm, noise reduction, compression wave removal, and inversion algorithm.

2.3.1 Local frequency estimation

With the local frequency estimation the frequency of the wave displacement is assessed locally [30, 31]. The shear modulus G (Pa) is deduced from its equation into a local homogeneous, linear, isotropic and elastic solid

$$G = \rho \left(\frac{\omega}{\nu} \right)^2,$$

where ρ is the material density (typically assumed to be 1,000 kg/m³), ω the angular frequency of the mechanical excitation (rad/s) and ν the shear wave spatial frequency (m⁻¹). The local frequency estimation method assumes homogeneity and no attenuation, thus only the real part of the complex stiffness is provided. The method has been used in patients to assess breast [32, 33], liver [34] and prostate cancer stiffness [35]. It has also been extended to estimate tissue viscosity by applying the local frequency estimation on the curl of the displacement field [36].

2.3.2 Direct inversion

The complex shear modulus (G^*) can be expressed by direct inversion of the Helmholtz wave equation,

$$G^*(r, \omega) = -\rho\omega^2 \frac{u_i(r, \omega)}{u_{i,11}(r, \omega) + u_{i,22}(r, \omega) + u_{i,33}(r, \omega)}$$

where r is the spatial coordinate system (1, 2, 3), u the complex harmonic shear wave displacement, ω the angular frequency of the mechanical excitation and i , the motion-encoding direction [37, 38]. Direct inversion is performed after application of a high-pass filter to remove the longitudinal wave [39] and assuming that the viscoelastic medium is linear, isotropic and homogeneous.

Another common method to remove the compression waves is to apply a discrete curl operator to the displacement field before the inversion [40]. In contrast to high-pass filtering, the curl is applied in the three-dimensional space and requires wave displacement encoding along the three spatial directions.

As the shear modulus is a complex quantity, it can be expressed as $G^* = G' + iG''$. The real part G' and the imaginary part G'' correspond respectively to the storage modulus representing tissue elasticity and to the loss modulus representing tissue viscosity [41, 42]. Other parameters that represent the visco-elastic ratio can be derived, including the damping ratio ($\zeta = \frac{G''}{2G'}$) and the shear modulus phase angle ($\varphi = \arctan(\frac{G''}{G'})$) which is also called fluidity, ranging from 0° for purely elastic materials to 90° for purely viscous materials [10, 43].

Direct inversion has been used in the clinics to assess the viscoelastic properties of breast [11, 44], liver [2] and prostate [25] tumors. It has also been used in preclinical MRE to assess subcutaneous [22] and orthotopic tumors [23].

2.3.3 Multifrequency direct inversion

With multifrequency direct inversion, multiple wave data sets acquired at different vibrational frequencies are combined [45]. With a variant of the method, called multifrequency dual viscoelastic reconstruction, frequency averaging is performed during the computation of the magnitude and the phase of the complex shear modulus rather than averaging the complex shear moduli obtained at each frequency [46].

More recently, tomoelastography has been introduced. It consists in using spatial filters in *k*-space to obtain shear waves in multiple directions. The shear wave speed and the attenuation are then averaged over all directions and frequencies. This method when used with multiple drivers placed around the explored area [19] is particularly promising to assess tumors located inside deep organs such as the pancreas. By increasing data quantity, multifrequency direct inversion increases the stability and the reliability of MRE. However, scan time can be long. Tomoelastography has already been used in the clinics to explore brain, liver, pancreas and prostate cancers [7, 20, 47, 48]. With the multifrequency direct inversion methods, the frequency dependence of the shear modulus is not considered.

2.3.4 Finite element method

The local frequency estimation and direct inversion assume local homogeneity. These methods work well in areas where the elasticity is almost constant. However, it has been reported that the homogeneity assumption may cause errors in regions where the elasticity variation is high [49].

There are finite element methods in which the local homogeneity assumption is not used. These methods are categorized into two groups: direct [50] and iterative methods [51, 52]. In the direct methods, the discretized equation of motion is rearranged to result in a system of equations with the shear modulus parameters as unknowns, which can be solved directly for a map of the shear modulus. Alternatively, for iterative methods, the inverse problem is considered as an optimization problem in which the shear modulus is changed iteratively to minimize the error between measured displacement and the simulated displacements [53]. The finite element method has been used to measure the mechanical properties of prostate cancer [12].

2.4 Comparisons of MRE methods

As described in this section, various MRE methods have been developed to reconstruct the tissue mechanical properties. Different mechanical actuators, MR imaging sequences, and reconstruction algorithms are used. How these various parameters influence the results of mechanical properties measurements is a topic of ongoing investigation. In chronic liver disease, Huwart et al. prospectively compared the performance of MRE using echo-planar and spin-echo

imaging for staging of hepatic fibrosis [27]. They showed that echo-planar imaging substantially decreased the data acquisition time, while maintaining the image quality and diagnostic performance for staging liver fibrosis.

More recently, the diagnostic performance of 2D gradient-echo and 3D echo-planar MRE has been compared to stage liver fibrosis. It was observed that the technical failure rate was lower with 3D echo-planar versus 2D gradient-echo MRE, but the diagnostic performance of both MRE sequences was similar when the same shear wave frequency was used [29, 54, 55]. The two MRE acquisitions differed regarding image acquisition (2D versus 3D, gradient-echo versus echo-planar), slice thickness, and reconstruction algorithm.

Moreover, lower stiffness values were observed with 3D echo-planar MRE than with 2D gradient-echo or 2D echo-planar MRE. This has been attributed to the fact that the 2D algorithm assumes all waves propagate in the imaging plane, which can lead to overestimation of the wavelengths of waves intersecting the imaging plane obliquely [55].

When comparing 2D echo-planar and gradient-echo MRE in the liver, the image quality scores were significantly higher and the failure rate substantially lower when using echo-planar imaging [56]. The higher quality with echo-planar MRE may be explained by the fact that the sequence is less sensitive to T2* decay than gradient-echo imaging and because its acquisition time is shorter, allowing single breath-hold acquisitions in the liver.

In the normal brain, it has been found that stiffness values obtained with a finite element model approach were 40% higher than those obtained with direct inversion using a curl method [57]. These higher values may be explained by lower noise sensitivity with a finite element model [58].

As the measured stiffness depends on the choice of data acquisition and image reconstruction, it has been recommended to normalize stiffness measurements to a reference tissue. In brain cancer patients, tissue stiffness has been measured relative to healthy white matter [14]. In a cohort of patients with solid pancreatic masses, it has been shown that the stiffness ratio (calculated as the ratio of mass stiffness to the parenchymal stiffness) outperformed stiffness for differentiating between pancreatic ductal adenocarcinomas and benign mass forming pancreatitis [59]. However, using a reference tissue for tumor MRE may be limited by the variations of reference stiffness related to age or disease (such as liver fibrosis in patients with liver cancer).

Various shear wave frequencies are used for MRE. In clinical studies, frequencies below 200 Hz (usually between 40 and 60 Hz) are used. In small animal studies, higher frequencies between 100 and 1,000 Hz are often used. Increasing the frequency improves the spatial resolution but decreases shear wave penetration. For the clinical MRE assessment of the deep-seated pancreas, it has been observed that more consistent results were obtained at 40 Hz than at 60 Hz [28].

Increasing the frequency increases the apparent stiffness [60]. This shear wave frequency dispersion may complicate comparisons between MRE studies performed at different frequencies. However, when probed with multifrequency MRE, the frequency dispersion coefficient may give additional information about tissue structure [61–63]. The potential value of multifrequency MRE in cancer remains to be determined.

One should also be aware of shear wave frequency differences when comparing stiffness results between MRE and ultrasound elastography. Indeed, whereas transient ultrasound elastography uses 50 Hz, acoustic radiation force impulse-based techniques have most of their energy between 120 and 160 Hz [64, 65]. Moreover, there are other differences between MRE and ultrasound elastography. First, ultrasound work is based on transient excitation and usually measures wave speed, but this is a group velocity rather than a phase velocity. Second, Young's modulus (compression modulus) is usually the quantity reported at ultrasound elastography, which is typically three-times the shear modulus that is reported with MRE [10].

3 Biophysical properties of cancer and elastography studies in animals

During the recent decades, our understanding of cancer has evolved, and malignant tumors are currently viewed as aberrant organs composed of tumor cells and their surrounding stroma, both of which influence the biomechanical properties of cancer [66–68]. These biomechanical properties include high stiffness, solid stress and interstitial fluid pressure. These abnormal physical traits affect tumor progression and treatment resistance [69, 70].

3.1 Tumor biomechanical components

3.1.1 Definitions and origins of high stiffness, solid stress and fluid pressure.

3.1.1.1 Stiffness

Stiffness or rigidity is an intrinsic property of tissue defined as the resistance of the tissue to the applied force. Stiffness is mainly determined by extracellular matrix composition and organization [71]. Particularly, tumor stiffness is related to collagen deposition and cross-linking [5]. It has been shown that hyaluronic acid swelling also increases tumor stiffness [72].

Moreover, tumor stiffness is influenced by extracellular matrix modifications caused by stromal cells. Indeed, it has been shown that the fraction of stroma cells and the activity of fibroblasts and immune cells correlate with stiffness [23, 73, 74]. In contrast, necrotic areas tend to decrease tumor stiffness [75].

In addition to the structural part of tissue stiffness related to matrix deposition, a functional part related to soft tissue water

content has been identified [60]. Indeed, several studies have reported elevated stiffness in tissues with inflammation and high interstitial or intravascular pressure [63, 76, 77].

3.1.1.2 Solid stress

The solid stress is a mechanical force influenced by the solid components of the tumor, including the cancer cells, the collagen fibers and the hyaluronan acid gel [68]. Cellular proliferation, matrix deposition, cell contraction, and abnormal growth patterns all lead to compressive and tensile solid stresses within tumors (1). Solid stress can thus be generated by both extracellular matrix and cells, in contrast to tumor stiffness which is mainly a property of the extracellular matrix [78, 79]. This difference between solid stress and stiffness is illustrated by the fact that primary and metastatic tumors can have substantially different levels of solid stress despite similar stiffness values [78].

3.1.1.3 Interstitial fluid pressure

The isotropic fluid pressure includes the pressure exerted by liquid within the interstitial space, blood and lymphatic vessels. In tumors, elevated interstitial fluid pressure is caused by leakage of plasma from abnormally permeable tumor blood vessels and insufficient lymphatic drainage. It adds to the solid stress to compose the total tumor pressure, which is dominated by the solid stress component, at least in pancreatic tumors [80].

3.2.1 Effects of abnormal physical traits on tumor progression.

High tumor stiffness, solid stress and interstitial fluid pressure promote tumor progression and resistance to treatment through activation of a large cascade of mechanoresponsive pathways in cancer cells and stromal cells, including endothelial, epithelial, mesenchymal and immune cells [1, 81, 82]. The mechanotransduction effect of high solid stress has been exemplified in a recent study showing that the application on the healthy mouse colon of an external pressure similar in magnitude to the stress induced by tumor growth, caused the stimulation of tumorigenic pathways without changes in tissue stiffness [83].

It should be noted that the physical traits favouring tumor progression are intricate. Physicists were tempted to consider the steps of the cancer metastasis cascade as single events caused by one mechanical alteration. However, this simple view has been challenged by the finding that several cancer parameters influence each other and contribute to tumor growth and cancer progression [84]. Indeed, by compressing blood and lymphatic vessels, solid stresses contribute to increased fluid pressure in tumors. Through strain-stiffening, solid stresses also increase tumor stiffness. Finally, fluid flow activates fibroblasts, which then contribute to increased solid stresses and stiffness values [1].

Globally, the changing biophysical properties in cancer are strongly coupled with abnormalities in the tumor vasculature and

extravascular compartments [79]. Indeed, high solid stresses related to high matrix stiffness and hyper-proliferative pressure in tumors are large enough to compress the blood and lymphatic vessels, impairing blood flow and the delivery of oxygen, drugs, and immune cells [85–87]. Impaired drug delivery is also promoted by the high interstitial fluid pressure secondary to the hyperpermeability of tumor angiogenic vessels [88]. Moreover, the pressure difference in the periphery of the tumor causes fluid leaking from the vessels at the tumor boundaries in the surrounding tissue. This facilitates the transport of cancer cells into the host tissue, increasing tumor progression and metastatic risk [89].

Both dysregulated angiogenesis which produces leaky blood vessels and desmoplasia which compresses the vessels, reduce the capacity of blood vessels to deliver oxygen to tumors. Hypoxia not only promotes disease progression and resistance to radiation and some chemotherapies, but also causes immunosuppression in the tumor microenvironment. Moreover, newly formed immature blood vessels cannot distribute infiltrating immune cells efficiently, whereas excessive fibrosis poses a physical barrier to immune cell migration in the tumor. Accordingly, hypoxia is associated with poor survival and resistance to treatment, especially to newly developed immunotherapies [90].

Improving drug delivery can be achieved by repairing the hyperpermeability of tumor blood vessels and/or decompressing tumor blood vessels by targeting tumor stromal components [79]. Restoration of the vascular function can be achieved with vascular normalization strategies, whereas decompression of tumor vessels can be obtained with stress-alleviating methods that target the tumor extracellular matrix and/or cancer associated fibroblasts. Most antiangiogenic agents target the vascular endothelial growth factor or its receptors. Recently, it was shown that metronomic chemotherapy and immunotherapy also can induce vascular normalization [90–92].

In contrast, stress alleviation strategies can be obtained with matrix-depleting agents, such as collagenase, relaxin, and matrix metalloproteinases. These agents have been used to reduce collagen or hyaluronic acid levels in tumors and have been shown to improve the efficacy of anticancer drugs in animal models [5, 93, 94]. Furthermore, the PEGylated human recombinant hyaluronidase (PEGPH20) has been used to reduce fibrosis and solid stress and increase survival in mouse models [95].

A second type of matrix normalization involves reversing vessel compression with cancer associated fibroblast reprogramming therapies. Hereby, cancer associated fibroblasts are turned quiescent such that they produce a smaller magnitude of forces. One such drug is losartan, which is an anti-hypertensive drug with decades of use in patients with high blood pressure. In preclinical models of pancreatic adenocarcinoma, losartan was used to decrease solid stress and decompress blood vessel, improving chemotherapy efficacy [96]. However, results in clinical trials are not yet

convincing. More precisely, if losartan has shown some promising clinical results in a phase II clinical trial [97], it is not the case for PEGPH20 [97, 98]. There are several reasons for this, including toxicity, complexity and heterogeneity of tumor microenvironment.

Indeed, matrix alleviating therapies may have contra-intuitive results. Whereas *in vivo* and *in vitro* studies have shown that extracellular matrix stiffness promotes breast, pancreas and brain cancer progression [99–102], it has recently been observed that fibrosis may have a dual role in tumor invasion as it produces stiffness induced mechano-signals for tumor progression but also mechanically restrains tumor spread [103]. The discordant results about the promoter versus protective role of fibrosis may at least be partially explained by tumor heterogeneity [5]. Indeed, heterogeneity poses the risk of obtaining inconsistent results. Moreover, the heterogeneous nature of pancreatic adenocarcinoma tissue has been reported to be a driver of aggression. Indeed, the periductal region surrounding tumor glands has been found to have increased stiffness which drives metastasis [92]. These findings suggest that the architecture and mechanics of collagen fibers adjacent to the epithelial lesion, and not bulk collagen abundance, may be an indicator of pancreatic adenocarcinoma aggression.

3.2.2 Measurement methods of cancer biophysical properties

Classically, tissue stiffness and viscoelasticity are measured *ex-vivo* with invasive methods, including atomic force microscopy and rheometry [104]. Currently, *in vivo* measurements of stiffness and viscoelasticity can be performed with ultrasound and MR elastography [105].

The measurement of solid stress remains more difficult, even with invasive methods. Stylianopoulos et al. [106] proposed to cut excised tumors and to measure the stress relaxation as the extent of tumor opening normalized to the diameter of the tumor. By using mathematical modelling, the growth-induced stress could be assessed [106, 107]. The method has been extended by Nia et al. [78] to quantify the deformation of the tumor cut plane with ultrasonography.

In vivo, the total tumor pressure, solid stress and interstitial fluid pressure can be quantified separately by inserting a piezoelectric pressure catheter inside the tumor [80, 95]. This invasive method is not routinely used in humans.

Non-invasive methods to assess tumor solid stress are being developed, using ultrasound or MR elastography measurements. Islam et al. have proposed a poroelastography model to quantify solid stress with ultrasound elastography [108, 109]. Fovargue et al. have suggested to investigate the mechanical properties of the tissue surrounding the tumor to estimate its pressure with MRE [110]. Based on the compression stiffening effect [111, 112], Pagé et al. have shown the potential of compression MRE to assess tumor solid stress in liver cancer [113, 114].

Non-invasive elastography may also be useful to assess fluid pressure by showing a correlation between fluid pressure and viscoelasticity. At ultrasound elastography, Rotemberg et al. and Gennisson et al. showed a relation between fluid pressure and tissue stiffness (measured with shear wave speed) in liver and kidney [115, 116]. Rotemberg et al. demonstrated a correlation between shear wave speed and portal venous pressure in excised canine liver. Gennisson et al. observed high kidney shear wave speed after renal vein or ureter ligation and low speed after renal artery ligation in pigs. With MRE, a correlation between liver and spleen viscoelasticity and portal venous pressure in small animals and in patients has been reported [77, 117–119].

Alternatively to elastography, dynamic-contrast enhanced MR imaging has been proposed to assess fluid flow and interstitial pressure in tumors [120, 121].

3.2.3 Non-invasive preclinical investigations with elastography

Elastography measurements have been performed in tumors implanted in mice to investigate the relation between stiffness and tumor progression [8, 73, 122]. In soft brain tumors (glioblastomas) Schregel et al. [8] observed decreased stiffness with tumor progression using MRE. In contrast, Ahmed et al. [122] found a stiffness increase during progression of liver metastases from pancreatic adenocarcinomas using shear wave elastography. In an ultrasound elastography study, Riegler et al. [73] observed a strong correlation between stiffness and tumor volume for slow growing tumors such as breast and pancreatic cancers.

Moreover, MRE studies have shown stiffness decrease with collagenase in breast cancer [5], anti-VEGF antibodies in glioblastoma [8], vascular disrupting agents in colon tumor and colorectal cancer [6, 22] and chemotherapeutic agents in glioma [26]. Several causes have been mentioned for this decreased stiffness. It has been related to the direct effect of collagenase on fibrosis, to the vascular normalization by anti-VEGF antibodies, and to the association between vascular disrupting agent effect and tumor necrosis. For the stiffness decrease with a chemotherapy treatment, it has been postulated that changes in interstitial pressures, cellularity, and extracellular components are involved [27]. However, the actual mechanism of the stiffness change is unknown.

The effect of radiation therapy was investigated on glioblastomas orthotopically implanted in mice [24]. In this study, the authors observed a decrease of tumor stiffness over time independently of radiation therapy.

In another study, the effect of radiation therapy was assessed in pancreatic cancer implanted in mice [123]. Here, a decrease of shear modulus was observed in pancreatic tumors after stereotactic body radiation therapy. It was concluded that shear modulus could help to differentiate between tumors responsive to radiation therapy and non-responsive tumors.

At ultrasound elastography, it has been observed that the tissue stiffness correlated with drug delivery in two orthotopic mouse models of pancreatic tumors [124]. However, as already discussed, drug delivery depends not only tumor stiffness, but also on solid and fluid pressure and on tumor heterogeneity [111].

It is expected that non-invasive measurements of solid stress with compression MRE may result in new elastography modalities to decouple stiffness from solid stress and improve our understanding of the roles of stiffness and stress in tumor progression and treatment response [111, 114]. Finally, high resolution ultrasound and MR elastography may be used to explore cancer heterogeneity [5, 125].

3.4. MRE for tumor assessment in patients

3.4.1 Liver

MRE is used to assess the biomechanical properties not only of hepatic tumors, but also of the liver parenchyma. Indeed, MRE offers good diagnostic accuracy to assess the features of chronic liver disease, including fibrosis, cirrhosis, and portal hypertension [118, 126–133]. These factors, especially liver cirrhosis, are promoters of hepatocellular carcinoma development [134].

3.4.1.1. Investigating the risk of cancer development

In a retrospective MRE study, the liver stiffness was measured in 301 patients with chronic liver disease of which 66 patients had hepatocellular carcinomas [135]. It was found that the patients with hepatocellular carcinomas had higher liver stiffness (mean: 5.0 kPa) than those without it (3.8 kPa). Moreover, at multivariate analysis, liver stiffness was the only significant predictor of hepatocellular carcinoma, in contrast to age, gender and blood markers.

In another study, two MRE acquisitions were performed at 12-months interval in 161 patients with chronic liver diseases [136]. The patients were divided into three groups according to sequential changes in liver stiffness as follows: high (> 4.7 kPa) on the first MRE (group A), low (< 3 kPa) on both MRE examinations (group C) and other combinations (group B). Group A classification was an independent risk factor for hepatocellular carcinoma development. The authors concluded that patients with chronic liver disease and high liver stiffness on previous MRE examinations were at high risk for developing hepatocellular carcinoma.

3.4.1.2 Characterization of liver tumors

Diffusion-weighted and dynamic contrast-enhanced MRI are routinely performed for tumor detection and characterization [137]. By investigating the mechanical properties, MRE offers a new opportunity for tumor characterization. Venkatesh et al. [16] studied the stiffness of 44 liver tumors. They found that the

malignant tumors had significantly higher stiffness than the liver (10.1 ± 3.6 kPa versus 2.3 ± 0.3 kPa), whereas benign tumor stiffness (2.7 ± 0.4) was similar to the liver stiffness.

In another study, MRE and diffusion-weighted imaging were performed in 79 patients with 124 focal liver lesions [138]. Again, mean stiffness of malignant tumors was significantly higher than that of benign lesions (7.9 vs. 3.1 kPa, $p < 0.0001$) and MRE had higher diagnostic performance than diffusion-weighted imaging for differentiating between benign and malignant tumors (AUC = 0.99 versus AUC = 0.87; $p = 0.002$).

Visco-elastic properties of 72 liver tumors were assessed [2]. In contrast to the two previous studies, 3D images were acquired, allowing for the reconstruction of the complex shear modulus from the curl reconstruction method. It was observed that the increase of complex shear modulus magnitude in malignant tumors was mainly caused by an increase of the loss modulus (viscosity) rather than the storage modulus (elasticity). These results have been confirmed in a recent study showing high tumor stiffness and fluidity in malignant liver tumors [7].

3.4.1.3 Recurrence

Tumor recurrence complicates 70% of hepatocellular carcinomas after hepatic resection at 5 years and is the expression of both early intrahepatic metastases and late development of *de novo* tumors. Predictive factors for early recurrence within 24 months of surgery, are mainly tumor-related (i.e., tumor size, tumor number, presence of tumor budding, microsatellites and vascular invasion). In contrast, late recurrence (>24 months after surgery) is probably related to the evolution of the underlying chronic liver disease, including fibrosis stage and portal hypertension severity [139, 140].

This difference in the cause of early and late recurrence explains that tumor stiffness has been explored as predictive marker of early recurrence, whereas liver and spleen stiffness has been assessed as marker of late recurrence [139, 141, 142]. In a cohort of 99 patients, Wang et al. found that high tumor stiffness and vascular invasion were two determinants associated with early hepatocellular carcinoma recurrence after hepatectomy [141].

For predicting late tumor recurrence, Marasco et al. measured liver and spleen stiffness in 175 patients before surgical resection and showed that spleen stiffness was an independent predictor of late hepatocellular carcinoma recurrence. Similarly, in a study performed in 192 patients, Cho et al. [142] observed that liver stiffness before treatment was a predictor of late hepatocellular carcinoma recurrence.

3.4.1.4 Therapy response

Few studies have been performed to assess the evolution of hepatocellular carcinoma mechanical properties after treatment. Gordic et al. [143] quantified hepatocellular carcinoma stiffness in patients after transarterial chemoembolization or yttrium radioembolization. Decrease of the mechanical properties was

observed in the treated patients. This decrease was paralleled by an increase of tumor necrosis.

Qayyum et al. [144] observed an increase of the hepatocellular stiffness after 6-weeks immunotherapy with pembrolizumab in patients with hepatocellular carcinomas. The stiffness was correlated with tumor T lymphocytes infiltration, overall survival and time to progression.

3.4.2 Breast

Manual palpation is an easy procedure to detect breast lumps but it has limited sensitivity of about 55% to detect breast cancer [145]. MRI can be used to improve breast cancer diagnosis. Dynamic contrast-enhanced MRI is routinely performed in the clinics for breast tumor detection and characterization, and more recently it has been associated with diffusion weighted and T₂-weighted imaging to perform a multiparametric analysis [146]. However, contrast-enhanced MRI still has many false-positive results [147].

Breast MRE represents an additional tool for assessing breast cancer. Early studies showed the feasibility to assess the mechanical properties of breast cancer, including stiffness, visco-elasticity, phase angle, anisotropy and frequency dispersion [11, 33, 40, 41, 148–150]. Globally, these studies and more recent clinical series [44, 151] showed increased viscosity and phase angle in malignant tumors with variable changes in elasticity, which may be increased or decreased relative to that in benign lesions. This variability may at least be partially explained by the type of breast lesions that are included in the studies.

Assessment of breast cancer is already performed in the clinics with ultrasound elastography [152, 153]. The accessibility of the organ and the fact that ultrasound elastography is easy to use and widely available are arguments in favour of this method. However, MRE provides better spatial resolution and encodes the three spatial directions of the shear wave displacement improving the accuracy of mechanical parameter quantification. This could be relevant to investigate the relation between mechanical parameters and tumor heterogeneity [5].

3.4.3 Brain

Surgical strategies to treat intracranial tumors depend on tumor consistency. Soft tumors generally require less invasive surgery, while firm fibrotic tumors may require more open surgery. It has been shown that determining brain tumor stiffness with MRE is feasible and correlates with surgical consistency [3, 154–157]. Another important factor in determining the difficulty of resection is the adherence of the tumor to the surrounding tissue, where tumors that are adherent are more difficult to remove. To address this problem, slip interface imaging has been developed. The basic idea is that if two adjacent compartments of tissue are free to move independently of one another, then as a shear wave

propagates across the boundary, a discontinuity will be created. This discontinuity can be detected on a shear strain map [158].

Regarding tumor characterization, several studies have suggested that benign meningiomas are the stiffest intracranial tumors and that they have the highest fluidity (phase angle) and viscosity (loss modulus) [3, 159, 160]. In contrast, malignant tumors, especially glioblastomas, are characterized by low stiffness (soft tumors) and low fluidity and viscosity [43]. However, discrimination between different tumor types based on the values of the mechanical parameters is currently limited because of substantial overlap of the distributions of each of the parameters from different tumors. To remove the bias in the reported values of the mechanical parameters caused by differences of hardware, reconstruction methods and signal to noise ratios, it has been recommended to compute the difference in each shear modulus parameter between the tumor and the contralateral normal appearing white matter. This has resulted in some improvement in defining the distribution of values in different tumors. Particularly, the distribution of delta loss modulus values in meningiomas is unique in that it is the only distribution with a predominantly unipolar positive distribution. Nevertheless, even after normalizing a wide range of values for essentially all tumors and all parameters remains [3].

Further characterization of gliomas with MRE has been shown by Pepin et al. [161]. In their study, gliomas were softer than healthy brain parenchyma, with grade IV tumors softer than grade II. Tumors with an isocitrate dehydrogenase 1 (IDH1) gene mutation were significantly stiffer than those with wild type IDH1. The authors conclude that noninvasive determination of tumor grade and IDH1 mutation may result in improved stratification of patients for different treatment options and the evaluation of novel therapeutics.

The potential role of fluidity in brain tumor aggressiveness has been described by Streitberger et al. The authors found that fluidity of neurotumors is anomalous in that it decreases as water content increases, enabling glioblastomas to infiltratively finger in normal surrounding brain matter [43]. The quantification of the fluidity as marker of invasiveness in brain cancer could potentially improve clinical prognosis and therapeutic management of patients.

Most applications of brain MRE assumes an isotropic tissue while brain white matter is likely to produce an anisotropic mechanical response [162]. Not taking the anisotropy into consideration can lead to misestimation of mechanical parameters in the normal brain, but this is probably less relevant in brain cancer. To measure the anisotropic effect, several MRE reconstruction methods have been developed [163–165].

Before the adoption of brain tumor MRE in routine clinical practice, more studies should be performed and the

reproducibility MRE parameters as imaging biomarkers of cancer should be assessed. Further studies on the relation between tumor mechanical parameters and extracellular matrix composition should also be performed.

3.4.4 Prostate

Multiparametric MRI including T₂-weighted imaging and dynamic contrast-enhanced MRI has shown its usefulness in prostate cancer diagnosis. However, the specificity remains low and prostate biopsy is still required [166]. Evaluation of stiffness could provide additional information about prostate cancer characterization. Indeed, ex-vivo studies have shown the high effect of collagen deposition in the evaluation of prostate tumor grade [167, 168].

A challenge in prostate cancer MRE is to propagate shear waves inside the organ. Specific drivers have been developed to respond to this need [169, 170]. In 2011, Li et al. have provided an *in vivo* study evaluating the viscoelastic properties in 12 patients with prostate cancer and 14 with prostatitis [35]. A transducer was placed above the pubis to transmit mechanical waves at 100 Hz. Prostate cancer was found to have significantly higher stiffness than the healthy peripheral zone (6.55 ± 0.47 kPa vs. 2.26 ± 0.45 kPa, $p < 0.01$) and a positive correlation was observed between elasticity of prostate cancer and Gleason score. Moreover, viscoelastic properties provided a good separation between malignant and benign prostate tissue (elasticity: 6.55 ± 0.47 kPa vs. 1.99 ± 0.66 kPa, $p < 0.01$; viscosity: 6.56 ± 0.99 Pa s vs. 2.13 ± 0.21 Pa s, $p < 0.01$).

In a later study, Sahebjavaheer et al. [12] used a transducer placed against the patient perineum. They have evaluated the repeatability in six volunteers and performed MRE acquisitions in 11 patients. Despite the poor repeatability results (25% variation in stiffness), a significant difference in stiffness was found between cancerous and normal tissue.

More recently, Dittmann et al. have shown the benefit of tomoelastography with drivers placed around the pelvis to assess prostate mechanical properties [19]. Twelve healthy volunteers and three subjects with a malignant prostate lesion were explored using three excitation frequencies (60 Hz, 70 and 80 Hz). Compared to the previous study, good reproducibility was found (3.7% and 2.7% in the peripheral and central prostate zone respectively). Consistent with the studies of Li and Sahebjavaheer [12, 35] the shear waves speed in prostate cancer was higher than in healthy tissue.

In a recent prospective study with 208 participants, it was shown with tomoelastography that prostate cancer stiffness and fluidity were higher in prostate cancer than in benign prostatic disease. Multiparametric MRI combined with stiffness and fluidity measurements enabled detection of prostate cancer with 95% specificity, which was significantly higher than with use of multiparametric MRI alone (77%) [48].

3.4.5 Pancreas

Pancreatic ductal adenocarcinoma is a highly aggressive cancer, the overall 5-years survival rate among patients with pancreatic cancer being less than 5 % [91, 171]. It is characterized by a dense and rich stroma which plays a critical but debated role in its progression, as explained above [103]. The quantification of pancreatic cancer mechanical properties could help to evaluate its severity. However, the pancreas is a deep-lying organ and it is difficult to transmit mechanical shear waves in the pancreas of obese patients who are at risk of pancreatic ductal adenocarcinoma development [172].

Improvement in sequence acquisition time (use of echo planar imaging, parallel imaging) and development of new actuators (with tomoelastography) to propagate shear waves in deeply located organs, has increased the capacity of MRE to estimate the mechanical parameters of the pancreas [28, 173–175]. Several recent studies have shown the good diagnostic performance of MRE to differentiate between benign and malignant pancreatic masses [4, 20, 28, 172]. Pancreatic adenocarcinomas and malignant endocrine tumors exhibit higher stiffness and fluidity than the normal pancreas and benign lesions, including autoimmune pancreatitis.

3.4.6 Kidney

MRE is increasingly used to investigate the renal mechanical parameters. Preliminary studies have shown the feasibility of MRE to estimate the renal stiffness [176] and to assess the renal mechanical parameter variations caused by hydration and urinary status [177]. In a study published in 2018, the viscoelastic parameters of small renal tumors were assessed in 19 patients [178]. Compared to dynamic contrast-enhanced and diffusion-weighted imaging, MRE viscoelastic parameters provided the best discrimination between renal oncocytomas and carcinomas. In accordance with the literature in ultrasound elastography [179], malignant tumors were stiffer than benign lesions.

4 Discussion

MRE is an emerging method to assess cancer. Increasing number of preclinical and clinical studies show the potential of MRE for cancer characterization and treatment follow-up. When evaluating the surrounding tissue, the usefulness of MRE to predict the risk of cancer occurrence or recurrence has also been shown [139, 180].

Generally, malignant tumors, except brain tumors, have elevated stiffness and mainly elevated fluidity relative to the surrounding parenchyma and relative to benign tumors and pseudo-tumors. This is a general trend and there is some overlap of the biomechanical properties between benign and malignant tumors. In the brain, glioblastomas have variable stiffness and low fluidity enabling them to infiltratively finger in normal surrounding brain matter [43].

4.1 Issues of MRE technique in cancer investigation

MRE is still a recent method that is not yet standardized. Differences in generation of mechanical vibrations (frequency of the mechanical shear waves), in acquisition and reconstruction methods between studies may bias the mechanical parameter values. There is thus a clear need for method standardization, reproducibility assessment and multicenter clinical validation.

From a technical point of view, MRE is still limited for the examination of deep-lying tumors or brain tumors. Pancreas cancer imaging is difficult because the pancreas is retroperitoneal and the shear waves are attenuated by more superficial structures of the abdomen including adipose tissue, colon, stomach and liver. Therefore, the wave amplitude may not be sufficient to obtain accurate estimation of the pancreatic tumor stiffness. Specific transducers have been developed with multiple drivers placed around the abdomen to increase the number of sources providing mechanical vibrations [173].

Ongoing research is also performed with passive elastography [181]. The method uses shear waves generated naturally from cardiac motion or blood pulsatility. Passive elastography might be interesting to investigate cancers located in organs where shear waves cannot be transmitted with sufficient amplitude from an external driver, such as the brain.

Another limitation of cancer MRE is related to small tumor size. In some studies, patients with lesions smaller than 10 mm in the liver [16] or 20 mm in the brain [161] have been excluded. The assessment of early cancer may be limited, as small tumors cannot be distinguished from surrounding parenchyma because of large point-spread function [182]. MRE acquisition with echo planar imaging may reduce the scan time and increase the matrix resolution [183, 184].

Still another limitation is related to the homogeneity assumption often used for stiffness assessment. At the tissue-tumor interface especially, a large difference between the mechanical properties can affect the homogeneity assumption. A wave inversion method, called heterogeneous multifrequency direct inversion has been developed to accommodate heterogeneous elasticity within a direct inversion by incorporating first-order gradients and combining results from a narrow band of multiple frequencies [185].

4.2 Perspectives

MRE may be used to assess the altered physical traits in cancer, i.e., high stiffness, interstitial fluid pressure and solid stress, and altered micro-architecture. This non-invasive assessment may help understanding cancer dynamics and diagnosing cancer aggressiveness.

Tumor stiffness and visco-elasticity which are related to collagen density and cross-linking but also to fluid pressure are measured with basal MRE [5, 6, 77, 118]. However, compression MRE assessing non-linear stiffness (compression stiffening) may be useful to

evaluate tumor solid stress which is influenced by the solid elements of the tumor, including cells and extracellular matrix [114, 186, 187]. Assessing tumor solid stress with compression MRE might have prognostic significance, as it has been recently reported that microvascular invasion in patients with hepatocellular carcinoma can be assessed with compression MRE [188].

Tumor heterogeneity which also plays an important role in cancer severity might be further assessed with high resolution MRE, radiomics and deep learning [189].

Further studies should investigate the relation between MRE parameters, including multifrequency MRE and MRE under compression, and altered physical parameters as determinants of cancer aggressiveness.

In conclusion, MRE is an emergent method to quantify tumor mechanical properties, offering a clinically usable method for determining cancer prognosis and assessing treatment response. Increasing research and clinical validation will improve the efficacy of MRE for cancer characterization.

Author contributions

GP, PG, and BVB conceived the manuscript. GP and BVB wrote the manuscript. PG and BVB revised and edited the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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