frontiers | Research Topics

Tendons and ligaments: development, pathogenesis, tissue engineering, and regenerative medicine

Edited by
Dai Fei Elmer Ker, Cheemeng Tan and
Sarah Harriet Cartmell

Published in
Frontiers in Bioengineering and Biotechnology
Frontiers in Medical Technology

ISSN 1664-8714
ISBN 978-2-8325-5493-7
DOI 10.3389/978-2-8325-5493-7

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Tendons and ligaments: development, pathogenesis, tissue engineering, and regenerative medicine

Topic editors

Dai Fei Elmer Ker — Hong Kong Polytechnic University, Hong Kong, SAR China

Cheemeng Tan — University of California, Davis, United States

Sarah Harriet Cartmell — The University of Manchester, United Kingdom

Citation

Ker, D. F. E., Tan, C., Cartmell, S. H., eds. (2024). *Tendons and ligaments: development, pathogenesis, tissue engineering, and regenerative medicine*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5493-7

Table of contents

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Editorial
PUBLISHED 17 September 2024
DOI 10.3389/fbioe.2024.1489256

OPEN ACCESS

EDITED AND REVIEWED BY
Sandra Van Vlierberghe,
Ghent University, Belgium

*CORRESPONDENCE
Dai Fei Elmer Ker,
elmer.ker@polyu.edu.hk

RECEIVED 31 August 2024
ACCEPTED 10 September 2024
PUBLISHED 17 September 2024

CITATION
Ker DFE, Tan C and Cartmell S (2024) Editorial: Tendons and ligaments: development, pathogenesis, tissue engineering, and regenerative medicine.
Front. Bioeng. Biotechnol. 12:1489256.
doi: 10.3389/fbioe.2024.1489256

Editorial: Tendons and ligaments: development, pathogenesis, tissue engineering, and regenerative medicine

Dai Fei Elmer Ker[1]*, Cheemeng Tan[2] and Sarah Cartmell[3]

[1]Department of Biomedical Engineering, The Hong Kong Polytechnic University, Hung Hom, Hong Kong SAR, China, [2]Department of Biomedical Engineering, University of California, Davis, CA, United States, [3]Department of Materials Science, Henry Royce Institute, School of Natural Sciences, Faculty of Science and Engineering, The University of Manchester, Manchester, United Kingdom

KEYWORDS

tendon, ligament, tissue engineering, regenerative medicine, animal model, stem cell, growth factors

Editorial on the Research Topic
Tendons and ligaments: development, pathogenesis, tissue engineering, and regenerative medicine

Globally, musculoskeletal diseases and injuries, such as tendon and ligament deficits, drastically reduce patient quality-of-life and increase national healthcare costs (WHO Scientific Group on the Burden of Musculoskeletal Conditions at the Start of the New Millennium, 2003; United States Bone and Joint Initiative, 2014; Global Burden of Disease, 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). In developed regions, such as the United States (Mather and Scommegna, 2024), United Kingdom (Centre for Aging Better, 2023), and Hong Kong (Census and Statistics Department of Hong Kong, 2015), this undesirable effect is exacerbated by rapid population aging. Addressing this challenge requires key advances to bridge both knowledge and technical gaps in musculoskeletal science, tissue engineering, and regenerative medicine. In this regard, the "*Tendons and Ligaments: Development, Pathogenesis, Tissue Engineering, and Regenerative Medicine*" Research Topic highlights some of the latest unique and groundbreaking preclinical and clinical studies.

This Research Topic features concise reviews and innovative research articles that summarize and/or showcase the latest advances in tendon/ligament basic and translational science.

The concise reviews herein present an overview of the tendon wound healing environment (Hart et al.) as well as pro-regenerative factors such as growth factors (Lin et al.), non-coding RNAs (Silva et al.), and tendon stem/progenitor cells (He et al.). Hart et al. discuss the importance of the Achilles tendon wound environment following injury along with surgical and non-surgical treatment options, state-of-the-art therapeutics under development, and the necessity of biomarkers that can be prognostic of long-term tendon healing outcomes to improve clinical research and clinical trials. Lin et al. summarize recent advances and limitations in growth factor-based tendon regenerative strategies, which include the combinatorial use of stem cells and scaffolds. The latter also highlights the need for more studies to support their use for routine management of tendon ailments. As an alternative to growth factors, Silva et al. describe the use of non-coding RNAs, such as siRNAs, miRNAs, and lncRNAs, as molecular tools for tendon tissue engineering. These efforts aim to knockdown proteins associated with

detrimental biological processes such as fibrosis and excessive inflammation or to beneficially enhance tendon healing. Providing an added dimension to these topics, He et al. examine cell-based strategies such as tendon-derived stem cells for creating tissue-engineered constructs, including the use of pro-regenerative cues, bioengineered cell sheets biomaterial scaffolds, and bioreactor-based mechanical conditioning.

Original research articles within this Research Topic include publications that advance the frontiers of scientific and clinical knowledge. These publications highlight trending tendon/ligament Research Topics (Zhang et al.), showcase current clinical opinions and practices (Xue et al.), elucidate the contribution of ECM in tendon/ligament biomechanics (Liu et al.), improve diagnosis of tendon pulley injuries (Iruretagoiena et al.), characterize novel drug delivery platforms (Shi et al.), and bioengineer stem cell-based tendon constructs (Taguchi et al.). Serving as a reference for the tendon/ligament field, Zhang et al. conducted bibliometric analyses to uncover researchers and the regions where tendon/ligament research is being actively conducted together with trending researching topics such as biomaterial scaffolds and immunomodulatory strategies. Meanwhile, Xue et al. conducted a survey among British hand surgeons to better understand the current treatments preferred by medical practitioners as well as their opinion on employing state-of-the-art tools and techniques such as minimally invasive instruments, biodegradable materials, and additive manufactured devices. The results reflect a conservative approach among clinicians with a weak preference for new techniques and advances. Altogether, such information is crucial for assessing the current state and future progress in tendon/ligament science and clinical practice. Liu et al. studied the contribution of elastin to patellar tendon biomechanics by subjecting porcine patellar tendons to elastin digestion followed by macroscopic mechanical testing. The study demonstrated that elastin plays an important role in the mechanical properties and fiber structure stability of patellar tendon, contributing towards our understanding of the structure-function relationship of patellar tendon. Iruretagoiena et al. performed high-resolution ultrasound measurements of tendon-to-bone distances. This work enabled the authors to uncover a minimum threshold for tendon-to-bone distance to diagnose partial and complete flexor tendon pulley injuries, which are common in rock climbers. Shi et al. characterized osteoadsorptive fluorogenic sentinel 3 (OFS-3), a novel drug delivery platform that comprises a bone-targeting bisphosphonate (BP) and cathepsin K (Ctsk)-triggered compound. In this study, the authors ruled out any potential negative effects on tendon biomechanical attributes and tendon healing efficacy, which paves the way for this drug delivery platform to undergo further development to specifically deliver therapeutic agents such as growth factors to bone-tendon sites. In Taguchi et al., the authors generated tendon tissue engineered constructs using a combination of adipose-derived stem cells. Collagen Type I sponge, tenogenic media, and tensile bioreactor conditioning. This work shows promise for contributing to the development of novel tendon therapies.

Collectively, the diverse works contained within the "*Tendons and Ligaments: Development, Pathogenesis, Tissue Engineering, and Regenerative Medicine*" Research Topic illustrate the latest conceptual and technical advances for tendon/ligament basic and clinical sciences. These publications highlight current challenges in tendon/ligament regeneration, describe recent scientific progress, and present potential solutions, offering a promising outlook for this field.

Author contributions

DK: Conceptualization, Writing–original draft, Writing–review and editing. CT: Writing–review and editing. SC: Conceptualization, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. DK is funded by the University's Startup Fund from The Hong Kong Polytechnic University. SC is funded by the Chinese University of Hong Kong-Manchester seed fund.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

Census and Statistics Department of Hong Kong (2015) *Hong Kong population projections*. Hong Kong.

Centre for Aging Better (2023). *State of ageing 2023*.

Global Burden of Disease 2016 Disease and Injury Incidence and Prevalence Collaborators (2017). Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 390 (10100), 1211–1259. doi:10.1016/s0140-6736(17)32154-2

Mather, M., and Scommegna, P. (2024). *Fact sheet: aging in the United States, population reference bureau*.

United States Bone and Joint Initiative (2014). *The burden of musculoskeletal diseases in the United States (BMUS)*. Rosemont, IL: Fourth Edition Advance Copy.

WHO Scientific Group on the Burden of Musculoskeletal Conditions at the Start of the New Millennium (2003). The burden of musculoskeletal conditions at the start of the new millennium. *World Health Organ. Tech. Rep. Ser.* 919, 218. Available at: https://iris.who.int/handle/10665/42721.

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Original Research
PUBLISHED 07 June 2023
DOI 10.3389/fbioe.2023.1123857

OPEN ACCESS

EDITED BY
Cheemeng Tan,
University of California, Davis,
United States

REVIEWED BY
David Andrew Bong,
University of Barcelona, Spain
Federico Zaottini,
University of Genoa, Italy

*CORRESPONDENCE
Xeber Iruretagoiena,
xeber.iruretagoiena@deusto.es

RECEIVED 17 February 2023
ACCEPTED 24 May 2023
PUBLISHED 07 June 2023

CITATION
Iruretagoiena X, Schöffl V, Balius R, Blasi M, Dávila F, Sala X, Sancho I and De La Fuente J (2023), High-resolution ultrasound tendon-to-bone distances in partial and complete finger flexor A2 pulley ruptures simulated in human cadaver dissection: toward understanding imaging of partial pulley ruptures.
Front. Bioeng. Biotechnol. 11:1123857.
doi: 10.3389/fbioe.2023.1123857

High-resolution ultrasound tendon-to-bone distances in partial and complete finger flexor A2 pulley ruptures simulated in human cadaver dissection: toward understanding imaging of partial pulley ruptures

Xeber Iruretagoiena[1,2,3]*, Volker Schöffl[4,5,6,7], Ramón Balius[8,9], Marc Blasi[10], Fernando Dávila[11], Xavier Sala[12,13], Igor Sancho[1] and Javier De La Fuente[14]

[1]Deusto Physical TherapIker, Physical Therapy Department, Faculty of Health Sciences, University of Deusto, San Sebastián, Spain, [2]Eskura Osasun Zentroa, Beasain, Spain, [3]Sputnik Investigación, Madrid, Spain, [4]Section Sportsorthopedics and Sportsmedicine, Department of Orthopedic and Trauma Surgery, Klinikum Bamberg, Bamberg, Germany, [5]Department of Trauma Surgery, Friedrich Alexander University of Erlangen-Nuremberg, Erlangen, Germany, [6]Section of Wilderness Medicine, Department of Emergency Medicine, University of Colorado School of Medicine, Denver, CO, United States, [7]School of Clinical and Applied Sciences, Leeds Becket University, Leeds, United Kingdom, [8]Consell Catala de l´Esport, Generalitat de Catalunya, Barcelona, Spain, [9]Sport Medicine and Imaging Department, Clínica Diagonal, Barcelona, Spain, [10]Department of Plastic Surgery, Hospital Germans Trias I Pujol, Barcelona, Spain, [11]Orthopedics Department, Hospital Bidasoa, Irun, Spain, [12]Anatomy and Embryology Department, School of Medicine, Universitat de Barcelona, Barcelona, Spain, [13]Department of Anesthesiology, Hospital Clínic de Barcelona, Barcelona, Spain, [14]Orthopedics Department, Clínica Pakea-Mutualía, San Sebastián, Spain

Introduction: The A2 pulley tear is the most common injury in rock climbing. Whereas complete A2 pulley ruptures have been extensively researched, studies focused on partial A2 pulley ruptures are lacking. A2 pulleys rupture distally to proximally. High-resolution ultrasound imaging is considered the gold-standard tool for diagnosis and the most relevant ultrasound measurement is the tendon-to-bone distance (TBD), which increases when the pulley ruptures. The purpose of this study was to establish tendon-to-bone distance values for different sizes of partial A2 pulley ruptures and compare these values with those of complete ruptures.

Material and methods: The sample consisted of 30 *in vitro* fingers randomly assigned to 5 groups: G1, no simulated tear (control); G2, simulated 5 mm tear (low-grade partial rupture); G3, simulated 10 mm tear (medium-grade partial rupture); G4, simulated 15 mm tear (high-grade partial rupture); and G5, simulated 20 mm or equivalent tear (complete rupture). A highly experienced sonographer blinded to the randomization process and dissections examined all fingers.

Results: The tendon-to-bone distance measurements (medians and interquartile ranges) were as follows: G1, 0.95 mm (0.77–1.33); G2, 2.11 mm (1.78–2.33); G3, 2.28 mm (1.95–2.42); G4, 3.06 mm (2.79–3.28); and G5, 3.66 mm (3.55–4.76). Significant differences were found between non-torn pulleys and simulated partial and complete pulley ruptures.

Discussion: In contrast, and inconsistent with other findings, no significant differences were found among the different partial rupture groups. In conclusion, the longer the partial pulley rupture, the higher the tendon-to-bone distance value. The literature is inconsistent regarding the tendon-to-bone distance threshold to diagnose a partial A2 pulley rupture. The minimum tendon-to-bone distance value for a partial rupture was 1.6 mm, and tendon-to-bone distance values above 3 mm suggest a high-grade partial pulley rupture (15 mm incision) or a complete pulley rupture.

KEYWORDS

A2 pulley, tear, climbing, partial rupture, ultrasound, tendon-bone distance

1 Introduction

Rupture of the finger flexor A2 pulley is the most common injury in rock climbers (Miro et al., 2021). The ring finger is most frequently affected, followed by the middle finger (Bollen, 1988). Pulley ruptures account for up to 33% of all rock climbing injuries (King and Lien, 2017). Climbing is rapidly gaining popularity, as reflected by its debut in the Olympics and a steep rise in climbing sport federation members (Lutter et al., 2017). As a result of this sport's growth and development, the frequency of climbing-related pulley injuries is increasing (Miro et al., 2021).

The annular pulleys are the retinaculum portions that form part of the fibro osseous sheath containing the tendons of the muscles flexor digitorum superficialis (FDS) and flexor digitorum profundus (FDP) at the finger level (Martinoli et al., 2005). Their main role is to hold these tendons close against the phalanges to optimize their biomechanical function and avoid a bowstring deformity (Lin et al., 1990; Schöffl et al., 2017). Of the five annular and three cruciform pulleys, with the A2 pulley is considered the most important (Doyle, 2001). The A2 pulley inserts into the periosteum of the proximal phalanx on both sides, encircling the anterior aspects of the FDS and FDP tendons. Cadaver measurements confirmed that it is the longest of the pulleys, varying from 16.8 mm (Doyle, 1988) to 20 mm (Moutet, 2003). The length of the A2 pulley varies between fingers; the order from longer to shorter is the middle finger (16.4–20.5 mm), then ring (15.1–18.9 mm), index (12.8–15.9 mm), and little (11.7 mm) finger (Doyle, 1988; Schöffl et al., 2017). It spans the proximal and middle third of the proximal phalanx. From the base of the middle phalanx, its proximal margin is located at 30.6 mm and its distal rim at 15.5 mm (Moutet, 2003). The thickness ranges from 0.3 (Martinoli et al., 2000) to 0.7 mm (Schreiber et al., 2015), and it tends to be thicker in climbers (1.2 mm) (Klauser et al., 2000). The A2 is the strongest of the finger pulleys (Schöffl et al., 2009b).

Annular pulley ruptures may be complete or partial (Mitsionis et al., 2000). As a physical examination is non-specific, imaging tests are needed to determine the grade of injury and the number and extent of injured pulleys (Iruretagoiena-Urbieta et al., 2020a). Ultrasound is considered the diagnostic procedure of choice, as it allows for both static and dynamic assessment (Schöffl et al., 2017). The most relevant ultrasound finding is an increased distance of the flexor tendons to the palmar aspect of the base of the phalanx during dynamic examination maneuvers, along with peritendinous fluid (Martinoli et al., 2005). This distance is referred to as the tendon-to-bone distance (TBD) (Klauser et al., 2002).

A complete A2 pulley rupture leads to a significant increase in the TBD, decreased strength in finger flexion (Iruretagoiena-Urbieta et al., 2020b), and reduced range of motion of the proximal interphalangeal joint (PIP) (Bowers et al., 1994). However, the heterogeneity of ultrasound examinations (probe frequency, coupling agent, anatomical landmark, finger position, and load) (Iruretagoiena-Urbieta et al., 2020b) means that the ultrasound TBD cutoffs that define a complete A2 pulley rupture vary among the different publications, namely, from 1.9 mm (Schöffl et al., 2017) to 5.1 mm (Bodner et al., 1999). Most researchers have used a 2 mm threshold to diagnose a complete pulley rupture (Miro et al., 2021).

Although complete A2 pulley ruptures have been extensively investigated, studies describing its partial rupture are scarce (Iruretagoiena-Urbieta et al., 2020b). Such ruptures begin from distal to proximal (Hauger et al., 2000) as a consequence of friction between the pulley and flexor tendon and eccentric stress (Schöffl et al., 2009a). A large partial rupture, spanning close to 75% of the total pulley length, is associated with a reduced capacity to tolerate the traction force of the flexor tendon against the pulley (Mitsionis et al., 2000), along with a slight reduction in the PIP range of motion (Mitsionis et al., 1999). Overall ultrasound is the most valuable diagnostic tool for pulley ruptures (Klauser et al., 2002); for the ultrasound diagnosis of a partial rupture, little evidence and much controversy exist over reference TBD values (Iruretagoiena-Urbieta et al., 2020b). The literature provides different cut offs for the diagnosis of partial ruptures, ranging from TBD values from >2.2 mm (Hauger et al., 2000) to <2 mm (Schöffl et al., 2003), which may even coincide with those indicated for complete ruptures (Bodner et al., 1999).

In a proposed classification system for A2 pulley lesions in climbers, four grades are defined: sprain, partial rupture, complete rupture, and multiple ruptures (Schöffl et al., 2003). Partial ruptures of the A2 pulley are graded as a grade 2 lesion and are associated with an estimated recovery time period (RTP) of 8–10 weeks (Lutter et al., 2021). In contrast, an isolated complete A2 rupture is described as a grade III lesion with an estimated RTP of 3 months (Lutter et al., 2021). Thus, distinguishing between a partial and complete rupture is crucial to plan exact patient management, time to recovery, and accurately determine prognosis.

The main aim of this study was to establish TBD values for partial A2 pulley ruptures compared with those of complete ruptures. We also sought to examine whether a higher grade of simulated partial rupture leads to an increase in TBD possibly contributing to the disparate values reported in the literature, sometimes even overlapping those proposed for complete tears.

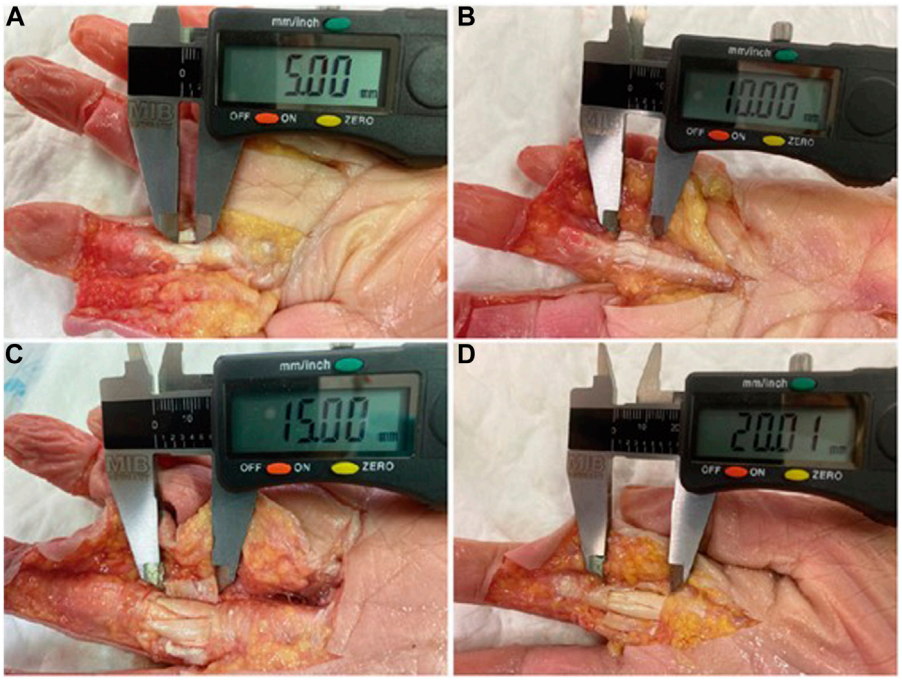


FIGURE 1
Simulated A2 pulley tears measurement: G2 **(A)**; G3 **(B)**; G4 **(C)**; G5 **(D)**.

2 Materials and methods

This was a cross-sectional study conducted on human cadavers. Partial and complete A2 pulley ruptures were simulated through surgical incision and evaluated with ultrasound. A total of 30 fingers (10 index, 10 middle, and 10 ring fingers) from 5 fresh frozen human cadaver arms (average age 78 years, range 75–82 years) were studied. The little fingers were excluded due to anatomic and biomechanical reasons. The specimens had no signs or history of finger, hand, or wrist injuries or surgery and were left to thaw at room temperature before dissection. All specimens were obtained from bodies donated to the Faculty of Medicine and Health Sciences (Clinic Campus) of the University of Barcelona. Institutional review board approval was obtained prior to the study. The used cadaver tissues were part of a body donation program and in compliance with current Spanish legislation about ethics in research. None of the specimens showed trauma, deformities, or surgical scars on the hand.

All fingers were initially dissected by performing a single unilateral longitudinal incision at the transition between the dorsal and palmar skin from the metacarpophalangeal (MCP) joint to the distal interphalangeal (DIP) joint. Then, the subcutaneous fat layer to the finger pulley system was dissected without disrupting it. All fingers were randomly assigned to one of the following injury simulation groups: G1, no simulated tear (control); G2, simulated 5 mm tear (low-grade partial rupture); G3, simulated 10 mm tear (medium-grade partial rupture); G4, simulated 15 mm tear (high-grade partial rupture); and G5, simulated 20 mm or equivalent tear (complete rupture) (Figure 1). Prior to sectioning the pulleys from distal to proximal on their volar aspect, the distances to be incised according to the group assignment were measured with a digital caliper (Qfun ® digital caliper, China, 0–150 mm, CN). After proper processing, abundant ultrasound gel was placed over the whole pulley system, which was then again covered with the previously raised skin and subcutaneous flap.

In addition, before ultrasound examination, the FDS and FDP tendons were exposed with a transverse incision at the forearm, proximal to the flexor retinaculum. Then, the FDS and FDP tendons in each finger were identified, and both flexor tendons corresponding to each finger were sutured using a polyglactin thread (Vicryl 2 ®, Ethicon, United States). To simulate flexor tendon tension, the sutured flexor tendons were isolated with a screw-locking carabiner clip and attached using a rigid aluminum wire to a Tindeq force sensory system (Tindeq®, sampling frequency: 80 Hz, design load: 150 kg, NO) (Figure 2).

For ultrasound examination, we used a Canon Aplio i800 ultrasound machine equipped with a 22 MHz ultra-high-frequency hockey stick (i22LH8) and a 24 MHz ultra-high frequency iDMS linear transducer (i24LX8) (Canon medical system ®, United States). A single sonographer (JDF, with over 25 years of experience in musculoskeletal ultrasound), blinded to the previous randomization process and dissections, examined and measured all fingers. Abundant ultrasound gel was used to avoid compression of the finger by the transducer. The finger examination position was 0° or neutral MCP joint, 40° of flexion of the PIP joint and 10° of flexion of the DIP joint with a constant traction force of 5 kg directly applied to the FDS and FDP tendons. First, the proximal phalanx was measured using a linear transducer to estimate the midpoint of the phalanx. Once this anatomic landmark was located, a stick transducer was used to measure

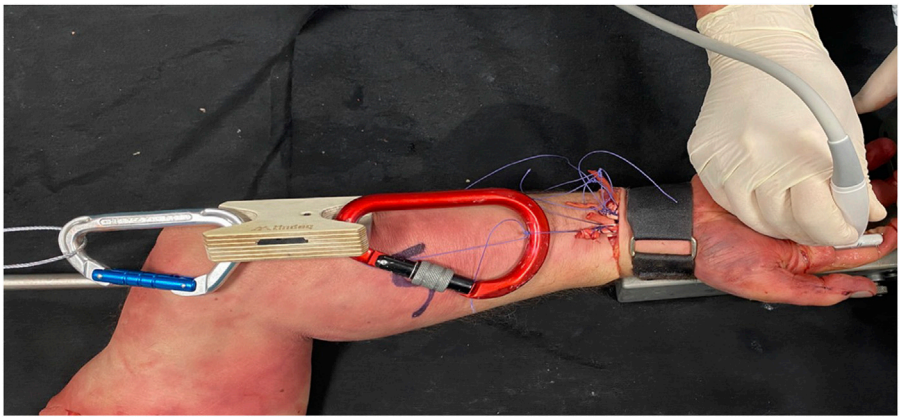


FIGURE 2
Finger flexor tendon tension system.

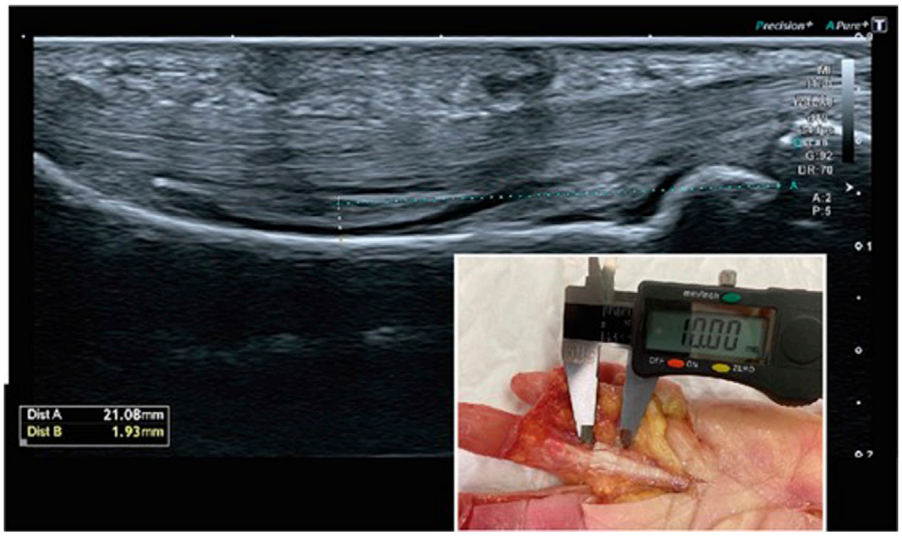


FIGURE 3
US TBD (1.9 mm) of a simulated 10 mm A2 pulley tear.

the TBD at the level of the midpoint of the proximal phalanx (Figure 3).

Data are described using the most appropriate statistics for the nature and scale of measurement of each variable: absolute and relative frequencies in percentages, mean and standard deviation for continuous variables, and median and interquartile range when appropriate according to the data distribution. The data are graphically represented through box plots. For quantitative variables, the Shapiro–Wilk test was used to check normality. To compare TDB means, we used ANOVA and Bonferroni correction for 2-by-2 pairwise comparisons of the different length sections incisions of the A2 pulleys. Due to the small sample size for each of the A2 pulley sections, the corresponding non-parametric test (Wilcoxon test) was employed along with the Jonckheere–Terpstra trend test. Correlations between variables were assessed with Spearman's correlation. The software used for data analysis was Stata SE for Windows (Stata Corp ®. 2021. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC, United States). Significance was set at $p < 0.05$.

3 Results

Of the 30 initial fingers included, 6 could not be examined as too much air had accumulated between the dissected tissues despite the abundant gel applied between the pulley system and the overlying dissected skin and subcutaneous flap.

For a final study sample of A2 pulleys of 24 fingers, TBD measurements (medians and interquartile ranges) were G1,

TABLE 1 Ultrasound TBD measurements.

Group	A2 pulley incision length	Sample size	D2	D3	D4	Total
	mm	n	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR) (Min-Max)
1	0	5	1.14 (0.95–1.33)	0.63 (0.49–0.77)	1.51 (1.51–1.51)	0.95 (0.77–1.33)
Intact tissue						(0.49–1.51)
2	5	6	1.88 (1.75–2)	2.06 (1.78–2.33)	2.44 (2.21–2.67)	2.11 (1.78–2.33)
Low-grade rupture						(1.75–2.67)
3	10	4	2.28 (2.23–2.33)	1.67 (1.67–1.67)	2.51 (2.51–2.51)	2.28 (1.95–2.42)
Medium-grade rupture						(1.67–2.51)
4	15	5	2.93 (2.79–3.06)	2.49 (2.29–2.49)	3.28 (3.28–3.28)	3.06 (2.79–3.28)
High-grade rupture						(2.49–3.28)
5	20	4	3.66 (3.62–3.69)	5.82 (5.82–5.82)	3.47 (3.47–3.47)	3.66 (3.55–4.76)
Complete rupture						(3.47–5.82)

D2, index digit; D3, middle digit; D4, annular digit; IQR, interquartile range; Min-Max, minimum–maximum.

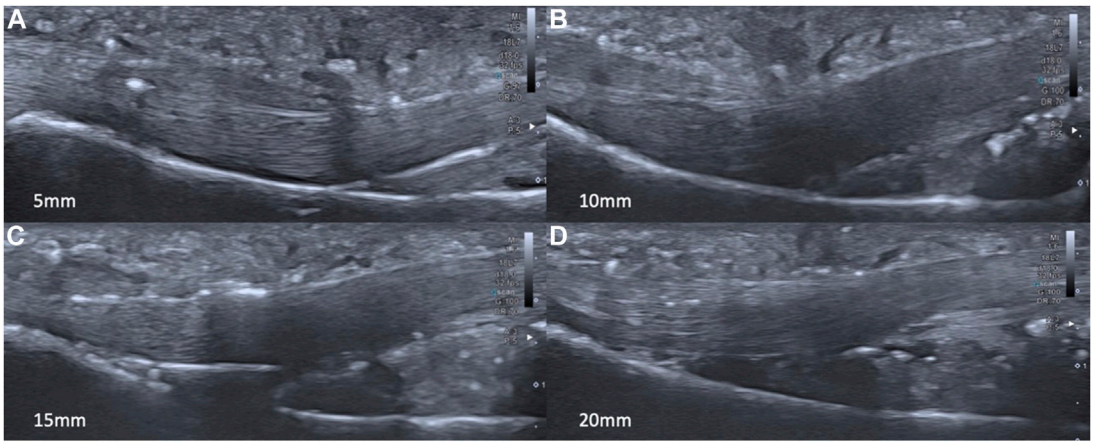


FIGURE 4
US TBD of each simulated tear size: G2 **(A)**; G3 **(B)**; G4 **(C)**; G5 **(D)**.

0.95 mm (0.77–1.33); G2, 2.11 mm (1.78–2.33); G3, 2.28 mm (1.95–2.42); G4, 3.06 mm (2.79–3.28); and G5, 3.66 mm (3.55–4.76). These measurements and the numbers of samples in each group are provided in Table 1.

The TBD values showed a significant increasing trend ($p < 0.05$): the larger the simulated pulley rupture, the larger the TBD (Figures 4, 5).

When comparing the TBD values recorded for the A2 pulleys among the groups, significant differences were found between the control non-torn pulleys (G1) and the simulated partial and complete pulley ruptures (vs. G2, $p < 0.05$; vs. G4 and G5, $p < 0.05$). In turn, TBD values were significantly different for the simulated partial and complete pulley ruptures (G2 vs. G5 $p < 0.05$; G3 vs. G5 $p < 0.05$). In contrast, no significant differences were found among the different partial rupture groups (G2 vs. G3 $p = 1.00$; G2 vs. G4 $p = 0.201$; G3 vs. G4 $p = 0.469$) or between the simulated high-grade partial and complete ruptures (G4 vs. G5, $p = 0.055$).

4 Discussion

This is only the second report to evaluate the effect on the TBD on US of variable length incisions of the A2 pulley simulating various degrees of partial rupture in a cadaver model. Our data support the notion that significant TBD differences exist between non-sectioned, partly sectioned, and fully sectioned pulleys (Leeflang and Coert, 2014). Inconsistent with the findings of Leeflang and Coert (2014), we found no significant differences in the TBD among different lengths of partial incisions. This difference

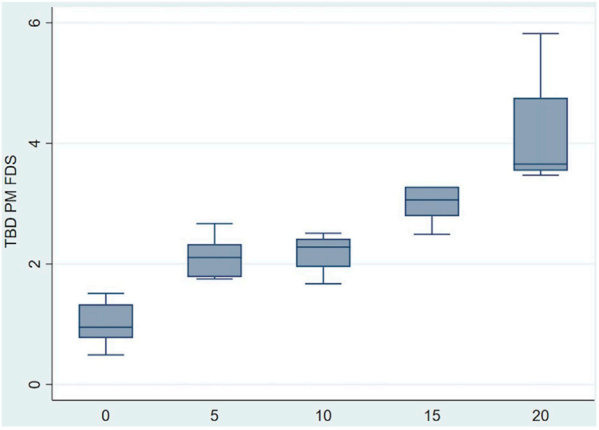


FIGURE 5
Boxplot of TBD values recorded for simulated different grades of ruptures.

could be related to the following technical differences. Leeflang and Coert (2014) progressively sectioned the same fingers whereas we chose to randomize our sample. In addition, pulley incision lengths differed, as we performed 5, 10, 15, and 20 mm incisions, whereas Leeflang and Coert (2014) incised the pulley in thirds. The ultrasound measurement method also varied. Leeflang and Coert (2014) recorded TBD measurements over a 5 mm section, we measured the TBD at the proximal phalanx's midpoint as recommended by several researchers (Schöffl et al., 2006; Bassemir et al., 2015; Schöffl et al., 2018).

The TBD values of the sectioned A2 pulleys obtained here are compatible with those reported in two *in vivo* studies (Bodner et al., 1999; Klauser et al., 2002) and one cadaveric trial (Hauger et al., 2000). Our minimum and maximum TBD values of the partially sectioned A2 pulleys varied between 1.67 and 3.28 mm, which is close to the ranges reported in the two studies of 1.8–3 mm (Bodner et al., 1999) and 1–3.1 mm (Klauser et al., 2002). The difference was caused by the following: in these two studies, the sample consisted of traumatic partial ruptures in climbers, and the partial rupture size were not specified. Our results are consistent with those of Hauger et al. (2000): partial A2 pulley ruptures can be diagnosed by ultrasound as a significant TBD increase that is nevertheless lower than that of complete A2 ruptures. However, our data are not in agreement with the distances detected: for a 10 mm distal-to-proximal incision in the A2 pulley, the same as the incision in our group 3, the mean TBD was 1.4 mm and ranged from 0 to 2 mm (Hauger et al., 2000), which is much lower than our mean of 2.18 mm and range of 1.67–2.51 mm. This difference might be explained by the degree of force of flexor activation of the finger. Hauger et al. (2000) applied a 500 g traction force attached to the common flexor tendon of each finger; in this study, a traction force of 5 kg was applied. No consensus has been reached regarding the finger position and the optimal amount of activation or traction force in the ultrasound assessment protocol (Iruretagoiena-Urbieta et al., 2020b).

In the literature, debate is ongoing regarding the TBD cutoff that should be used to diagnose a partial A2 pulley rupture: >1.4 mm (Hauger et al., 2000), >1.5 mm (Klauser et al., 2002), <2 mm (Schöffl et al., 2003; Schöffl et al., 2018), or >2.2 mm (Bodner et al., 1999). The explanation for these differences could be that these values have not been related to a specific partial rupture size. As such, our distances fell between these limits but were always associated with different lengths of A2 pulley incisions, as we detected TBD values under 2 mm for 5 mm sections or above 2.2 mm for 10 mm sections. Consensus is also lacking regarding the anatomy landmark for TBD measurement (Iruretagoiena-Urbieta et al., 2020b), distal third of the proximal phalanx (Bowers et al., 1994; Klauser et al., 2002), or distal end of A2 pulley (Bodner et al., 1999) versus midpoint of the proximal phalanx (Schöffl and Schöffl, 2006). Therefore, the similarities with these values are not valid.

In contrast to the findings of others (Schöffl et al., 2003), we found partial ruptures of the A2 pulley with a TBD greater than 2 mm (in 66.6% of G2, 75% of G3, and 100% of G4). Accordingly, we think that this value cannot serve to directly diagnose a complete rupture of the A2 pulley. Conversely, rarely did we record a TBD > 3 mm for partial ruptures of small or medium ruptures (in 0% of G2 and G3, and 60% of G4), suggesting this value as a good cutoff for the diagnosis of large partial ruptures and especially, complete ruptures.

The clinical aim of detecting partial ruptures of the A2 pulley is to obtain a more accurate diagnosis to allow a precise classification of the injury degree (Schöffl and Schöffl, 2006; Lutter et al., 2021); hence, the return to climbing period can be estimated with increased accuracy. This will also help in the conservative treatment choice and to decide whether to use a thermoplastic ring (Schneeberger and Schweizer, 2016).

For a correct understanding of this section, caution should be exercised when comparing *in vivo* and *in vitro* specimens. This might distort the comparisons of TBD values among studies. The main limitations of the present study are its small sample size and the distortion due to the artifacts produced in the ultrasound images because of prior dissection. A possible solution to this problem may be taking ultrasound measurements in a water tank, but this would hinder the study of large numbers of fingers, and the visibility is higher using gel (Schöffl et al., 2018). The main limitation was that no statistically significant differences were found among different size partial rupture groups and future studies should measure TBD at more anatomic landmarks of the proximal phalanx to be more accurate for small partial rupture diagnosis. Another possible limitation could be that we did not use a fixation device to ensure finger position during US examinations, as performed in previous studies (Marco et al., 1998). However, we did monitor at all times finger joints position using a goniometer to ensure the accuracy of measurements. Further study is needed on a larger sample to confirm the TBD differences detected here between different sized partial ruptures and possible differences between the fingers. Further research is also needed with direct and indirect US manifestations to distinguish between high-grade partial ruptures and complete ruptures. Additional investigation could focus on setting subdivisions within grade II pulley ruptures to obtain more detailed information about treatment and time to recovery for each partial rupture size of A2 pulley. Furthermore, these results need to be compared with *in vivo* findings, despite the difficulty involved in finding control reference values.

The main conclusion of this study is that significant TBD differences were found between non-torn, simulated partial, and simulated complete rupture pulleys. This means that when partially

sectioning the A2 pulley, clear separation is produced between the flexor tendons and the proximal phalanx even for incisions as short as 5 mm, representing one-third or even less of the total pulley length. The minimum TBD value for a partial rupture was 1.67 mm. Furthermore, the increase in TBD observed progressively increased the longer the pulley incision. The mean distance for the fingers examined was 2.11 (5 mm incision) to 3.66 mm (15 mm incision), which confirmed the capacity of ultrasound to diagnose small partial lesions. Additionally, for the different lengths of incisions, we did not find TDB values greater than 3 mm in low- or medium-grade partial ruptures (5 and 10 mm incision groups). This means that TBD values below 3 mm suggest a partial rupture, whereas values above this indicate a suspected complete lesion of the A2 pulley or a high-grade partial pulley rupture (15 mm incision). However, no significant differences were found among the 5, 10, and 15 mm simulated partial ruptures, which suggests that more research is needed.

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

Ethical approval was not provided for this study on human participants because all specimens were obtained from bodies donated to the Faculty of Medicine and Health Sciences (Clinic Campus) of the University of Barcelona. Institutional review board approval was obtained prior to the study. The cadaver tissues used were part of a body donation program and in compliance with current Spanish Legislation about ethics in research. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

XI and MB designed the study and conducted the literature search. XI, FD, RB, JDF and XS were responsible for data acquisition. XI, VS and MB were involved in data analysis, data interpretation and in writing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

We acknowledge support by the Open Access Publication Fund of the University of Deusto. The funding body was independent of the design of the study and collection, analysis, and interpretation of data and of writing the manuscript.

Acknowledgments

The authors wish to thank Jose Aramendi (MD, Ph.D.) for his support with the design and statistics of this study.

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.

Publisher's note

References

Bassemir, D., Unglaub, F., Hahn, P., Müller, L. P., Bruckner, T., and Spies, C. K. (2015). Sonographical parameters of the finger pulley system in healthy adults. *Arch. Orthop. Trauma. Surg.* 135, 1615–1622. doi:10.1007/s00402-015-2304-9

Bodner, G., Rudisch, A., Gabl, M., Judmaier, W., Springer, P., and Klauser, A. (1999). Diagnosis of digital flexor tendon annular pulley disruption: Comparison of high frequency ultrasound and MRI. *Ultraschall. Med.* 20, 131–136. doi:10.1055/s-1999-8904

Bollen, S. R. (1988). Soft tissue injury in extreme rock climbers. *Br. J. Sports Med.* 22, 145–147. doi:10.1136/bjsm.22.4.145

Bowers, W. H., Kuzma, G. R., and Bynum, D. K. (1994). Closed traumatic rupture of finger flexor pulleys. *J. Hand. Surg. Am.* 19, 782–787. doi:10.1016/0363-5023(94)90183-X

Doyle, J. R. (1988). Anatomy of the finger flexor tendon sheath and pulley system. *J. Hand. Surg. Am.* 13, 473–484. doi:10.1016/s0363-5023(88)80082-0

Doyle, J. R. (2001). Palmar and digital flexor tendon pulleys. *Clin. Orthop. Relat. Res.* 383, 84–96. doi:10.1097/00003086-200102000-00011

Hauger, O., Chung, C. B., Lektrakul, N., Botte, M. J., Trudell, D., Boutin, R. D., et al. (2000). Pulley system in the fingers: Normal anatomy and simulated lesions in cadavers at MR imaging, CT, and US with and without contrast material distention of the tendon sheath. *Radiology* 217, 201–212. doi:10.1148/radiology.217.1.r00oc40201

Iruretagoiena-Urbieta, X., De la Fuente-Ortiz de Zarate, J., Blasi, M., Obradó-Carriedo, F., Ormazabal-Aristegi, A., and Rodríguez-López, E. S. (2020a). Grip force measurement as a complement to high-resolution ultrasound in the diagnosis and follow-up of A2 and A4 finger pulley injuries. *Diagn. (Basel)* 10, 206. doi:10.3390/diagnostics10040206

Iruretagoiena-Urbieta, X., De la Fuente-Ortiz de Zarate, J., Rodríguez-López, E. S., Barceló-Galíndez, P., Oliva-Pascual-Vaca, A., Otero-Campos, A., et al. (2020b). Ultrasonographic diagnosis of A2 or A4 flexor tendon pulley injury: A systematic review. *Wilderness. Environ. Med.* 31, 498–505. doi:10.1016/j.wem.2020.07.007

King, E. A., and Lien, J. R. (2017). Flexor tendon pulley injuries in rock climbers. *Hand. Clin.* 33, 141–148. doi:10.1016/j.hcl.2016.08.006

Klauser, A., Frauscher, F., Bodner, G., Cihak, C., Gabl, M., Schocke, M., et al. (2000). Value of high-resolution ultrasound in the evaluation of finger injuries in extreme sport climbers. *Ultraschall. Med.* 21, 73–78. doi:10.1055/s-2000-316

Klauser, A., Frauscher, F., Bodner, G., Halpern, E. J., Schocke, M. F., Springer, P., et al. (2002). Finger pulley injuries in extreme rock climbers: Depiction with dynamic US. *Radiology* 222, 755–761. doi:10.1148/radiol.2223010752

Leeflang, S., and Coert, J. H. (2014). The role of proximal pulleys in preventing tendon bowstringing: Pulley rupture and tendon bowstringing. *J. Plast. Reconstr. Aesthet. Surg.* 67, 822–827. doi:10.1016/j.bjps.2014.01.041

Lutter, C., El-Sheikh, Y., Schöffl, I., and Schöffl, V. (2017). Sport climbing: Medical considerations for this new olympic discipline. *Br. J. Sports. Med.* 51, 2–3. doi:10.1136/bjsports-2016-096871

Lutter, C., Tischer, T., and Schöffl, V. (2021). Olympic competition climbing – the beginning of a new era: A narrative review. *Br. J. Sports. Med.* 55, 857–864. doi:10.1136/bjsports-2020-102035

Martinoli, C., Bianchi, S., and Cotton, A. (2005). Imaging of rock climbing injuries. *Semin. Musculoskelet. Radiol.* 9, 334–345. doi:10.1055/s-2005-923378

Martinoli, C., Bianchi, S., Nebiolo, M., Derchi, L., and Garcia, J. (2000). Sonographic evaluation of digital annular pulley tears. *Skelet. Radiol.* 29, 387–391. doi:10.1007/s002560000226

Miro, P. H., vanSonnenberg, E., Sabb, D. M., and Schöffl, V. (2021). Finger flexor pulley injuries in rock climbers. *Wilderness Environ. Med.* 32, 247–258. doi:10.1016/j.wem.2021.01.011

Mitsionis, G., Bastidas, J. A., Grewal, R., Pfaeffle, H. J., Fischer, K. J., and Tomaino, M. M. (1999). Feasibility of partial A2 and A4 pulley excision: Effect on finger flexor tendon biomechanics. *J. Hand. Surg. Am.* 24, 310–314. doi:10.1053/jhsu.1999.0310

Mitsionis, G., Fischer, K. J., Bastidas, J. A., Grewal, R., Pfaeffle, H. J., and Tomaino, M. M. (2000). Feasibility of partial A2 and A4 pulley excision: Residual pulley strength. *J. Hand. Surg. Br.* 25, 90–94. doi:10.1054/jhsb.1999.0332

Moutet, F. (2003). Flexor tendon pulley system: Anatomy, pathology, treatment. *Chir. Main.* 22, 1–12. doi:10.1016/s1297-3203(02)00010-0

Schneeberger, M., and Schweizer, A. (2016). Pulley ruptures in rock climbers: Outcome of conservative treatment with the pulley-protection splint-A series of 47 cases. *Wilderness. Environ. Med.* 27, 211–218. doi:10.1016/j.wem.2015.12.017

Schöffl, I., Deeg, J., Lutter, C., Bayer, T., and Schöffl, V. (2018). Diagnosis of A3 pulley injuries using ultrasound. *Sportverletz. Sportschaden.* 32, 251–259. doi:10.1055/a-0598-7655

Schöffl, I., Hugel, A., Schöffl, V., Rascher, W., and Jüngert, J. (2017). Diagnosis of complex pulley ruptures using ultrasound in cadaver models. *Ultrasound. Med. Biol.* 43, 662–669. doi:10.1016/j.ultrasmedbio.2016.10.005

Schöffl, I., Oppelt, K., Jüngert, J., Schweizer, A., Bayer, T., Neuhuber, W., et al. (2009a). The influence of concentric and eccentric loading on the finger pulley system. *J. Biomech.* 42, 2124–2128. doi:10.1016/j.jbiomech.2009.05.033

Schöffl, I., Oppelt, K., Jüngert, J., Schweizer, A., Neuhuber, W., and Schöffl, V. (2009b). The influence of the crimp and slope grip position on the finger pulley system. *J. Biomech.* 42, 2183–2187. doi:10.1016/j.jbiomech.2009.04.049

Schöffl, V., Hochholzer, T., Winkelmann, H. P., and Strecker, W. (2003). Pulley injuries in rock climbers. *Wilderness. Environ. Med.* 14, 94–100. doi:10.1580/1080-6032(2003)014[0094:piirc]2.0.co;2

Schöffl, V. R., Einwag, F., Strecker, W., and Schöffl, I. (2006). Strength measurement and clinical outcome after pulley ruptures in climbers. *Med. Sci. Sports. Exerc.* 38, 637–643. doi:10.1249/01.mss.0000210199.87328.6a

Schöffl, V. R., and Schöffl, I. (2006). Injuries to the finger flexor pulley system in rock climbers: Current concepts. *J. Hand. Surg. Am.* 31, 647–654. doi:10.1016/j.jhsa.2006.02.011

Schreiber, T., Allenspach, P., Seifert, B., and Schweizer, A. (2015). Connective tissue adaptations in the fingers of performance sport climbers. *Eur. J. Sport. Sci.* 15, 696–702. doi:10.1080/17461391.2015.1048747

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Review
PUBLISHED 14 September 2023
DOI 10.3389/fbioe.2023.1250533

OPEN ACCESS

EDITED BY
Sibylle Grad,
AO Research Institute, Switzerland

REVIEWED BY
Mohammad El Khatib,
University of Teramo, Italy
Burhan Gharaibeh,
University of Pittsburgh, United States

*CORRESPONDENCE
Chenchao Wang,
wangchenchao@139.com
Miao Jiang,
jiangmiao@cmu.edu.cn
Yongping Lu,
valensu423@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 03 July 2023
ACCEPTED 04 September 2023
PUBLISHED 14 September 2023

CITATION
Lin M, Li W, Ni X, Sui Y, Li H, Chen X, Lu Y, Jiang M and Wang C (2023), Growth factors in the treatment of Achilles tendon injury.
Front. Bioeng. Biotechnol. 11:1250533.
doi: 10.3389/fbioe.2023.1250533

Growth factors in the treatment of Achilles tendon injury

Meina Lin[1†], Wei Li[1,2†], Xiang Ni[1], Yu Sui[1], Huan Li[1], Xinren Chen[1], Yongping Lu[1*], Miao Jiang[1*] and Chenchao Wang[3*]

[1]Liaoning Research Institute of Family Planning, China Medical University, Shenyang, China, [2]Medical School, Shandong Modern University, Jinan, China, [3]Department of Plastic Surgery, The First Hospital of China Medical University, Shenyang, China

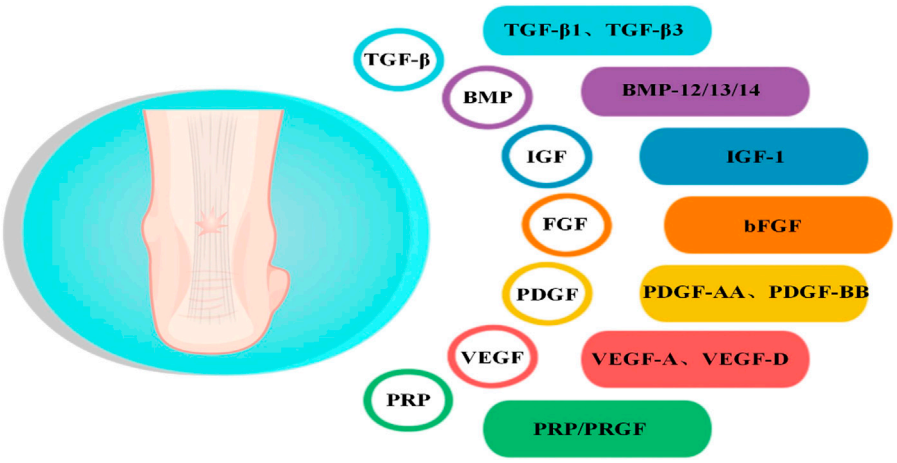
Achilles tendon (AT) injury is one of the most common tendon injuries, especially in athletes, the elderly, and working-age people. In AT injury, the biomechanical properties of the tendon are severely affected, leading to abnormal function. In recent years, many efforts have been underway to develop effective treatments for AT injuries to enable patients to return to sports faster. For instance, several new techniques for tissue-engineered biological augmentation for tendon healing, growth factors (GFs), gene therapy, and mesenchymal stem cells were introduced. Increasing evidence has suggested that GFs can reduce inflammation, promote extracellular matrix production, and accelerate AT repair. In this review, we highlighted some recent investigations regarding the role of GFs, such as transforming GF-β(TGF-β), bone morphogenetic proteins (BMP), fibroblast GF (FGF), vascular endothelial GF (VEGF), platelet-derived GF (PDGF), and insulin-like GF (IGF), in tendon healing. In addition, we summarized the clinical trials and animal experiments on the efficacy of GFs in AT repair. We also highlighted the advantages and disadvantages of the different isoforms of TGF-β and BMPs, including GFs combined with stem cells, scaffolds, or other GFs. The strategies discussed in this review are currently in the early stages of development. It is noteworthy that although these emerging technologies may potentially develop into substantial clinical treatment options for AT injury, definitive conclusions on the use of these techniques for routine management of tendon ailments could not be drawn due to the lack of data.

KEYWORDS

Achilles tendon injury, growth factors, tendon healing, clinical trial, combined application

1 Introduction

Achilles tendon (AT) injury is a clinically intractable tendon disorder that severely affects patients' daily activities, imposing a significant clinical burden on healthcare systems worldwide. Naturally healed AT typically have poor quality, as they are prone to developing fibrotic scars and tendon sheath adhesion. These tendons lack the necessary biomechanical properties for proper function and are susceptible to re-injury and re-rupture during exercise and daily activities. This is primarily due to the low number of cells, poor blood supply, and low tendon metabolism (Jiang et al., 2014; Veronesi et al., 2015; Li et al., 2021b). At present, there is a lack of effective clinical treatments to promote functional and anatomical recovery for AT injury (Steinmann et al., 2020). The goal of any therapeutic intervention is to expedite the restoration of complete mechanical strength and deliver a regenerated tendon that closely resembles the original, uninjured state. The healing process of tendons is facilitated and directed by a diverse range of GFs that are produced within the local area. So, recently, the focus



GRAPHICAL ABSTRACT

has been on the biological pathways by which tendons heal and the GFs involved. GFs involved in cell differentiation, angiogenesis, and extracellular matrix (ECM) production play a vital role in regulating cellular life activities. Therefore, GFs may be promising therapeutics for skin wounds, burns, and nonhealing chronic and diabetic wounds (Legrand and Martino, 2022). GFs also play a critical role in the natural regeneration of tendons as they participate in cell recruitment and stimulation of ECM synthesis. Studies have shown that cells within the paratendinous tissue promote healing in AT injury when stimulated by GFs (Muller et al., 2019). Based on this finding, many studies have focused more closely on how to effectively incorporate GFs into damaged tissues and determine their effects. GFs such as TGF-β, BMP, FGF, VEGF, IGF, and PDGF have shown promising applications in AT repair.

GFs can be administered percutaneously, during open surgery, or through carriers such as scaffolds or coated suture materials. These methods are intended to maintain the concentration of GFs in local areas. However, apart from platelet-rich plasma (PRP), none of these GFs have been successfully implemented in clinical practice due to a lack of compelling preclinical evidence. Single GFs have limited effectiveness in promoting tendon healing, both in laboratory settings and animal models. This suggests that a combination of different GFs/stem cells/ scaffolds may be required to significantly enhance the intricate process of tendon healing. There is ample evidence supporting the use of GFs to improve tendon healing. However, several unresolved issues still exist, such as determining which specific GFs to utilize and the optimal timing and method of their delivery.

In this review, we summarized the advantages and disadvantages of the different isoforms of TGF-β and BMP in AT healing, including GFs combined with stem cells, scaffolds, or other GFs. In addition, we summarized the clinical trials and animal experiments on the efficacy of GFs in AT repair. The findings of studies using PRP had controversial conclusions. Although these emerging basic studies provided extensive information on the therapeutic effects of GFs in AT repair, there is still a long way before they are implemented in clinical use.

2 Anatomical characteristics of the AT

The AT is the strongest and largest tendon and is subjected to the highest loads in the human body (McCartney et al., 2019). The tendon has good elasticity and extensibility and plays a vital role in daily life, especially in sports. The AT begins at the musculotendinous junction of the gastrocnemius and soleus muscles and consists of the combined tendons of the soleus, gastrocnemius, and plantaris muscles. The plantaris muscle varies in size and is absent in approximately 6%–8% of individuals (O'Brien, 2005). Generally, the length and thickness of the AT vary between subjects. In adults, the average size of the AT is 15 cm (range, 11–26 cm), and the mean width is 6.8 cm at its origin, 1.8 cm at the midsection, and 3.4 cm at its insertion to the midpoint of the posterior surface of the calcaneus (Doral et al., 2010). Furthermore, the proportion of gastrocnemius and soleus tendons involved in the composition of the AT slightly varies in different individuals (Wasniewska et al., 2022). At approximately 8–10 cm above the termination, the gastrocnemius and soleus tendons fuse completely (Ahmed et al., 1998). Spiral torsion occurs when the tendon fibers move to the termination. The gastrocnemius tendon attaches to the lateral and posterior sides of the calcaneus, while the soleus tendon attaches to the medial and anterior sides of the calcaneus (Dayton, 2017; Winnicki et al., 2020). Studies have confirmed that spiral torsion results in less fiber bending when the ankle is in plantar flexion and less deformation when tension is applied to the AT (Pekala et al., 2017). However, the spiral torsion site, 2–6 cm from the termination, has a relatively poor blood supply and is most prone to rupture and degeneration (Wolff et al., 2012; Nagelli et al., 2022). The AT is encased in the *paratenon*, which is rich in blood vessels, rather than a tendon sheath (Lohrer et al., 2008). A synovial bursa is present between the AT and the skin and calcaneus, which is essential in reducing friction and ensuring relative sliding between tissues (Campanelli et al., 2011). The tendon is also surrounded by the epitenon, a well-defined layer of connective tissue that extends inward to become the endotenon surrounding the fiber bundle (Sharma and Maffulli, 2006). Tendon composition

varies slightly in different parts of the body, which may be attributed to the physiological environment (Walia and Huang, 2019). In general, tendons in the human body have similar tissue structures, consisting mainly of collagen and scattered cells (Waggett et al., 1998). The AT mainly contains collagen I (Col I) and collagen III (Col III) (Fukuta et al., 1998; Im and Kim, 2020). Three peptide chains form into soluble tropocollagen after enzymatic excision of the terminal peptide. Tropocollagen forms collagen fibrils through lateral connections. Collagen fibril then aggregates to form collagen fiber. Many collagen fibers gather into bundles, creating primary, secondary, and tertiary fiber bundles (Andarawis-Puri et al., 2015). In addition to collagen fibers, the ECM of the AT includes elastin, fibronectin, decorin, biglycan, and fibromodulin (Walia and Huang, 2019). Tissue-resident tendon cells play a vital role in the composition of AT, exhibiting significant heterogeneity. In mature tendons, tenocytes and tenoblasts make up approximately 90%–95% of the tendon cell population, with tenoblasts being predominant in young tendons. Tissue-resident tendon stem/progenitor cells (TSPCs), which share similar characteristics with MSCs, constitute 1%–4% of the tendon resident cells. Telocytes, found in the equine inter-fascicular tendon matrix near blood vessels, express stem cell markers and are localized near the tendon stem cell niche. Additionally, new populations of tenocytes, including tendon fibroblasts 1 and 2, and junctional fibroblasts, have been recently reported. The remaining cells consist of chondrocytes, synovial cells, capillary endothelial cells, and smooth muscle cells (Sharma and Maffulli, 2005). The heterogeneity of tenocytes, along with the interaction between different cell types in tendon tissue, is crucial for the maintenance and repair of tendon tissue during homeostasis and tendinopathies (Russo et al., 2022). In normal tendons, the collagen fibers are continuous, parallel, and closely arranged (Angrisani et al., 2022). Tenocytes are arranged following the direction of the fibers, extending a few thin-winged pseudopodia embedded in fiber bundles (Millar et al., 2021). The AT exhibits a hierarchical structure (Figure 1) composed of fascicles, fibers, and fibrils. These components are encompassed by a delicate collagen membrane known as endotenon, which houses lymphatics, blood vessels, and nerves. Within this intricate arrangement, certain cells, including tenocytes, fibroblasts, and TSPCs, align themselves along the direction of the fibers.

3 Pathophysiology of AT injury

AT injury can lead to loss of tissue integrity and dysfunction, causing pain, swelling, and stiffness (Martin et al., 2018). In tendinopathy, tenocytes become more extended, thinner, smaller in size, and produce less ECM (Millar et al., 2021), and the collagen fibers are broken and arranged irregularly (Maffulli et al., 2008). Simultaneously, inflammatory cells infiltrate the tissue, triggering an inflammatory response (Ye et al., 2023). During tendon injury, the activation of inflammatory mechanisms and the innate immune system is evident within the tendon matrix microenvironment and probably attributed to the dysregulated homeostasis. The resident tenocytes secrete cytokines and chemokines in both autocrine or paracrine manner and can be activated towards an inflammatory phenotype, they influence on the reactions that submerge after tendon damage by communicating with immune-sensing cells, attracting, and activating the infiltrating immune cells into the injury site, or modulating the secreted implicated cytokines (Russo et al., 2022). In addition, supporting tissues including vascular and nervous play an important in modulating the inflammatory response of the injured tissue and in tissue regeneration. So, new nerves are frequently found in diseased tendons (Millar et al., 2021). A normal AT is not rich in blood vessels; however, in AT injury, there are apparent vascular proliferations (Chen et al., 2011). Furthermore, the increased density of Col III in the ECM leads to an abnormal Col I/Col III ratio, thus severely affecting the mechanical properties of the AT (Millar et al., 2017).

4 Etiology of AT injury

The AT is the most frequently ruptured tendon in the human body, accounting for 20% of all large tendon ruptures. AT injury is classified into closed and open injuries. Closed injury, such as rupture, tendinitis, and degeneration, is mainly caused by strain and often occurs in the middle, insertion, and peritendinous parts of the AT. Aseptic inflammation accompanied by symptoms such as redness, pain, and weakness may present at the local injured site. Open injury is mainly caused by direct violence, such as chopping and slashing with sharp objects. Other factors that can influence AT injury include age, sex, use of fluoroquinolone antibiotics, and uneven force on the AT during exercise (Seeger et al., 2006; Slane et al., 2015; Ganestam et al., 2016; Tramer et al., 2021). Furthermore, with improved quality of life, obesity has gradually become an essential factor affecting AT injury.

5 GFs in AT injury

GFs are a class of cytokines secreted by cells and participate in various biological activities by binding to specific, high-affinity receptors to maintain and regulate the growth and metabolism of the body. A study retrieved 2,332 publications on early tendon development, draw a comparative map of molecules that control tenogenesis, and found several hub genes that plays an important role in tendon development, including TGF-βs, BMPs, FGFs, IGFs (Peserico et al., 2023). These GFs related to tenogenesis paths belonging to macro-categories such as (1) growth, differentiation and survival, (2) morphogenesis and cell motility, (3) nervous system, (4) and endocrine system. GFs such as FGF-4 and TGF-β2, which are critical during embryonic development, influence tendon development. FGF-4 regulates the expression of Scleraxis (Scx), an early marker for tendon cell fate (Glass et al., 2014). As the upstream molecules of Scx, TGF-β and FGF coordinately induce the development of axial and limb tendon progenitors via Scx action. TGF-β plays a central role in tendon development, and TGFB receptors are expressed in tendon progenitor cells, the genetic deletion of TGFBR2 or TGFB2 results in failure of tendon development (Peserico et al., 2023). TGF-β1, -β2, and -β3 have distinct spatiotemporal developmental protein localization patterns in the developing tendon and may probably have independent roles in tendon development (Kuo et al., 2008). TGF-β2 was noted to be tenogenic for tendon progenitor cells at all developmental stages *in vitro* (Titan et al., 2019). BMP-12 guides the expression of Scx,

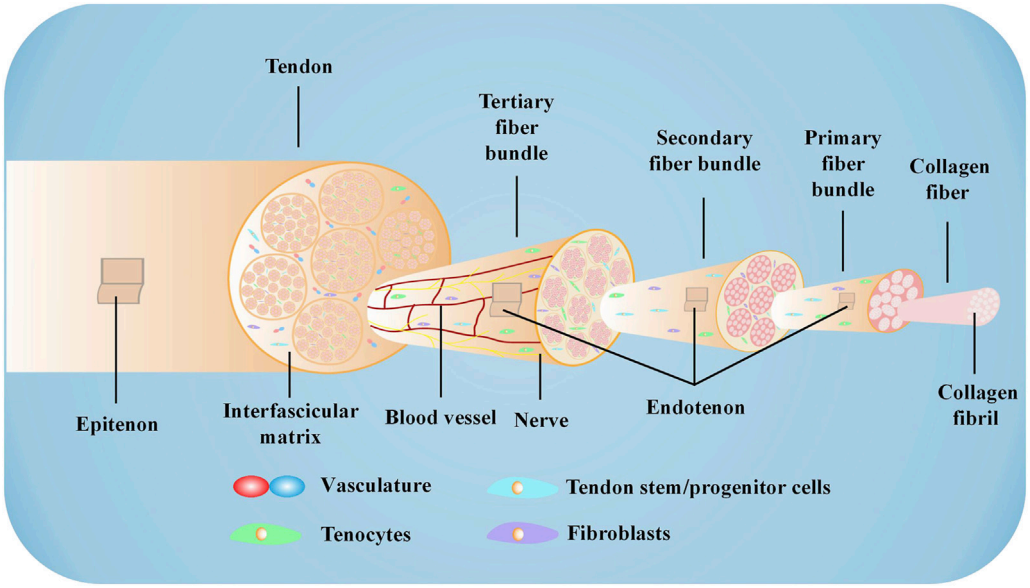


FIGURE 1
Microstructure of the AT The AT comprises collagen fibers, cells, and a small number of blood vessels. Specific proteases hydrolyze the terminal peptide of procollagen formed by three peptide chains to form tropocollagen. Triple-stranded tropocollagen forms collagen fibril through lateral connections. Collagen fibril further aggregates to form collagen fiber. Collagen fiber converges into a primary fiber bundle surrounded by the endotendon to create a secondary fiber bundle. The secondary fiber bundle also assembles into a tertiary fiber bundle, which is surrounded by the epitenon to constitute the tendon.

tenomodulin (Tnmd), Col 1, and tenascin-C (TNC) in tendon progenitor cells *in vitro* (Liu et al., 2015b). VEGF signaling is vital during tendon development, specifically within developing tendons under traction, and gliding tendons maintain an avascular zone even from the fetal period (Petersen et al., 2002). GFs such as TGF-β, BMPs, PDGF, VEGFs, FGFs, IGF, and PRP have been studied extensively in various tendon healing models (Table Supplementary S1). These GFs play a role in all three stages of AT healing, which include the inflammatory, proliferative, and remodeling phases (Figure 2). Briefly, In the first phase, the focus is on the inflammatory activities, which involve the infiltration of inflammatory cells and fibroblasts from outside the injury site, and various GFs are released during this phase, including VEGF, PDGF, bFGF, TGF-β, and IGF. The proliferative phase is a crucial stage in the healing process, characterized by the proliferation of fibroblasts, synthesis of ECM, activation and differentiation of TSPCs, and extensive growth of blood vessels and nerves. Various growth factors, including VEGF, BMPs, bFGF, TGF-β, and IGF, play important roles in this phase. In the final remodeling phase, the newly synthesized collagen undergoes rearrangement to form mature tissue. During this phase, GFs such as BMPs, bFGF, TGF-β, and PDGF play a crucial role in guiding and regulating the remodeling process.

5.1 TGF-β and AT injury

TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) are the prototypical members of the TGF-β superfamily. They are responsible for many cellular activities, including proliferation, differentiation, migration, adhesion, ECM synthesis, immune response, and cell death (Aschner and Downey, 2016; Acharya et al., 2022). The healing response in natural tendon injury is triphasic: inflammation, proliferation, and remodeling phases (Ruiz-Alonso et al., 2021). In the acute inflammation phase, which is the first phase that occurs after tendon injury, proinflammatory factors and inflammatory cell infiltration resist harmful stimuli, mediating the inflammatory response. In contrast, anti-inflammatory and proinflammatory factors act together to control the progression of inflammation (D'Addona et al., 2017). TGF-β expresses at all stages of tendon healing, particularly during the inflammatory and proliferative phases. TGF-β maintains immune homeostasis in several tissues, and the lack of TGF-β exacerbates inflammation, leading to tissue damage and cellular transformation. Its activity decreases with the relief of inflammatory response (Tauriello et al., 2022). Specific genes are selectively expressed during different tendon formation and repair stages to promote normal tendon development (Perucca Orfei et al., 2019). TGF-β isoforms have other effects during wound healing and scarring. While TGF-β3 is a significant inducer of Scx, which is expressed early in tendon development initiating tendon differentiation, it is also an inhibitor of collagen fiber maturation during tenogenic differentiation, especially in the late stage (Perucca Orfei et al., 2019; Zhou et al., 2021a). TGF-β3 alone or combined with other GFs acts as an essential tenogenic inducer in many cell types, such as AD-MSCs (Shojaee et al., 2022), human tenocytes (Tsiapalis et al., 2021), BMSCs (Bottagisio et al., 2017), embryo-derived stem cells (ESCs) (Barsby et al., 2014), tonsil-derived MSCs (TMSCs) (Wee et al., 2022). Contrary to the effects of TGF-β3, TGF-β1 inhibits the expression of Scx, promotes the expression of Tnmd, which is a marker of mature tendon cells, and accelerates tendon development (Hyun et al., 2017).

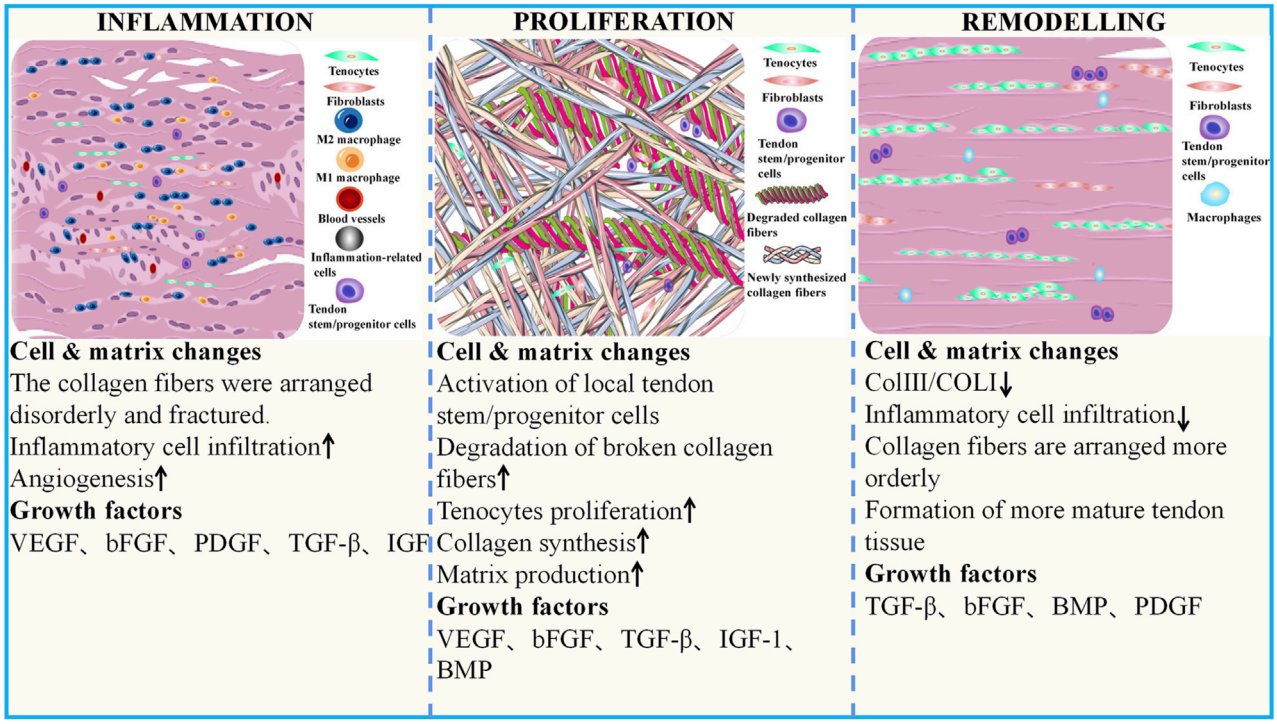


FIGURE 2
Cell and matrix changes during different phases of AT healing and the GFs involved The recovery process following AT injury is triphasic: acute inflammation, proliferation, and remodeling phases. During the inflammatory phase, collagen fibers are disorganized and broken. There is a considerable appearance of inflammatory cells in the AT and a significant increase in neovascularization. With the activation and proliferation of *in situ* cells, tenocytes and fibroblasts increase significantly, and the recovery phase gradually transitions to the proliferation phase. Here, the content of ECM fractions also gradually increases. The damaged tissue begins to degrade, and new tissues fill the defect site. As rehabilitation progresses, the number of inflammatory cells decreases sharply, the arrangement of collagen fibers becomes more regular, and Col I gradually replace Col III. The biomechanical properties of the AT tissue significantly improve. AT, Achilles tendon; GFs, growth factors.

Proper application of TGF-β1 at the early stage of injury can reduce inflammation and accelerate wound healing (Sun et al., 2021). Collagen content and cross-linking are significant determinants of tendon structural integrity and function. Col Ⅰ and Col Ⅲ are the main contents of the AT. TGF-β1 may participate in matrix remodeling by modulating collagen synthesis (Sun et al., 2015). AT injury treated with TGF-β1-bone marrow mesenchymal stem cells (BMMSCs) healed more rapidly and completely. TGF-β1 accelerates collagen protein synthesis, cross-link formation, and matrix remodeling in tendon healing, thus enhancing mechanical strength (Hou et al., 2009b). Low-magnitude, low-frequency 10 Hz vertical vibration training enhances TGF-β1 expression, subsequently increasing the expression of Tnmd and synthesis of Col Ⅰ and increasing AT stiffness in rats. This treatment improves tendon properties and minimizes the risk of ligament/tendon reinjury during rehabilitation (Chen et al., 2018b). A study demonstrated that in patients with AT rupture, TGF-β1 and VEGF 3 expressions significantly increased 3 months after treatment and significantly decreased 6 months after surgery. TGF-β1 and VEGF expressions decreased after surgery with improvement in the efficacy; therefore, TGF-β1 and VEGF can be considered as observational indexes and predictors of clinical efficacy in patients with AT rupture before and after surgery (Cui et al., 2019). Collagen sponges loaded with GFs, such as basic FGF (bFGF), BMP-12, and TGF-β1, implanted in a rat with transected AT showed a rapid increase in mechanical strength and faster tendon remodeling (Majewski et al., 2018). The TGF-β1 and TGF-β3 changes in the coursing of AT healing in a rat model was higher than that in the sham operation group at all-time points (2w, 4w, 6 weeks after injury), reaching its peak at 2 weeks, decreased at 4 weeks, and significantly reduced at 6 weeks after the operation. Therefore, the expression levels of these two factors may be used as indicators to determine the degree of recovery following an AT injury (Wu et al., 2021). Rat muscle biopsies transduced with recombinant adenovirus-TGF-β1 were transplanted to surgically transected AT of recipient animals, which accelerated the healing, and the repair tissue gained nearly normal histological appearance at 2 weeks post operation (Majewski et al., 2012). In addition, the treatment notably alleviated the inflammatory responses *in vivo* via downregulation of IL-1β, TNF-α, and IL-6 and promoted the tube formation in tissues through upregulating VEGF, bFGF, TGF-β1, and CD31 (Gong et al., 2022).

Although TGF-β fosters recovery after AT injury, its overexpression can result in abnormal deposition of ECM proteins, leading to tissue fibrosis, induced excessive scar hyperplasia in the injured area, altered tissue structure, and decreased anatomical function (Schroer and Merryman, 2015). In addition, elevated active TGF-β promotes ectopic bone formation. Huang et al. demonstrated that injured AT of rats transfected with TGF-β short hairpin RNA using ultrasound-targeted microbubble

destruction technique attenuated AT adhesions and scar formation, and the decreased TGF-β helped to alleviate tendon adhesion by reducing the number of inflammatory cells (Hung et al., 2022). Although attenuating the effect of TGF-β1 can diminish the grading of adhesions, the ultimate strength of repaired tendons was significantly impaired (Zhou et al., 2013).

In contrast, TGF-β3 is well known for its antifibrotic effects. Jiang reported that adding TGF-β3 to tenocytes significantly downregulated the expression of Smad3 and upregulated the expression of Smad7 (Jiang et al., 2016b), minimizing extrinsic scarring via antagonizing the TGF-β1/Smad3 signaling pathway (Jiang et al., 2016a; Deng et al., 2017). This result provides a new therapeutic approach for reducing scar tissue and promoting tendon healing. Cetik et al. used PLGA-b-PEG NPs [poly (lactic-co-glycolic acid)-b-poly (ethylene glycol) nanoparticles (NPs)] loaded TGF-β3 as a sustained-release system to treat rat models with unilateral AT transection and demonstrated that TGF-β3 may positively affect AT midsubstance repair, especially during the remodeling phase, and the NP form will achieve better outcomes (Cetik et al., 2022).

TGF-β2 was the only isoform detected in tenocytes within the fibrillar matrix, and its expression was significantly higher in patient tendons compared to normal cadaver tendons. This elevated presence of TGF-β2 in pathological AT suggests its potential role in regulating cellular activity during the advancement of this disease (Fenwick et al., 2001). TGF-β2 and TGF-β3 expressions fluctuated during bone formation. TGF-β2 was significantly upregulated during HO formation in animal model for HO induced by Achilles tenotomy in rats (Lin et al., 2010). The zinc finger transcription factor (EGR1) plays a role in the production of type I collagen in postnatal tendons. In a rat model of AT injury, the application of EGR1-producing MSCs resulted in an increase in the formation of tendon-like tissues. This effect is partially mediated by TGF-β2 (Guerquin et al., 2013). Mohawk (Mkx) is expressed in developing tendons and is an important regulator of tenogenic differentiation, its expression level was dramatically lower in human tendinopathy tissue and it is activated at specific stages of tendon development. Mkx dramatically upregulated Scx through binding to the TGF-β2 promoter. Mkx activates Scx and tendon ECM genes expression partially through direct activation of TGF-β2 (Liu et al., 2015a).

In conclusion, the TGF-β isoforms have different effects during AT healing and scarring (Table 1), even if the same subtype has a double-sided effect. However, further studies regarding the effective ways to use these factors to promote AT healing and prevent scar formation or their role as indicators of the degree of recovery following injury are warranted.

5.2 BMPs and AT injury

BMPs constitute the largest subdivision of the TGF-β family with nearly 30 different proteins (Lowery and Rosen, 2018). However, significant differences among BMPs regarding their effects on AT repair exist. BMP-1 is significantly upregulated at each time point of traumatic heterotopic ossification (HO). On the contrary, BMP-4 is significantly downregulated in the early stages of traumatic HO. This indicates that BMP-1 plays an essential role in the formation of traumatic HO, whereas BMP-4 plays a protective role in the early stage of HO (Yu et al., 2021a). The BMP-2/4/7 expression in Sprague-Dawley (SD) rats AT increased with aging, especially BMP-2, and this expression remained consistent with the increasing trend of HO and osteogenesis-related gene expression in the tissue. BMP-4/7 might play an essential role in forming ectopic ossification at the early stage and a weaker function at the late phase (Dai et al., 2020). BMP-2 negatively regulates the expression of Prospero homeobox protein 1, thereby inhibiting the formation of lymphatic endothelial cells, aggravating the inflammatory response, and promoting the formation of HO (Dunworth et al., 2014).

BMP-12/13/14 can induce tendon differentiation *in vivo*. BMP-12/14 induces tendon differentiation of adipose tissue-derived mesenchymal stromal/stem cells (ADMSCs), and BMP-12 induces tendon differentiation of ADMSCs via the Smad1/5/8 pathway in a dose- and time-dependent manner (Shen et al., 2013) (Figure 3). BMP-14 may induce the tenogenic differentiation of BMMSCs via the Sirt1-JNK/Smad1-PPARγ signaling pathway (Wang et al., 2018a) (Figure 3). Adenovirus-mediated gene therapy of BMP-14 expedited tendon healing in SD rats model with transected AT; BMP-14 significantly increased the Col II expression and enhanced the mechanical properties of the AT (Wang et al., 2018a). In another study, BMP-12 was tethered on a book-shaped decellularized tendon matrix and implanted into a rat AT defect model, which proved more beneficial than autograft for AT healing (Xiao et al., 2021). Exogenous BMP-7 in a rat AT has been shown to enhance fibro-chondrocyte differentiation of tendon cells, induce ectopic cartilage formation, promote meniscus regeneration, and prevent cartilage degeneration (Ozeki et al., 2013). Biopsies of autologous skeletal muscle transduced with Ad. BMP-12 and surgically implanted around experimentally transected AT in a rat model improved and accelerated tendon healing and influenced early tissue regeneration, leading to quicker recovery and improved biomechanical properties of the AT (Majewski et al., 2008). Lee et al. showed that BMMSCs implanted into an animal model after loading onto a three-dimensional collagen sponge scaffold and induction by BMP-12 promoted tendon-like tissue formation (Lee et al., 2011). In addition, the number of tenocytes increased, which were arranged regularly along the tension axis. Engineered tendon matrix containing BMMSCs and BMP-13 implanted in an AT injury model significantly improved the fiber alignment, increased the tensile modulus, and increased the ultimate load of the AT (Jiang et al., 2016a). Park et al. showed that 100 ng/mL of BMP-14 significantly promoted the proliferation of ADMSCs and expression of the tendon marker genes Scx, Tnmd, and TNC (Park et al., 2010). Although BMPs combined with stem cells can promote AT healing, differences in the therapeutic effects of different sources of stem cells on AT injury exist. BMMSCs, ADMSCs, and synovial membrane-derived MSCs (SMMSCs) induced with BMP-12 all showed similar fibroblast morphology and expressed typical tendon marker genes, such as Scx, TNC, and Tnmd (Dai et al., 2015). Nonetheless, BMMSCs showed superior tendon differentiation ability, followed by SMMSCs, while ADMSCs showed the least tendon differentiation ability. However, compared with BMMSCs, BMP-14-induced muscle-derived MSCs were more effective in promoting tendon healing (Ozasa et al., 2014). The advantages/disadvantages of BMPs are presented in Table 2.

In summary, BMP isoforms exhibit different expression patterns in various stages of AT repair and play various roles in the AT

TABLE 1 The advantages and disadvantages of TGF-β isoforms in AT repair.

TGF- β isoforms	Advantages	Disadvantages	Summary
TGF- β1	①Increases the collagen synthesis You et al. (2020).	Its overexpression will result in abnormal deposition of ECM proteins, alteration in tissue structure and anatomical hyperplasia Schroer and Merryman (2015), lead to tissue fibrosis, excessive scarring, and HO Wang et al. (2018b).	TGF-β isoforms function in the regulation of collagen synthesis in tendon fibroblasts. Proper application of TGF-β1 at the early stage of injury can reduce inflammation and accelerate wound healing. Attenuating the effect of TGF-β1 at the late stage will diminish the extent of scar formation. TGF-β3 exerted antagonistic effects to TGF-β1.
	②Promotes tube formation Gong et al. (2022).		
	③Increases Tnmd, Col I and the AT stiffness Chen et al. (2018a).		
	④Reduces the inflammatory response Pakshir and Hinz (2018).		
	⑤Increases the proliferation, migration, and differentiation of TSCs Li et al. (2021a).		
TGF- β2	① Increases Scx, Col I and Eln in TPCs and MSCs Brown et al. (2014); Brown et al. (2015).	Induces HO formation Lin et al. (2010).	
	② Induces Tnmd expression, alters cadherins and connexin-43 protein expression Theodossiou et al. (2019).		
	③ Increases paracrine factors and ECM molecules in tendon healing and promotes tenocytes migration Koch et al. (2022).		
	④ Promotes tenogenesis Chien et al. (2018),Tnmd-expressing mature tenocytes differentiation Yoshimoto et al. (2022) and collagen synthesis Font Tellado et al. (2018)		
TGF- β3	① Induces Scx expression, initiates tendon differentiation Hyun et al. (2017).	NA	
	② Anti-fibrotic effects, minimizes scarring via antagonizing the TGF-β1/smad3 signaling Jiang et al. (2016b); Deng et al. (2017).		

NA, no data available.

healing process. BMP-2, -12, −13, and −14 are potent inducers of tenogenic differentiation in different MSCs, promoting AT recovery. BMP-1, -2, -7, and -14 induce HO, whereas BMP-4 inhibits HO. Therefore, to enhance AT healing and decrease HO, the effective use of BMP isoforms is important.

5.3 IGFs and AT injury

The IGF family plays a critical role in normal growth and development. It comprises (i) ligands (IGF-I, IGF-II, and insulin), (ii) six well-characterized high affinity binding proteins (IGF binding protein [IGFBP]-1–6), (iii) IGFBP proteases, and (iv) cell surface receptors that mediate the biological functions of IGFs (IGF-1 receptor [IGF-1R], IGF-2R, and insulin receptor substrates) (Stuard et al., 2020). IGF-1 is critical for tenocyte migration, division, matrix expression, collagen synthesis, phenotypic maintenance, and tendon repair after injury (Muller et al., 2015). A study showed that after regular training, the cross-sectional area of tendons increased significantly in men than in women, which may be related to IGF-1 secretion and sex; the cumulative effect of the amount of IGF-1 secretion makes men more superior to women due to motor-adapted hypertrophy of the AT (Astill et al., 2017). IGF-1 accelerates tendon recovery and promotes tendon-derived stem cell (TDSC) phenotype proliferation and maintenance (Holladay et al., 2016). IGF-1 combined with TGF-β1 induces ADMSCs to differentiate into stable tenocytes (Schneider et al., 2011). IGF-I injections stimulated collagen synthesis in Ehlers-Danlos patients (Nielsen et al., 2014). Tang et al. showed that IGF-1 significantly improved the mechanical properties of the AT, which may be attributed to the promotion of collagen synthesis by IGF-1 (Tang et al., 2015). IGF-1 signaling is required for proper tendon growth in response to mechanical loading through a coordinated induction of collagen synthesis and cell proliferation. Disser et al. demonstrated that IGF-I induces tenocyte proliferation via the RAS/RAF/MEK/ERK signal transduction pathway and stimulates ECM protein synthesis via the PI3K/Akt signaling pathway (Hortensius and Harley, 2013; Disser et al., 2019). After AT injury, resident cells, such as macrophages and mastocytes, recruit immune cells from circulation to build a protective system. Tenocytes participate in the inflammatory response and tissue remodeling through autocrine or paracrine secretion. While low-level inflammation clears damaged tissues and promotes tissue recovery, a too-strong inflammatory response can lead to tissue fibrosis, resulting in poor remodeling and seriously affecting the healing of the AT. IGF-1 may alleviate the functional impairment after AT injury by reducing the inflammatory response (Kurtz et al., 1999). As there are few studies on the effects of IGFs on AT healing, it would be interesting to analyze whether different concentrations of IGF-1 can generate different results.

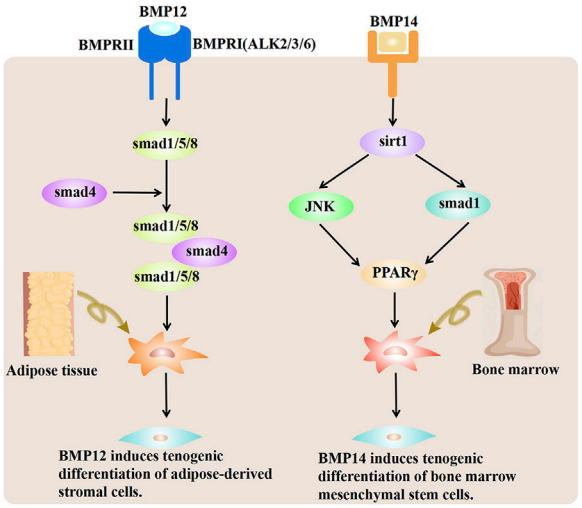


FIGURE 3
BMP-12 and BMP-14 mechanisms promoting tenogenic differentiation of MSCs. BMP-12 induces tenogenic differentiation of ADMSCs via the Smad1/5/8 pathway. BMP-12 triggers the phosphorylation of Smad1/5/8 in ADMSCs. The effect is likely conveyed by type I receptors ALK2/3/6. BMP-14 induces the deacetylation of PPARγ through activation of the Sirt1-Smad1/JNK pathway. Deacetylated PPARγ further promotes the tendon differentiation of BMMSCs. BMP-12, bone morphogenetic protein 12; BMP-14, bone morphogenetic protein 14; MSCs, mesenchymal stem cells; ADMSCs, adipose tissue-derived mesenchymal/stromal stem cells; BMMSCs, bone marrow mesenchymal stem cells.

5.4 FGFs and AT injury

FGFs are broad-spectrum mitogens and play a critical role in development, metabolism, and tissue homeostasis by regulating a wide range of cellular functions, including migration, proliferation, differentiation, and survival. FGFs exert pleiotropic effects by binding to and activating high-affinity tyrosine kinase receptors (FGFR) (Xie et al., 2020). Fibrin clots and vitamin C produce a more robust tendon structure and better quality tendon healing in the surgical treatment of AT ruptures, which may be attributed to the proper stimulation of FGFs secretion during the first phase of AT injury (Celik et al., 2021).

FGF2, also known as bFGF, promotes cell proliferation and migration and accelerates wound healing (Zhang et al., 2018a). bFGF stimulates collagen production, tendon development, tenocyte proliferation, and tendon tissue differentiation and promotes the expression of a series of GF genes in AT repair. bFGF stimulates ECM secretion and slows down ECM degradation by upregulating the expression of tissue inhibitors of metalloproteinase (MMP) (Tang et al., 2016). A study demonstrated that human TDSCs transfected with a lentivirus carrying the FGF2 gene promoted the expression of Col 3A1 and Scx *in vitro* (Guo et al., 2020). In addition, the rat AT defect model transplanted with FGF2-hTDSCs demonstrated more ECM production and a more orderly arrangement of collagen fibers. Hyun et al. found that FGF2 dose-dependently increased Scx and Tnmd expressions, which are markers of tendinogenesis, in human periodontal ligament stem cells. Simultaneously, FGF2 counteracted the inhibition of early tendinogenic marker expression by TGF-β1 and attenuated the ossification effect of BMP-2/4 (Hyun et al., 2017). The lasting time of GFs used in tendon injury is key in determining their effects. Tissue-engineered scaffolds can sustainably release GFs to promote cell infiltration and tissue formation. bFGF-loaded multiscale fibrous scaffolds (polycaprolactone [PCL]/Col/bFGF) with appropriate porosity provide physical and biochemical cues to facilitate tenocyte proliferation and differentiation. *In vivo* study of cell-seeded scaffold after dynamic stimulation in the AT defect model showed tendon tissue regeneration, with aligned collagen morphology within 12 weeks of implantation, and inhibition of scar formation, thus playing an essential role in maintaining the tendon's ultimate tensile strength (UTS) (Jayasree et al., 2019). Chen et al. demonstrated that FGF gene expression was associated with improved patient-reported outcomes and suggested that FGF expression in surgical biopsies could potentially be used as a predictor for healing (Chen et al., 2021).

HO, an extraskeletal bone formation, is a common complication after trauma; however, its underlying mechanisms remain unclear. The degree of ossification depends on the level of damage/inflammation. Zhang et al. revealed that conditional knockout FGFR3 in $Col2^{+}$ cells promote acquired HO development. Knockdown of FGFR3 in lymphatic endothelial cells inhibits local lymphatic formation in a BMPR1a-pSmad1/5-dependent manner, exacerbating inflammatory levels in the repaired tendon, leading to HO. Therefore, activating FGFR3 in lymphatic endothelial cells may be a therapeutic strategy to inhibit HO formation by increasing local lymphangiogenesis (Zhang et al., 2021). However, further studies evaluating its plausibility and the role of FGF in AT healing are warranted.

5.5 PDGFs and AT injury

PDGF, a serum-derived GF, is essential for the maturation of multiple cells. It includes four isoforms: PDGFA, PDGFB, PDGFC, and PDGFD. These isoforms dimerize to form homodimers PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD and heterodimers PDGF-AB (Paolini et al., 2022). PDGF receptor (PDGFR) is a transmembrane glycoprotein belonging to class III receptor tyrosine kinases, including two subtypes, PDGFRα and PDGFRβ, which can polymerize with each other to form three dimers: PDGFRαα, PDGFRαβ, and PDGFRββ (Guerit et al., 2021). Five distinct PDGFs can bind to one or both PDGFRs in a dimeric state contributing to the normal development of different tissues. PDGF-AA, which only activates PDGFRα, drives both tenogenesis and fibrosis. Tubulin polymerization-promoting protein family member 3-expressing (Tppp3+) cell population can generate new tenocytes and self-renew upon injury. PDGF-AA induces new tenocyte production while inactivating PDGFRα in Tppp3+ cells block tendon regeneration. However, PDGF-AA can also act on fibro-adipogenic progenitors and lead to tendon fibrotic scar formation (Harvey et al., 2019). Therefore, studies regarding the use of PDGF-AA to accelerate AT recovery while reducing scar formation are worth conducting.

PDGF-BB is considered the universal isoform of PDGF and approved by the Food and Drug Administration because of its ability to bind to all three PDGF receptors and trigger different signaling pathways (Evrova and Buschmann, 2017). PDGF-BB expresses

TABLE 2 The advantages and disadvantages of BMPs isoforms in AT repair.

	Advantages	Disadvantages	Summary
BMP-1	NA	①Degrades collagen precursor, inactivates BMP antagonists, activates TGF-β1, and induces HO Yu et al. (2021a).	Overall, different BMPs play different roles in various stages of AT healing. BMP-2/12/13/14 are potent inducers of tenogenic differentiation in MSCs, BMP-1/2/7/14 induce HO, otherwise, BMP-4 inhibits HO.
BMP-2	① Improves tendon healing and biomechanical parameters Pelled et al. (2012).	Promotes chondrogenic differentiation TSCs Xu et al. (2016), and induces HO *in vivo*.	
	② Increases human tendon cell growth and viability Arslan et al. (2016).		
	③ Mediates TC differentiation and tendon-like tissue formation of MSCs Ker et al. (2011); Noack et al. (2014).		
	④ Promotes the woven bone in tendon-bone junctions and increases the mean maximal load Kim et al. (2011).		
BMP-4	① Enhances tendon-to-bone attachments healing, promotes the regeneration of fibrocartilaginous enthesis and mineralization Chen et al. (2021).	NA	
	② Inhibits HO Yu et al. (2021a).		
	③ Enhances human TCs growth and viability Arslan et al. (2016).		
BMP-7	① Enhances human TCs growth and viability Arslan et al. (2016).	Induces fibro-chondrocyte differentiation of TCs, and HOOzeki et al. (2013).	
BMP-12	①Induces MSCs to differentiate into tenocytes and promotes tendon healing Xiao et al. (2021).	NA	
	②Improves collagen organization, reduce adhesions, decreases cell numbers Chamberlain et al. (2015).		
	③Regulates ingrowth at the enthesis		
	④Accelerates tendon remodeling by increasing Col1 and shifting fibroblasts to fibrocytes.		
	⑤Improves tendon healing, leading to regenerates Majewski et al. (2018).		
BMP-13	①Induces MSCs to differentiate into tenocytes.	NA	
	②Induces elastin and Col I, resulting in stronger tendons;		
	③Increases tendon fibroblasts proliferation, matrix remodeling, tissue regeneration, COL1, COL3 expression, and the strength in healing tendon Eliasson et al. (2008); Mikic et al. (2009).		
BMP-14	①Induces MSCs to differentiate into tenocytes Bottagisio et al. (2017); Wang et al. (2018a)	Induces cartilage formation Rickert et al. (2001)	
	②Increases tendon resistance, stiffness, tensile strength, and neotenocytes nummber at the site of healing Bolt et al. (2007); Rickert (2008).		

NA, no data available.

predominantly during tendon healing. It induces tissue repair through its generic chemotactic, mitogenic, and angiogenic properties and synergistic actions with other GFs. The supplementation of tenocyte culture with PDGF-BB increased tenocyte proliferation in a rabbit AT model, leading to the upregulation of fibronectin, biglycan, and TNC in the cultured tenocytes (Evrova et al., 2020). Chen et al. demonstrated that recombinant human PDGF-BB promoted hADMSC proliferation via the miR-363/PI3K/Akt pathway. PDGF-BB and hADMSC can improve the biomechanical indices of Achilles tendinitis separately, such as stiffness, stress, and maximum load-to-failure, and upregulate Col Ⅰ, Scx, and TNC expressions. PDGF-BB and hADMSC can also enhance these effects further (Chen et al., 2018b). The combined application of two or more GFs can overcome the defects of a single GF and even have a cumulative effect, thereby promoting healing. PDGF-BB combined with growth differentiation factor-6 (GDF-6) can stimulate the tenogenic differentiation of ADMSCs, with results better than that of a

single GF (Younesi Soltani et al., 2022). ADMSCs cultured with GDF5/PDGF before implantation can promote tendon repair by improving cellular proliferation, differentiation, tenogenesis, vascular infiltration, proteinogenesis gene SOX9 expression, and tissue remodeling (Fitzgerald et al., 2021).

Degradable biological scaffolds are often used to deliver GFs continuously at the site of injury for long-term effects. Evrova et al. found that PDGF-BB loaded with polyester urethane scaffold significantly increased the expression of α-smooth muscle actin, promoted the proliferation of tenocytes, and improved the UTS of the tendons, with no significant local scar hyperplasia (Evrova et al., 2020). Kang et al. suggested that the long-term local PDGF delivery by porous microspheres modified with heparin has a great potential to enhance tendon healing in a rat model of Achilles tendinitis by suppressing inflammation responses (Kang et al., 2019). PDGF-AA-modified poly (lactide-co-glycolide) acid (PLGA) electrospun fibers (PLGA-PDGF-AA) effectively promoted tendon healing by stimulating collagen synthesis, deposition, and mechanical strength of tendon tissue (Wang et al., 2022). The sustained delivery of PDGF-BB via an electrospun DegraPol tube to the wound site in a full-transection rabbit AT model accelerated tendon wound healing by causing a more uniform cell distribution, with higher proteoglycan content and less fibrotic tissue (Meier Burgisser et al., 2020). Liu et al. constructed a multilayer composite membrane and a GF sustained-release system conforming to the tendon-healing cycle by coating two surfaces of freeze-dried amnions with PCL nanofibers (Liu et al., 2020). In the study, the rabbit tendon injury model treated with the composite membrane effectively isolated the exogenous adhesion tissue and promoted endogenous tendon healing by slowly releasing TGF-β1, bFGF, VEGF, and PDGF and regulating the ERK1/2 and SMAD2/3 pathways.

5.6 VEGFs and AT injury

The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental GF, and endocrine gland-derived VEGF. VEGF promotes endothelial cell mitosis, improves vascular permeability, and stimulates cell migration. In addition, VEGFs binding to VEGF receptors (VEGFR) promote tyrosine kinase enzyme activation and several intracellular signaling pathways (Melincovici et al., 2018).

Although tendons are relatively hypovascular, they become hypervascular during injury and degeneration, and this may be attributed to the formation of new blood vessels in the injured tissue. Neovascularization facilitates healing by controlling the immune response, delivering oxygen and nutrients, removing waste products, and transporting regulatory factors. Vascular ingrowth is necessary for tendon healing; prolonged hypervascularization following tendon injury may not be beneficial. VEGF plays a vital role in angiogenesis. Cui et al. found that during the inflammatory phase of tendon healing, M2 macrophages can release VEGF and promote endothelial cell sprouting, contributing to angiogenesis (Cui et al., 2022). As a potent regulator of angiogenesis, VEGF dose-dependently promotes tenocyte proliferation, enhances the expression of tendon-related genes (Kraus et al., 2018), facilitates the formation of microvessels, and improves the UTS of the AT when used appropriately. A study has shown that reducing VEGF-signaling leads to tendon healing (Tempfer et al., 2018). VEGFR1, VEGFR2, and VEGFR3 expressed in murine and human tendon cells *in vivo* and VEGFR1, VEGFR3, and VEGF-D expressed in tenocytes respond to inflammatory stimuli and injury both *in vitro* and *in vivo* and can affect tenocyte proliferation, stromal disruption, cell migration, and degenerative changes in the AT (Tempfer et al., 2022). After a tendon injury, tenocytes secrete hypoxia-inducible factor 1 (HIF-1) in response to mechanical overload and hypoxia. Simultaneously, HIF-1 promotes VEGF expression, resulting in increased vascularization and accelerated tendon healing (Tempfer and Traweger, 2015). A study showed that the serum TGF-β1 and VEGF expressions in patients with AT rupture significantly increased 3 months and significantly decreased 6 months after surgery, as compared to results before treatment. Therefore, TGF-β1 and VEGF may be considered observational indexes and predictors for clinical efficacy in patients with AT rupture (Cui et al., 2019). A study indicated that compared with normal controls, VEGF and corresponding protein levels significantly downregulated in the healing AT of type 2 diabetic rats 2 weeks post injury; the vascular remodeling ability decreased, and the recovery was slow (Ahmed et al., 2014). Therefore, upregulation of VEGF levels may be a strategy to improve tendon repair in diabetic rats during the early stages of AT injury.

VEGF-111 is a biologically active and proteolysis-resistant splice variant of VEGF-A. Local injection of VEGF-111 significantly improved the UTS of the healing ATs 15 and 30 days after surgery and the mechanical stress in the late phase (30 days) of the repair compared to the control group (Kraus et al., 2014). Many studies linked heparin injection to poor outcomes in rat AT repair due to its role in the inhibition of thrombin activity and anticoagulation effect. However, suture loading with heparin has achieved good results. Poly-l-lactic acid/polyamide sutures loaded with heparin can reduce inflammation and accelerate the healing and regeneration of the AT by promoting VEGF secretion (Ye et al., 2018).

Vascularization in healthy tendons is low; however, the production of new vessels after an injury is not necessarily a sign of functional tissue repair. Instead, it may be associated with degeneration. Some studies have demonstrated that antiangiogenic treatment in tendon models may cause improvements in tissue organization and mechanical properties. Bevacizumab, an antiangiogenic drug that is a recombinant humanized monoclonal antibody blocking VEGF-A signaling, can alter tendon vascularity and dose-dependently improve tendon healing (Riggin et al., 2019). In addition, the drug can significantly improve tendon healing in a rat model by reducing angiogenesis, cross-sectional area, stiffness, and Young's modulus, thereby improving matrix organization and increasing maximum load and stress. The gait pattern of the rat model also improved (Tempfer et al., 2018). ADMSC transplantation into injury sites during tendon repair in a mice model significantly increased VEGF and CD31 positive vessels, repairing the tendinopathy and preventing HO (Kokubu et al., 2020). VEGF can also cause scarring by stimulating the formation of a hypofunctional vascular connective tissue at the injury site, disrupting the typical molecular structure. Using VEGF to promote recovery from AT injury can be complicated. Hence, further studies are needed to assess whether VEGF is appropriate for tendon healing.

5.7 PRPs and AT injury

PRP is an autologous blood product containing high concentrations of GFs and cytokines, such as PDGF, IGF, VEGF, and TGF. It alters the biological processes in many pathogeneses, promotes injured tissue regeneration, accelerates anabolism, and improves healing by stimulating cell proliferation, migration, and angiogenesis (Boesen et al., 2017; Bennell et al., 2021; Park et al., 2021; Xu et al., 2021). Depending on their leukocyte and fibrin content, PRPs can be classified into pure PRP (P-PRP), leukocyte-rich PRP (LR-PRP), pure platelet-rich fibrin (P-PRF), and leucocyte- and platelet-rich fibrin (L-PRF) (Dohan Ehrenfest et al., 2014). Recent clinical and experimental studies have demonstrated the successful application of PRP in treating chronic tendinopathy and acute tendon injury. PRP can promote tissue recovery in the early phase of tendon healing by stimulating tendon cell proliferation and collagen production while inhibiting apoptosis and macrophage infiltration (Yu et al., 2021b). Zou et al. injected PRP into the paratenon sheath and around the ruptured tissue of patients with AT tendon rupture after surgery, and the results demonstrated that PRP improved the short- and mid-term functional outcomes after surgical repair (Zou et al., 2016). Platelets are a major high mobility group box1 (HMGB1) source. Platelets HMGB1 within PRP play a vital role in tendon healing by decreasing inflammation, increasing local HMGB1 levels, and recruiting stem cells to the wound area, suggesting that the efficacy of PRP treatment for tendon injuries in clinics may depend on platelets (Zhang et al., 2021).

Compared to LR-PRP, leukocyte-poor PRP (LP-PRP) is a better choice for treating tendinopathies. The high amounts of proinflammatory factors and catabolic enzymes in leukocytes exacerbate the inflammatory response after injury. LP-PRP may promote AT healing by modulating the balance of MMPs and tissue inhibitors of metalloproteinases and reducing proinflammatory catabolic cytokines release. In addition, the leukocytes present in LR-PRP may provoke further chronic inflammation (Yan et al., 2017). PRP combined with a variety of therapies can enhance AT healing. MSCs combined with PRP significantly increased the inflammatory cell density, mean maximum breaking force, and tendon strength force (Uyar et al., 2022). The primary use of glucocorticoid enhanced the regenerative effects of PRP in early inflammatory tendinopathy (Ruan et al., 2021). However, PRP combined with eccentric training in chronic Achilles tendinopathy proved more effective in improving activity levels and reducing pain, tendon thickness, and intratendinous vascularity (Boesen et al., 2017). A study indicated that PRP injection may be an intensive treatment for patients with AT rupture (Padilla et al., 2021). However, studies have shown that the application of PRP in the nonsurgical treatment of AT rupture showed no special clinical effect or functional improvement and is not as effective as percutaneous fixation in reducing pain (Boesen et al., 2017; Kearney et al., 2021; Kirschner et al., 2021). This difference in results may be attributed to varying treatment regimens. Nonetheless, despite the controversial results of PRP use for tendinopathy, it remains the most used biological treatment (Kirschner et al., 2021).

In conclusion, PRP is the only GF used in clinical studies because of its apparent advantages, such as self-sufficiency, convenient extraction, and high safety. However, the results are inconsistent, and studies regarding using PRP for routine management of tendon ailments are limited to allow definitive conclusions.

5.8 Combination of GFs with other GFs/stem cells/ scaffold in AT injury

As mentioned above, various GFs activate cellular processes, ECM deposition, and tissue regeneration during the different phases of tendon healing. Specific GFs critical in tendon healing are TGF-β; BMP-12, -13, −14; bFGF; PDGF; IGF-1; and VEGF; however, some GFs present complex effects on AT recovery. To improve AT healing and reduce complications, most studies mainly use GFs, often combined with scaffolds or stem cells, to surgically create tendon defects (Table 3). Several types of stem cells, including BMSCs, AD-MSCs, TDSCs, ESCs, and terminated differentiated cells (Muscle cells), were utilized in combination with GFs and scaffolds for AT treatment. These stem cells were derived from various sources. The clinical application of ESCs has been limited due to ethical concerns. On the other hand, MSCs are extensively utilized in musculoskeletal repair due to their ability to self-renew and differentiate into various mesoderm-derived tissues, such as bone, cartilage, muscle, tendon, and fat. BMSCs and AD-MSCs are the two main types of MSCs used in AT injury, and both have shown significant improvements in AT healing in animal models. However, AD-MSCs have certain advantages such as ease of harvesting, ready availability, and low donor site morbidity. On the other hand, the use of BMSCs is limited due to the painful technique of bone marrow aspiration, which can also cause donor site morbidity (Pillai et al., 2017). TDSCs, or tendon-derived stem cells, possess a similar proliferative capacity to other types of stem cells. However, TDSCs have demonstrated higher clonogenicity, faster proliferation, and increased expression of TnmD, Scx, and Col1A1 compared to BMSCs (Tan et al., 2012). TDSCs may be a more favorable cell source for musculoskeletal tissue regeneration. However, obtaining autologous TDSCs without causing donor site morbidity can be challenging. Many results indicated that combining GFs with SCs or scaffolds may be a promising approach to treating AT injury. However, further studies investigating whether the combination of GFs/SCs/scaffolds can achieve the best efficacy with the least adverse effects in AT injury are warranted.

6 Clinical applications and limitations of GFs

GFs play a critical role in AT injury repair. GFs accelerate wound site restoration and improve functional recovery. However, they have some limitations, such as short effective half-life, instability, and promotion of scar and adhesion formation. PRP is the only GF to enter clinical trials for AT repair because of its apparent advantages, such as self-sufficiency, convenient extraction, and high safety. Based on the results of many basic research and animal experiments, some clinicians treated AT injury patients with local injections of autologous PRP. Nonetheless, the conclusions drawn from numerous clinical studies on the efficacy and safety of PRP remain inconsistent (Table 4). The three different isoforms of TGF (TGF-β1, -β2, and -β3) upregulate the production

TABLE 3 GFs combined with other GFs/SCs/Scaffolds in the treatment of AT injury.

	GFs	Combined application	Outcomes	Animal models/defect type	References
GFs/ other GFs	TGF-β	TGF-β+ IGF-1	Reduces Col III, increase Col I, ML, and ensile stress.	Rat/Transect	Xiang et al. (2018)
	BMP	TGF-β1+BMP-12+bFGF	Enhances AT healing.	Rat/4-mm defect	Muller et al. (2019)
		+paratenon			
	FGF	bFGF+VEGF165+rPDGF	Promotes blood vessel densities.	Rabbit/Transverse hole (3 mm diameter)	Hou et al. (2009b)
GFs/SCs					
	TGF-β	TGF-β1+BMSCs	Promotes Col I synthesis, ECM remodeling and fiber bundles.	Rabbit/Transverse hole (3 mm diameter)	Hou et al. (2009b)
		TGF-β1+Muscle cell	Accelerates AT healing, restore mechanical strength, and increases tendon thickness.	Rat/Transect	Majewski et al. (2012)
		TGF-β1/VEGF (165)	Improves AT healing and biomechanical properties, leading to quicker recovery.	Rabbits/Transverse hole (3-mm diameter)	Hou et al. (2009b)
		+BMSCs			
	BMP	BMP-2/Smad8+MSC	Increases the stiffness, elastic modulus, and functional recovery.	Murine/2-mm full thickness defect	Hoffmann et al. (2006), Pelled et al. (2012)
			Induces tendon regeneration.	Rat/3-mm-long partial-resection defect	
		BMP-12+skeletal muscle	Improves AT healing and biomechanical properties, influences early tissue regeneration, leading to quicker recovery.	Rat/Transect	Majewski et al. (2008)
	FGF	bFGF+hTDSCs	Increases ECM, orderly collagen fibers, Col III, Col I and biomechanical properties.	Rat/3 mm gap	Guo et al. (2020)
		bFGF+MSC	Increases Col I production.	Rat/punch (2.4 mm)	Kraus et al. (2014); Kraus et al. (2016)
	PDGF	PDGF+GDF5+ADSCs	Improves cellular proliferation, tenogenesis, and vascular infiltration, tendon repair.	Rat/cut transversely 1.5 cm	Fitzgerald et al. (2021)
	VEGF	TGF-beta1/VEGF (165) +BMSCs	Accelerates tendon healing, improve biomechanical properties of AT.	Rabbits/A complete transverse hole (3 mm diameter)	Hou et al. (2009a)
		VEGF (165)+TGFβ1	Promotes angiogenesis of the reconstructed ligament and its mechanical properties.	Rabbits/ACL reconstruction	Wei et al. (2011).
		+BMSCs			
	PRP	PRP	Increases tensile strengths, Col I, FGF, and VEGF, decrease the	Rabbits/Incision	Uysal et al. (2012)
			TGF-β.		
		PRP+rBM-MSC	Promotes tendon repair and increase its structural strength.	Rat/Transverse cut	Yuksel et al. (2016).
		PRP+TSCs	Improves tendon healing.	Rat/3-mm long segment was removed	Chen et al. (2012)
		PRP +MSCs	Increases the inflammatory cell density and the mean maximum breaking force.	Rat/Cut	Uyar et al. (2022)
GFs/ scaffolds	TGF-β	TGF-β1+BMSC	Improves tendon healing and tendon regeneration.	Rat/A defect of 5 mm in length and 1 mm in width	Zhang et al. (2018a).
		+collagen sponge			
		TGF-β3+PLGA-b-PEG NPs	Sustained-release TGF-β3 accelerates AT healing and remodeling.	Rat/A 3–4 mm gap	Cetik et al. (2022).

(Continued on following page)

TABLE 3 (*Continued*) GFs combined with other GFs/SCs/Scaffolds in the treatment of AT injury.

	GFs	Combined application	Outcomes	Animal models/defect type	References
		TGF-β +Fibrin glue	Promotes fibrocartilage formation, the tensile strength.	Rabbit/Transect	Kim et al. (2007)
		TGF-β1+BMP12+bFGF +collagen sponge	Increases mechanical strength and tendon remodeling.	Rat/Transect	Majewski et al. (2018).
	BMP				
		BMP-12+mineral-coated sutures/ collagen sponge	Improves collagen organization, reduce adhesions, and decrease total cell numbers.	Rat/Transect	Chamberlain et al. (2015)
		BMP-2+fibrin glue	Accelerates bone-tendon healing, and improves histological/biomechanical properties.	Rabbit/Transect	Kim et al. (2007)
		BMP-12+collagen	Increases biomechanical properties, spindle-shaped fibroblasts.	Rat/A defect 6 mm in length	Xiao et al. (2021)
		BMP-2+PRP+fibrin	Accelerates bone-tendon junction repair, and increase the junction holding strength.	Rabbit/Transect	Kim et al. (2011).
		BMP-2+Osteoprotegerin +collagen sponge	Increases Col I and Col II, promote fibrocartilage attachment.	Rabbit/ACL reconstruction	Wei et al. (2023)
	IGF	IGFBP4+PLLA electrospun membrane	Sustained release of IGFBP-4 promotes tendon healing in functional performance, ultrastructure, and biomechanical properties.	Rat/Transect	Wang et al. (2023)
	FGF	bFGF+BMP-12+TGFβ1 +collagen sponge	Increases mechanical strength and tendon remodeling.	Rat/Transect	Majewski et al. (2018).
		bFGF+VEGFA+nanoparticle-coated sutures	Enhances tendon healing, improves tendon gliding function, Inhibits adhesion.	Rat/Transect	Zhou et al. (2021b)
		bFGF+VEGF+nanostructured mineral coating	Increases vascularization, collagen fiber organization, and mechanical properties, improve functional healing.	Rabbit/Transect	Yu et al. (2017)
		bFGF-fibrin gel +PLGA	Stimulates the proliferation and tenogenic differentiation of MSCs and synergistically enhance the injured tendon reconstruction.	Rat/A defect of 7 mm in length	Zhao et al. (2019)
		bFGF+ GPH nanofiber membranes	Provides a niche for inducing tendon tissue regeneration, and restoring the tendon tissue structure and function.	Rabbit/15-mm defect	Darshan et al. (2022)
		bFGF+ PCL micro/collagen	Enhances cellular proliferation and tenogenic marker expression, stimulates tissue regeneration with aligned collagen morphology.	Rabbit/Defect	Jayasree et al. (2019)
	PDGF	PDGF-BB+electrospun DegraPol® tub	Accelerates tendon wound healing, causing a more uniform cell distribution with higher proteoglycan content and less fibrotic tissue; Increases the tensile strength, cell hyperproliferation, and α-smooth muscle actin expression at the wound site.	Rabbi/sliced	Meier Burgisser et al. (2020)
		PDGF-B+mesoporous silica nanoparticles	Accelerates AT healing.	Rat/NA	Suwalski et al. (2010)

(Continued on following page)

TABLE 3 (*Continued*) GFs combined with other GFs/SCs/Scaffolds in the treatment of AT injury.

	GFs	Combined application	Outcomes	Animal models/defect type	References
		PDGF+microneedle patch	Enhances tendon healing quality, angiogenesis, stiffness, maximum load, and stress.	Rat/Transect	Liu et al. (2022)
		PDGF-BB+ DegraPol tube	Decrease adhesion formation.	Rabbit/sliced perpendicularly	Burgisser et al. (2021)
	VEGF	VEGF+bFGF+nanostructured mineral coating	Improves vascularization, collagen fiber organization, mechanical properties, and functional healing.	Rabbit/Transect	Yu et al. (2017)
	PRP	Bone-tendon quadriceps tendon graft+PRP	Enhances healing capability.	Patient/Chronic tendinosis	Kirschner et al. (2021)
		PRP+decellularized bovine tendon sheets	Remodel and integrate into the AT and improves the healing process	Rabbit/Transect	((Zhang et al., 2018b)
		PRP+ASCs+ hydrogel	Increases total photon flux, Mean ultimate failure load, ECM, and cellularity.	Rat/0.5- mm in width and 5-mm in length defect	Chiou et al. (2015)
Summary	Up to date, the tissue engineering technology combined with GFs, stem cells and scaffolds has been widely applied *in vitro* and *in vivo* studies of AT repair, and showed a superior therapeutic effect, which may be a promising treatment method for AT repair. But further studies need to be carried out to determine the optimal scheme.				

TABLE 4 The clinical application of PRP for AT healing.

Growth factors	Application	Outcome	References
PRP			
	PRP	Not superior to placebo treatment in chronic AT diseases.	Liu et al. (2019); Boesen et al. (2020); Keene et al. (2022)
	PRP	Improves short and midterm functional outcomes in acute AT rupture.	Zou et al. (2016)
	PRP	Enhanced the maturity of the healing tendon tissues, promotes better ColI deposition, decreases cellularity, less vascularity, and higher glycosaminoglycan content.	Alsousou et al. (2015)
	PRP	Modest improvement in functional outcome measures, MRI appearance of diseased AT remained largely unchanged.	Owens et al. (2011)
	PRP	Not result in greater improvement in pain and activity.	de Vos et al. (2010)
	PRP	Injecting PRP for the treatment of chronic midportion Achilles tendinopathy does not contribute to an increased tendon structure or alter the degree of neovascularisation, compared with a placebo.	de Vos et al. (2011)
	PRP	PRP is not useful for the treatment of AT ruptures.	Schepull et al. (2011)
	PRP+endoscopyassisted percutaneous	did not yield superior functional and clinical outcomes.	Hung et al. (2022)
	PRP+Endoscopic debridement	The addition of PRP did not improve outcomes compared to debridement alone.	Thermann et al. (2020).
	FD-PFC+surgery	The patient could return to play at the pre-injury level at 3 months after surgery of rupture.	Morimoto et al. (2021)

of Col I and Col III, playing an essential role in tendon healing. Contrarily, TGF-β1 appears to be responsible for scar and adhesion formation. A clinical trial of TGF-β for indications outside of the tendon had to be halted due to excessive scar formation; therefore, TGF-β was not tested in AT healing in the clinic. PDGF-AA drives both tenogenesis and fibrosis. However, further studies regarding using PDGF-AA to accelerate AT injury recovery while reducing scars are warranted. VEGF plays a vital role in angiogenesis during AT healing, and vascular ingrowth is necessary for tendon healing. However, hypervascularization may be associated with degeneration. Therefore, the role of GFs in AT repair is a double-edged sword, and the question of how to better apply it remains unsolved. As previously indicated, there is still a long way from basic research to clinical application.

7 Conclusion

Repair and healing after AT injury are low due to the tendon's low cellularity, vascularity, and metabolic activity. In general, injured tendons rarely regain the structural integrity and mechanical strength of healthy tendons and are, therefore, prone to reinjury. GFs play a significant role in natural tendon regeneration as they participate in cell recruitment and stimulation of ECM synthesis. In recent years, GFs have been a popular treatment option for tendon injuries, which provided new perspectives on the healing of tendon injuries. The combination of multiple GFs with stem cells/scaffolds/other GFs also showed promising efficacy compared with individual GFs.

The GFs discussed in this review have important and varied roles in AT healing, affecting their functions and molecular changes. TGF-β2, IGF-I, and bFGF are involved in multiple signaling pathways and promote the synthesis of ECM components, cell proliferation, migration, and directed differentiation of MSCs. VEGF regulates angiogenesis within the wound site. PDGF shows different effectiveness in promoting tendon healing in varying stages of the injury. TGF-β2 and BMP-2 sometimes hinder AT injury recovery and even cause more severe complications. The effectiveness of PRP in AT repair remains controversial. There are also complex interactions between different cytokines, showing synergistic or antagonistic effects. Although clinical studies have confirmed the definite efficacy of some GFs, insignificant therapeutic effects have also been reported.

Several challenges must be overcome before GF delivery can translate into clinical practice, and further research is warranted (Prabhath et al., 2018). For instance, it is important to (1) further define the roles of each GF in AT healing and selecting the optimal GF(s) or a combination of GFs, (2) identify and clarify the synergistic and antagonistic influences they have on each other when combined, (3) confirm the most efficient stage and duration of delivery of each GF, (4) determine the effective dose of the selected GF and its retention time at the injured site, and (5) determine the most appropriate strategy for GF administration, including using gene therapy, recombinant GFs, or biomaterial scaffolds. If biomaterial scaffolds are used for GF sustained release, the most appropriate material and scaffold form must be selected.

Adhesion and fibrosis, which are critical problems affecting the degree of healing, are essential difficulties to overcome in repairing AT injury. GFs can simultaneously promote healing and cause fibrosis. The direction for further research should be how to effectively use GFs or combine GFs/stem cells/tissue engineering scaffolds in AT repair.

Author contributions

Conceptualization, WL and ML; writing-original draft preparation, ML, WL, XN, HL, and XC; writing-review and editing, YL, MJ, and CW; funding acquisition, ML; All authors contributed to the article and approved the submitted version.

Funding

This research was supported by the following funding: The young and middle-aged science and technology innovation talents support the program of Shenyang, No: RC210454. Basic Research Project of the Education Department of Liaoning Province, No. LJKZ0740.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2023.1250533/full#supplementary-material

References

Acharya, B., Miah, S., and Frett, B. (2022). Targeting TGF-β: triumphs and challenges. *Future Med. Chem.* 14, 455–458. doi:10.4155/fmc-2021-0344

Ahmed, A. S., Li, J., Schizas, N., Ahmed, M., Ostenson, C. G., Salo, P., et al. (2014). Expressional changes in growth and inflammatory mediators during achilles tendon repair in diabetic rats: new insights into a possible basis for compromised healing. *Cell Tissue Res.* 357, 109–117. doi:10.1007/s00441-014-1871-3

Ahmed, I. M., Lagopoulos, M., McConnell, P., Soames, R. W., and Sefton, G. K. (1998). Blood supply of the Achilles tendon. *J. Orthop. Res.* 16, 591–596. doi:10.1002/jor.1100160511

Alsousou, J., Thompson, M., Harrison, P., Willett, K., and Franklin, S. (2015). Effect of platelet-rich plasma on healing tissues in acute ruptured achilles tendon: A human immunohistochemistry study. *Lancet* 385 (1), S19. doi:10.1016/s0140-6736(15)60334-8

Andarawis-Puri, N., Flatow, E. L., and Soslowsky, L. J. (2015). Tendon basic science: development, repair, regeneration, and healing. *J. Orthop. Res.* 33, 780–784. doi:10.1002/jor.22869

Angrisani, N., Willbold, E., Kampmann, A., Derksen, A., and Reifenrath, J. (2022). Histology of tendon and enthesis - suitable techniques for specific research questions. *Eur. Cell Mater* 43, 228–251. doi:10.22203/ecm.v043a16

Arslan, E., Nellesen, T., Bayer, A., Prescher, A., Lippross, S., Nebelung, S., et al. (2016). Effect of platelet mediator concentrate (PMC) on achilles tenocytes: an *in vitro* study. *BMC Musculoskelet. Disord.* 17, 307. doi:10.1186/s12891-016-1160-2

Aschner, Y., and Downey, G. P. (2016). Transforming growth factor-β: master regulator of the respiratory system in health and disease. *Am. J. Respir. Cell Mol. Biol.* 54, 647–655. doi:10.1165/rcmb.2015-0391tr

Astill, B. D., Katsma, M. S., Cauthon, D. J., Greenlee, J., Murphy, M., Curtis, D., et al. (2017). Sex-based difference in Achilles peritendinous levels of matrix metalloproteinases and growth factors after acute resistance exercise. *J. Appl. Physiol. (1985)* 122, 361–367. doi:10.1152/japplphysiol.00878.2016

Barsby, T., Bavin, E. P., and Guest, D. J. (2014). Three-dimensional culture and transforming growth factor beta3 synergistically promote tenogenic differentiation of equine embryo-derived stem cells. *Tissue Eng. Part A* 20, 2604–2613. doi:10.1089/ten.tea.2013.0457

Bennell, K. L., Paterson, K. L., Metcalf, B. R., Duong, V., Eyles, J., Kasza, J., et al. (2021). Effect of intra-articular platelet-rich plasma vs placebo injection on pain and medial tibial cartilage volume in patients with knee osteoarthritis: the RESTORE randomized clinical trial. *JAMA* 326, 2021–2030. doi:10.1001/jama.2021.19415

Boesen, A. P., Boesen, M. I., Hansen, R., Barfod, K. W., Lenskjold, A., Malliaras, P., et al. (2020). Effect of platelet-rich plasma on nonsurgically treated acute achilles tendon ruptures: A randomized, double-blinded prospective study. *Am. J. Sports Med.* 48, 2268–2276. doi:10.1177/0363546520922541

Boesen, A. P., Hansen, R., Boesen, M. I., Malliaras, P., and Langberg, H. (2017). Effect of high-volume injection, platelet-rich plasma, and sham treatment in chronic midportion achilles tendinopathy: A randomized double-blinded prospective study. *Am. J. Sports Med.* 45, 2034–2043. doi:10.1177/0363546517702862

Bolt, P., Clerk, A. N., Luu, H. H., Kang, Q., Kummer, J. L., Deng, Z. L., et al. (2007). BMP-14 gene therapy increases tendon tensile strength in a rat model of Achilles tendon injury. *J. Bone Jt. Surg. Am.* 89, 1315–1320. doi:10.2106/00004623-200706000-00021

Bottagisio, M., Lopa, S., Granata, V., Talo, G., Bazzocchi, C., Moretti, M., et al. (2017). Different combinations of growth factors for the tenogenic differentiation of bone marrow mesenchymal stem cells in monolayer culture and in fibrin-based three-dimensional constructs. *Differentiation* 95, 44–53. doi:10.1016/j.diff.2017.03.001

Brown, J. P., Finley, V. G., and Kuo, C. K. (2014). Embryonic mechanical and soluble cues regulate tendon progenitor cell gene expression as a function of developmental stage and anatomical origin. *J. Biomech.* 47, 214–222. doi:10.1016/j.jbiomech.2013.09.018

Brown, J. P., Galassi, T. V., Stoppato, M., Schiele, N. R., and Kuo, C. K. (2015). Comparative analysis of mesenchymal stem cell and embryonic tendon progenitor cell response to embryonic tendon biochemical and mechanical factors. *Stem Cell Res. Ther.* 6, 89. doi:10.1186/s13287-015-0043-z

Burgisser, G. M., Evrova, O., Heuberger, D. M., Wolint, P., Rieber, J., Miescher, I., et al. (2021). Electrospun tube reduces adhesion in rabbit Achilles tendon 12 weeks post-surgery without PAR-2 overexpression. *Sci. Rep.* 11, 23293. doi:10.1038/s41598-021-02780-4

Campanelli, V., Fantini, M., Faccioli, N., Cangemi, A., Pozzo, A., and Sbarbati, A. (2011). Three-dimensional morphology of heel fat pad: an *in vivo* computed tomography study. *J. Anat.* 219, 622–631. doi:10.1111/j.1469-7580.2011.01420.x

Celik, M., Bayrak, A., Duramaz, A., Basaran, S. H., Kizilkaya, C., Kural, C., et al. (2021). The effect of fibrin clot and C vitamin on the surgical treatment of Achilles tendon injury in the rat model. *Foot Ankle Surg.* 27, 681–687. doi:10.1016/j.fas.2020.09.006

Cetik, R. M., Yabanoglu Ciftci, S., Arica, B., Baysal, I., Akarca Dizakar, S. O., Erbay Elibol, F. K., et al. (2022). Evaluation of the effects of transforming growth factor–beta 3 (TGF-β3) loaded nanoparticles on healing in a rat achilles tendon injury model. *Am. J. Sports Med.* 50, 1066–1077. doi:10.1177/03635465211073148

Chamberlain, C. S., Lee, J. S., Leiferman, E. M., Maassen, N. X., Baer, G. S., Vanderby, R., et al. (2015). Effects of BMP-12-releasing sutures on Achilles tendon healing. *Tissue Eng. Part A* 21, 916–927. doi:10.1089/ten.tea.2014.0001

Chen, C. H., Lin, Y. H., Chen, C. H., Wang, Y. H., Yeh, M. L., Cheng, T. L., et al. (2018a). Transforming growth factor beta 1 mediates the low-frequency vertical vibration enhanced production of tenomodulin and type I collagen in rat Achilles tendon. *PLoS One* 13, e0205258. doi:10.1371/journal.pone.0205258

Chen, H., Wang, Z., Zhou, L., Wu, B., Lu, H., Zhang, C., et al. (2021). Recombinant human bone morphogenetic protein-4 enhances tendon-to-bone attachment healing in a murine model of rotator cuff tear. *Ann. Transl. Med.* 9, 565. doi:10.21037/atm-20-6761

Chen, J., Yu, Q., Wu, B., Lin, Z., Pavlos, N. J., Xu, J., et al. (2011). Autologous tenocyte therapy for experimental Achilles tendinopathy in a rabbit model. *Tissue Eng. Part A* 17, 2037–2048. doi:10.1089/ten.tea.2010.0492

Chen, L., Dong, S. W., Liu, J. P., Tao, X., Tang, K. L., and Xu, J. Z. (2012). Synergy of tendon stem cells and platelet-rich plasma in tendon healing. *J. Orthop. Res.* 30, 991–997. doi:10.1002/jor.22033

Chen, Q. J., Chen, L., Wu, S. K., Wu, Y. J., and Pang, Q. J. (2018b). rhPDGF-BB combined with ADSCs in the treatment of Achilles tendinitis via miR-363/PI3 K/Akt pathway. *Mol. Cell Biochem.* 438, 175–182. doi:10.1007/s11010-017-3124-8

Chien, C., Pryce, B., Tufa, S. F., Keene, D. R., and Huang, A. H. (2018). Optimizing a 3D model system for molecular manipulation of tenogenesis. *Connect. Tissue Res.* 59, 295–308. doi:10.1080/03008207.2017.1383403

Chiou, G. J., Crowe, C., McGoldrick, R., Hui, K., Pham, H., and Chang, J. (2015). Optimization of an injectable tendon hydrogel: the effects of platelet-rich plasma and adipose-derived stem cells on tendon healing *in vivo*. *Tissue Eng. Part A* 21, 1579–1586. doi:10.1089/ten.tea.2014.0490

Cui, J., Chen, Z., and Wu, W. (2019). Expression of TGF-β1 and VEGF in patients with Achilles tendon rupture and the clinical efficacy. *Exp. Ther. Med.* 18, 3502–3508. doi:10.3892/etm.2019.7968

Cui, J., Ning, L. J., Wu, F. P., Hu, R. N., Li, X., He, S. K., et al. (2022). Biomechanically and biochemically functional scaffold for recruitment of endogenous stem cells to promote tendon regeneration. *NPJ Regen. Med.* 7, 26. doi:10.1038/s41536-022-00220-z

D'Addona, A., Maffulli, N., Formisano, S., and Rosa, D. (2017). Inflammation in tendinopathy. *Surgeon* 15, 297–302. doi:10.1016/j.surge.2017.04.004

Dai, G., Li, Y., Liu, J., Zhang, C., Chen, M., Lu, P., et al. (2020). Higher BMP expression in tendon stem/progenitor cells contributes to the increased heterotopic ossification in achilles tendon with aging. *Front. Cell Dev. Biol.* 8, 570605. doi:10.3389/fcell.2020.570605

Dai, L., Hu, X., Zhang, X., Zhu, J., Zhang, J., Fu, X., et al. (2015). Different tenogenic differentiation capacities of different mesenchymal stem cells in the presence of BMP-12. *J. Transl. Med.* 13, 200. doi:10.1186/s12967-015-0560-7

Darshan, G. D., Chen, C. H., Kuo, C. Y., Shalumon, K. T., Chien, Y. M., Kao, H. H., et al. (2022). Development of high resilience spiral wound suture-embedded gelatin/PCL/heparin nanofiber membrane scaffolds for tendon tissue engineering. *Int. J. Biol. Macromol.* 221, 314–333. doi:10.1016/j.ijbiomac.2022.09.001

Dayton, P. (2017). Anatomic, vascular, and mechanical overview of the achilles tendon. *Clin. Podiatr. Med. Surg.* 34, 107–113. doi:10.1016/j.cpm.2016.10.002

de Vos, R. J., Weir, A., Tol, J. L., Verhaar, J. A., Weinans, H., and van Schie, H. T. (2011). No effects of PRP on ultrasonographic tendon structure and neovascularisation in chronic midportion Achilles tendinopathy. *Br. J. Sports Med.* 45, 387–392. doi:10.1136/bjsm.2010.076398

de Vos, R. J., Weir, A., van Schie, H. T., Bierma-Zeinstra, S. M., Verhaar, J. A., Weinans, H., et al. (2010). Platelet-rich plasma injection for chronic achilles tendinopathy: A randomized controlled trial. *JAMA* 303, 144–149. doi:10.1001/jama.2009.1986

Deng, L., Huang, L., Guo, Q., Shi, X., and Xu, K. (2017). CREB1 and Smad3 mediate TGF-β3-induced Smad7 expression in rat hepatic stellate cells. *Mol. Med. Rep.* 16, 8455–8462. doi:10.3892/mmr.2017.7654

Disser, N. P., Sugg, K. B., Talarek, J. R., Sarver, D. C., Rourke, B. J., and Mendias, C. L. (2019). Insulin-like growth factor 1 signaling in tenocytes is required for adult tendon growth. *FASEB J.* 33, 12680–12695. doi:10.1096/fj.201901503r

Dohan Ehrenfest, D. M., Andia, I., Zumstein, M. A., Zhang, C. Q., Pinto, N. R., and Bielecki, T. (2014). Classification of platelet concentrates (Platelet-Rich plasma-PRP, platelet-rich fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. *Muscles Ligaments Tendons J.* 4, 3–9.

Doral, M. N., Alam, M., Bozkurt, M., Turhan, E., Atay, O. A., Donmez, G., et al. (2010). Functional anatomy of the Achilles tendon. *Knee Surg. Sports Traumatol. Arthrosc.* 18, 638–643. doi:10.1007/s00167-010-1083-7

Dunworth, W. P., Cardona-Costa, J., Bozkulak, E. C., Kim, J. D., Meadows, S., Fischer, J. C., et al. (2014). Bone morphogenetic protein 2 signaling negatively modulates lymphatic development in vertebrate embryos. *Circ. Res.* 114, 56–66. doi:10.1161/circresaha.114.302452

Eliasson, P., Fahlgren, A., and Aspenberg, P. (2008). Mechanical load and BMP signaling during tendon repair: A role for follistatin? *Clin. Orthop. Relat. Res.* 466, 1592–1597. doi:10.1007/s11999-008-0253-0

Evrova, O., Burgisser, G. M., Ebnother, C., Adathala, A., Calcagni, M., Bachmann, E., et al. (2020). Elastic and surgeon friendly electrospun tubes delivering PDGF-BB positively impact tendon rupture healing in a rabbit Achilles tendon model. *Biomaterials* 232, 119722. doi:10.1016/j.biomaterials.2019.119722

Evrova, O., and Buschmann, J. (2017). *In vitro* and *in vivo* effects of PDGF-BB delivery strategies on tendon healing: A review. *Eur. Cell Mater* 34, 15–39. doi:10.22203/ecm.v034a02

Fenwick, S. A., Curry, V., Harrall, R. L., Hazleman, B. L., Hackney, R., and Riley, G. P. (2001). Expression of transforming growth factor-beta isoforms and their receptors in chronic tendinosis. *J. Anat.* 199, 231–240. doi:10.1046/j.1469-7580.2001.19930231.x

Fitzgerald, M. J., Mustapich, T., Liang, H., Larsen, C. G., Nellans, K. W., and Grande, D. A. (2021). Tendon transection healing can Be improved with adipose-derived stem cells cultured with growth differentiation factor 5 and platelet-derived growth factor. *Hand (N Y)* 18, 436–445. doi:10.1177/15589447211028929

Font Tellado, S., Chiera, S., Bonani, W., Poh, P. S. P., Migliaresi, C., Motta, A., et al. (2018). Heparin functionalization increases retention of TGF-β2 and GDF5 on biphasic silk fibroin scaffolds for tendon/ligament-to-bone tissue engineering. *Acta Biomater.* 72, 150–166. doi:10.1016/j.actbio.2018.03.017

Fukuta, S., Oyama, M., Kavalkovich, K., Fu, F. H., and Niyibizi, C. (1998). Identification of types II, IX and X collagens at the insertion site of the bovine achilles tendon. *Matrix Biol.* 17, 65–73. doi:10.1016/s0945-053x(98)90125-1

Ganestam, A., Kallemose, T., Troelsen, A., and Barfod, K. W. (2016). Increasing incidence of acute Achilles tendon rupture and a noticeable decline in surgical treatment from 1994 to 2013. A nationwide registry study of 33,160 patients. *Knee Surg. Sports Traumatol. Arthrosc.* 24, 3730–3737. doi:10.1007/s00167-015-3544-5

Glass, Z. A., Schiele, N. R., and Kuo, C. K. (2014). Informing tendon tissue engineering with embryonic development. *J. Biomech.* 47, 1964–1968. doi:10.1016/j.jbiomech.2013.12.039

Gong, F., Li, X., Zhang, H., Wu, J., Ma, G., Zhang, B., et al. (2022). Comparison of the effects of open surgery and minimally invasive surgery on the achilles tendon rupture healing based on angiogenesis. *Comput. Intell. Neurosci.* 2022, 1–8. doi:10.1155/2022/1447129

Guerit, E., Arts, F., Dachy, G., Boulouadnine, B., and Demoulin, J. B. (2021). PDGF receptor mutations in human diseases. *Cell Mol. Life Sci.* 78, 3867–3881. doi:10.1007/s00018-020-03753-y

Guerquin, M. J., Charvet, B., Nourissat, G., Havis, E., Ronsin, O., Bonnin, M. A., et al. (2013). Transcription factor EGR1 directs tendon differentiation and promotes tendon repair. *J. Clin. Invest.* 123, 3564–3576. doi:10.1172/jci67521

Guo, D., Li, H., Liu, Y., Yu, X., Zhang, X., Chu, W., et al. (2020). Fibroblast growth factor-2 promotes the function of tendon-derived stem cells in Achilles tendon restoration in an Achilles tendon injury rat model. *Biochem. Biophys. Res. Commun.* 521, 91–97. doi:10.1016/j.bbrc.2019.10.082

Harvey, T., Flamenco, S., and Fan, C. M. (2019). A Tppp3(+)Pdgfra(+) tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis. *Nat. Cell Biol.* 21, 1490–1503. doi:10.1038/s41556-019-0417-z

Hoffmann, A., Pelled, G., Turgeman, G., Eberle, P., Zilberman, Y., Shinar, H., et al. (2006). Neotendon formation induced by manipulation of the Smad8 signalling pathway in mesenchymal stem cells. *J. Clin. Invest.* 116, 940–952. doi:10.1172/jci22689

Holladay, C., Abbah, S. A., O'Dowd, C., Pandit, A., and Zeugolis, D. I. (2016). Preferential tendon stem cell response to growth factor supplementation. *J. Tissue Eng. Regen. Med.* 10, 783–798. doi:10.1002/term.1852

Hortensius, R. A., and Harley, B. A. (2013). The use of bioinspired alterations in the glycosaminoglycan content of collagen-GAG scaffolds to regulate cell activity. *Biomaterials* 34, 7645–7652. doi:10.1016/j.biomaterials.2013.06.056

Hou, Y., Mao, Z., Wei, X., Lin, L., Chen, L., Wang, H., et al. (2009a). Effects of transforming growth factor-β1 and vascular endothelial growth factor 165 gene transfer on Achilles tendon healing. *Matrix Biol.* 28, 324–335. doi:10.1016/j.matbio.2009.04.007

Hou, Y., Mao, Z., Wei, X., Lin, L., Chen, L., Wang, H., et al. (2009b). The roles of TGF-β1 gene transfer on collagen formation during Achilles tendon healing. *Biochem. Biophys. Res. Commun.* 383, 235–239. doi:10.1016/j.bbrc.2009.03.159

Hung, C. Y., Lin, S. J., Yeh, C. Y., and Yeh, W. L. (2022). Effect of platelet-rich plasma augmentation on endoscopy-assisted percutaneous achilles tendon repair. *J. Clin. Med.* 11, 5389. doi:10.3390/jcm11185389

Hyun, S. Y., Lee, J. H., Kang, K. J., and Jang, Y. J. (2017). Effect of FGF-2, TGF-beta-1, and BMPs on teno/ligamentogenesis and osteo/cementogenesis of human periodontal ligament stem cells. *Mol. Cells* 40, 550–557. doi:10.14348/molcells.2017.0019

Im, G. I., and Kim, T. K. (2020). Stem cells for the regeneration of tendon and ligament: A perspective. *Int. J. Stem Cells* 13, 335–341. doi:10.15283/ijsc20091

Jayasree, A., Kottappally Thankappan, S., Ramachandran, R., Sundaram, M. N., Chen, C. H., Mony, U., et al. (2019). Bioengineered braided micro-nano (multiscale) fibrous scaffolds for tendon reconstruction. *ACS Biomater. Sci. Eng.* 5, 1476–1486. doi:10.1021/acsbiomaterials.8b01328

Jiang, D., Gao, P., Zhang, Y., and Yang, S. (2016a). Combined effects of engineered tendon matrix and GDF-6 on bone marrow mesenchymal stem cell-based tendon regeneration. *Biotechnol. Lett.* 38, 885–892. doi:10.1007/s10529-016-2037-z

Jiang, D., Xu, B., Yang, M., Zhao, Z., Zhang, Y., and Li, Z. (2014). Efficacy of tendon stem cells in fibroblast-derived matrix for tendon tissue engineering. *Cytotherapy* 16, 662–673. doi:10.1016/j.jcyt.2013.07.014

Jiang, K., Chun, G., Wang, Z., Du, Q., Wang, A., and Xiong, Y. (2016b). Effect of transforming growth factor-β3 on the expression of Smad3 and Smad7 in tenocytes. *Mol. Med. Rep.* 13, 3567–3573. doi:10.3892/mmr.2016.4944

Kang, S., Yoon, J. S., Lee, J. Y., Kim, H. J., Park, K., and Kim, S. E. (2019). Long-term local PDGF delivery using porous microspheres modified with heparin for tendon healing of rotator cuff tendinitis in a rabbit model. *Carbohydr. Polym.* 209, 372–381. doi:10.1016/j.carbpol.2019.01.017

Kearney, R. S., Ji, C., Warwick, J., Parsons, N., Brown, J., Harrison, P., et al. (2021). Effect of platelet-rich plasma injection vs sham injection on tendon dysfunction in patients with chronic midportion achilles tendinopathy: A randomized clinical trial. *JAMA* 326, 137–144. doi:10.1001/jama.2021.6986

Keene, D. J., Alsousou, J., Harrison, P., O'Connor, H. M., Wagland, S., Dutton, S. J., et al. (2022). Platelet-rich plasma injection for acute achilles tendon rupture: two-year follow-up of the PATH-2 randomized, placebo-controlled, superiority trial. *Bone Jt. J.* 104-B, 1256–1265. doi:10.1302/0301-620x.104b11.bjj-2022-0653.r1

Ker, E. D., Nain, A. S., Weiss, L. E., Wang, J., Suhan, J., Amon, C. H., et al. (2011). Bioprinting of growth factors onto aligned sub-micron fibrous scaffolds for simultaneous control of cell differentiation and alignment. *Biomaterials* 32, 8097–8107. doi:10.1016/j.biomaterials.2011.07.025

Kim, H. J., Kang, S. W., Lim, H. C., Han, S. B., Lee, J. S., Prasad, L., et al. (2007). The role of transforming growth factor-beta and bone morphogenetic protein with fibrin glue in healing of bone-tendon junction injury. *Connect. Tissue Res.* 48, 309–315. doi:10.1080/03008200701692610

Kim, H. J., Nam, H. W., Hur, C. Y., Park, M., Yang, H. S., Kim, B. S., et al. (2011). The effect of platelet rich plasma from bone marrow aspirate with added bone morphogenetic protein-2 on the Achilles tendon-bone junction in rabbits. *Clin. Orthop. Surg.* 3, 325–331. doi:10.4055/cios.2011.3.4.325

Kirschner, J. S., Cheng, J., Hurwitz, N., Santiago, K., Lin, E., Beatty, N., et al. (2021). Ultrasound-guided percutaneous needle tenotomy (pnt) alone versus pnt plus platelet-rich plasma injection for the treatment of chronic tendinosis: A randomized controlled trial. *PM R.* 13, 1340–1349. doi:10.1002/pmrj.12583

Koch, D. W., Schnabel, L. V., Ellis, I. M., Bates, R. E., and Berglund, A. K. (2022). TGF-β2 enhances expression of equine bone marrow-derived mesenchymal stem cell paracrine factors with known associations to tendon healing. *Stem Cell Res. Ther.* 13, 477. doi:10.1186/s13287-022-03172-9

Kokubu, S., Inaki, R., Hoshi, K., and Hikita, A. (2020). Adipose-derived stem cells improve tendon repair and prevent ectopic ossification in tendinopathy by inhibiting inflammation and inducing neovascularization in the early stage of tendon healing. *Regen. Ther.* 14, 103–110. doi:10.1016/j.reth.2019.12.003

Kraus, A., Sattler, D., Wehland, M., Luetzenberg, R., Abuagela, N., and Infanger, M. (2018). Vascular endothelial growth factor enhances proliferation of human tenocytes and promotes tenogenic gene expression. *Plast. Reconstr. Surg.* 142, 1240–1247. doi:10.1097/prs.0000000000004920

Kraus, T. M., Imhoff, F. B., Reinert, J., Wexel, G., Wolf, A., Hirsch, D., et al. (2016). Stem cells and bFGF in tendon healing: effects of lentiviral gene transfer and long-term follow-up in a rat achilles tendon defect model. *BMC Musculoskelet. Disord.* 17, 148. doi:10.1186/s12891-016-0999-6

Kraus, T. M., Imhoff, F. B., Wexel, G., Wolf, A., Hirsch, D., Lenz, L., et al. (2014). Stem cells and basic fibroblast growth factor failed to improve tendon healing: an *in vivo* study using lentiviral gene transfer in a rat model. *J. Bone Jt. Surg. Am.* 96, 761–769. doi:10.2106/jbjs.l.01794

Kuo, C. K., Petersen, B. C., and Tuan, R. S. (2008). Spatiotemporal protein distribution of TGF-βs, their receptors, and extracellular matrix molecules during embryonic tendon development. *Dev. Dyn.* 237, 1477–1489. doi:10.1002/dvdy.21547

Kurtz, C. A., Loebig, T. G., Anderson, D. D., DeMeo, P. J., and Campbell, P. G. (1999). Insulin-like growth factor I accelerates functional recovery from Achilles tendon injury in a rat model. *Am. J. Sports Med.* 27, 363–369. doi:10.1177/03635465990270031701

Lee, J. Y., Zhou, Z., Taub, P. J., Ramcharan, M., Li, Y., Akinbiyi, T., et al. (2011). BMP-12 treatment of adult mesenchymal stem cells *in vitro* augments tendon-like tissue formation and defect repair *in vivo*. *PLoS One* 6, e17531. doi:10.1371/journal.pone.0017531

Legrand, J. M. D., and Martino, M. M. (2022). Growth factor and cytokine delivery systems for wound healing. *Cold Spring Harb. Perspect. Biol.* 14, a041234. doi:10.1101/cshperspect.a041234

Li, M., Jia, J., Li, S., Cui, B., Huang, J., Guo, Z., et al. (2021a). Exosomes derived from tendon stem cells promote cell proliferation and migration through the TGF beta signal pathway. *Biochem. Biophys. Res. Commun.* 536, 88–94. doi:10.1016/j.bbrc.2020.12.057

Li, P., Zhou, H., Tu, T., and Lu, H. (2021b). Dynamic exacerbation in inflammation and oxidative stress during the formation of peritendinous adhesion resulted from acute tendon injury. *J. Orthop. Surg. Res.* 16, 293. doi:10.1186/s13018-021-02445-y

Lin, L., Shen, Q., Xue, T., and Yu, C. (2010). Heterotopic ossification induced by achilles tenotomy via endochondral bone formation: expression of bone and cartilage related genes. *Bone* 46, 425–431. doi:10.1016/j.bone.2009.08.057

Liu, C., Tian, S., Bai, J., Yu, K., Liu, L., Liu, G., et al. (2020). <p>Regulation of ERK1/2 and SMAD2/3 pathways by using multi-layered electrospun PCL–amnion nanofibrous membranes for the prevention of post-surgical tendon adhesion</p>. *Int. J. Nanomedicine* 15, 927–942. doi:10.2147/ijn.s231538

Liu, C. J., Yu, K. L., Bai, J. B., Tian, D. H., and Liu, G. L. (2019). Platelet-rich plasma injection for the treatment of chronic achilles tendinopathy: A meta-analysis. *Med. Baltim.* 98, e15278. doi:10.1097/md.0000000000015278

Liu, H., Zhang, C., Zhu, S., Lu, P., Zhu, T., Gong, X., et al. (2015a). Mohawk promotes the tenogenesis of mesenchymal stem cells through activation of the TGFβ signaling pathway. *Stem Cells* 33, 443–455. doi:10.1002/stem.1866

Liu, J., Tao, X., Chen, L., Han, W., Zhou, Y., and Tang, K. (2015b). CTGF positively regulates BMP12 induced tenogenic differentiation of tendon stem cells and signaling. *Cell Physiol. Biochem.* 35, 1831–1845. doi:10.1159/000373994

Liu, X., Li, Y., Wang, S., Lu, M., Zou, J., Shi, Z., et al. (2022). PDGF-loaded microneedles promote tendon healing through p38/cyclin D1 pathway mediated angiogenesis. *Mater Today Bio* 16, 100428. doi:10.1016/j.mtbio.2022.100428

Lohrer, H., Arentz, S., Nauck, T., Dorn-Lange, N. V., and Konerding, M. A. (2008). The achilles tendon insertion is crescent-shaped: an *in vitro* anatomic investigation. *Clin. Orthop. Relat. Res.* 466, 2230–2237. doi:10.1007/s11999-008-0298-0

Lowery, J. W., and Rosen, V. (2018). The BMP pathway and its inhibitors in the skeleton. *Physiol. Rev.* 98, 2431–2452. doi:10.1152/physrev.00028.2017

Maffulli, N., Longo, U. G., Franceschi, F., Rabitti, C., and Denaro, V. (2008). Movin and Bonar scores assess the same characteristics of tendon histology. *Clin. Orthop. Relat. Res.* 466, 1605–1611. doi:10.1007/s11999-008-0261-0

Majewski, M., Betz, O., Ochsner, P. E., Liu, F., Porter, R. M., and Evans, C. H. (2008). *Ex vivo* adenoviral transfer of bone morphogenetic protein 12 (BMP-12) cDNA improves Achilles tendon healing in a rat model. *Gene Ther.* 15, 1139–1146. doi:10.1038/gt.2008.48

Majewski, M., Heisterbach, P., Jaquiery, C., Durselen, L., Todorov, A., Martin, I., et al. (2018). Improved tendon healing using bFGF, BMP-12 and TGFβ1 in a rat model. *Eur. Cell Mater* 35, 318–334. doi:10.22203/ecm.v035a22

Majewski, M., Porter, R. M., Betz, O. B., Betz, V. M., Clahsen, H., Fluckiger, R., et al. (2012). Improvement of tendon repair using muscle grafts transduced with TGF-β1 cDNA. *Eur. Cell Mater* 23, 94–102. doi:10.22203/ecm.v023a07

Martin, R. L., Chimenti, R., Cuddeford, T., Houck, J., Matheson, J. W., McDonough, C. M., et al. (2018). Achilles pain, stiffness, and muscle power deficits: midportion achilles tendinopathy revision 2018. *J. Orthop. Sports Phys. Ther.* 48, A1–A38. doi:10.2519/jospt.2018.0302

McCartney, W., Ober, C., Benito, M., and MacDonald, B. (2019). Suturing achilles tendon and mesh simultaneously in augmented repair resists gap formation foremost: an experimental study. *J. Orthop. Surg. Res.* 14, 332. doi:10.1186/s13018-019-1390-8

Meier Burgisser, G., Evrova, O., Calcagni, M., Scalera, C., Giovanoli, P., and Buschmann, J. (2020). Impact of PDGF-BB on cellular distribution and extracellular matrix in the healing rabbit Achilles tendon three weeks post-operation. *FEBS Open Bio* 10, 327–337. doi:10.1002/2211-5463.12736

Melincovici, C. S., Bosca, A. B., Susman, S., Marginean, M., Mihu, C., Istrate, M., et al. (2018). Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Rom. J. Morphol. Embryol.* 59, 455–467.

Mikic, B., Rossmeier, K., and Bierwert, L. (2009). Sexual dimorphism in the effect of GDF-6 deficiency on murine tendon. *J. Orthop. Res.* 27, 1603–1611. doi:10.1002/jor.20916

Millar, N. L., Murrell, G. A., and McInnes, I. B. (2017). Inflammatory mechanisms in tendinopathy - towards translation. *Nat. Rev. Rheumatol.* 13, 110–122. doi:10.1038/nrrheum.2016.213

Millar, N. L., Silbernagel, K. G., Thorborg, K., Kirwan, P. D., Galatz, L. M., Abrams, G. D., et al. (2021). Tendinopathy. *Nat. Rev. Dis. Prim.* 7, 1. doi:10.1038/s41572-020-00234-1

Morimoto, S., Iseki, T., Nakayama, H., Shimomura, K., Nishikawa, T., Nakamura, N., et al. (2021). Return to the original sport at only 3 months after an achilles tendon rupture by a combination of intra-tissue injection of freeze-dried platelet-derived factor concentrate and excessively early rehabilitation after operative treatment in a male basketball player: A case report. *Regen. Ther.* 18, 112–116. doi:10.1016/j.reth.2021.05.002

Muller, S. A., Quirk, N. P., Muller-Lebschi, J. A., Heisterbach, P. E., Durselen, L., Majewski, M., et al. (2019). Response of the injured tendon to growth factors in the presence or absence of the paratenon. *Am. J. Sports Med.* 47, 462–467. doi:10.1177/0363546518814534

Muller, S. A., Todorov, A., Heisterbach, P. E., Martin, I., and Majewski, M. (2015). Tendon healing: an overview of physiology, biology, and pathology of tendon healing and systematic review of state of the art in tendon bioengineering. *Knee Surg. Sports Traumatol. Arthrosc.* 23, 2097–2105. doi:10.1007/s00167-013-2680-z

Nagelli, C. V., Hooke, A., Quirk, N., De Padilla, C. L., Hewett, T. E., van Griensven, M., et al. (2022). Mechanical and strain behaviour of human Achilles tendon during *in vitro* testing to failure. *Eur. Cell Mater* 43, 153–161. doi:10.22203/ecm.v043a12

Nielsen, R. H., Holm, L., Jensen, J. K., Heinemeier, K. M., Remvig, L., and Kjaer, M. (2014). Tendon protein synthesis rate in classic Ehlers-Danlos patients can be stimulated with insulin-like growth factor-I. *J. Appl. Physiol. (1985)* 117, 694–698. doi:10.1152/japplphysiol.00157.2014

Noack, S., Seiffart, V., Willbold, E., Laggies, S., Winkel, A., Shahab-Osterloh, S., et al. (2014). Periostin secreted by mesenchymal stem cells supports tendon formation in an ectopic mouse model. *Stem Cells Dev.* 23, 1844–1857. doi:10.1089/scd.2014.0124

O'Brien, M. (2005). The anatomy of the Achilles tendon. *Foot Ankle Clin.* 10, 225–238. doi:10.1016/j.fcl.2005.01.011

Owens, R. F., Ginnetti, J., Conti, S. F., and Latona, C. (2011). Clinical and magnetic resonance imaging outcomes following platelet rich plasma injection for chronic midsubstance Achilles tendinopathy. *Foot Ankle Int.* 32, 1032–1039. doi:10.3113/fai.2011.1032

Ozasa, Y., Gingery, A., Thoreson, A. R., An, K. N., Zhao, C., and Amadio, P. C. (2014). A comparative study of the effects of growth and differentiation factor 5 on muscle-derived stem cells and bone marrow stromal cells in an *in vitro* tendon healing model. *J. Hand Surg. Am.* 39, 1706–1713. doi:10.1016/j.jhsa.2014.05.005

Ozeki, N., Muneta, T., Koga, H., Katagiri, H., Otabe, K., Okuno, M., et al. (2013). Transplantation of Achilles tendon treated with bone morphogenetic protein 7 promotes meniscus regeneration in a rat model of massive meniscal defect. *Arthritis Rheum.* 65, 2876–2886. doi:10.1002/art.38099

Padilla, S., Sanchez, M., Vaquerizo, V., Malanga, G. A., Fiz, N., Azofra, J., et al. (2021). Platelet-rich plasma applications for achilles tendon repair: A bridge between biology and surgery. *Int. J. Mol. Sci.* 22, 824. doi:10.3390/ijms22020824

Pakshir, P., and Hinz, B. (2018). The big five in fibrosis: macrophages, myofibroblasts, matrix, mechanics, and miscommunication. *Matrix Biol.* 68-69, 81–93. doi:10.1016/j.matbio.2018.01.019

Paolini, C., Agarbati, S., Benfaremo, D., Mozzicafreddo, M., Svegliati, S., and Moroncini, G. (2022). PDGF/PDGFR: A possible molecular target in scleroderma fibrosis. *Int. J. Mol. Sci.* 23, 3904. doi:10.3390/ijms23073904

Park, A., Hogan, M. V., Kesturu, G. S., James, R., Balian, G., and Chhabra, A. B. (2010). Adipose-derived mesenchymal stem cells treated with growth differentiation factor-5 express tendon-specific markers. *Tissue Eng. Part A* 16, 2941–2951. doi:10.1089/ten.tea.2009.0710

Park, Y. B., Kim, J. H., Ha, C. W., and Lee, D. H. (2021). Clinical efficacy of platelet-rich plasma injection and its association with growth factors in the treatment of mild to moderate knee osteoarthritis: A randomized double-blind controlled clinical trial as compared with hyaluronic acid. *Am. J. Sports Med.* 49, 487–496. doi:10.1177/0363546520986867

Pekala, P. A., Henry, B. M., Ochala, A., Kopacz, P., Taton, G., Mlyniec, A., et al. (2017). The twisted structure of the achilles tendon unraveled: A detailed quantitative and qualitative anatomical investigation. *Scand. J. Med. Sci. Sports* 27, 1705–1715. doi:10.1111/sms.12835

Pelled, G., Snedeker, J. G., Ben-Arav, A., Rigozzi, S., Zilberman, Y., Kimelman-Bleich, N., et al. (2012). Smad8/BMP2-engineered mesenchymal stem cells induce accelerated recovery of the biomechanical properties of the Achilles tendon. *J. Orthop. Res.* 30, 1932–1939. doi:10.1002/jor.22167

Perucca Orfei, C., Vigano, M., Pearson, J. R., Colombini, A., De Luca, P., Ragni, E., et al. (2019). *In vitro* induction of tendon-specific markers in tendon cells, adipose- and bone marrow-derived stem cells is dependent on TGFβ3, BMP-12 and ascorbic acid stimulation. *Int. J. Mol. Sci.* 20, 149. doi:10.3390/ijms20010149

Peserico, A., Barboni, B., Russo, V., Bernabo, N., El Khatib, M., Prencipe, G., et al. (2023). Mammal comparative tendon biology: advances in regulatory mechanisms through a computational modeling. *Front. Vet. Sci.* 10, 1175346. doi:10.3389/fvets.2023.1175346

Petersen, W., Pufe, T., Kurz, B., Mentlein, R., and Tillmann, B. (2002). Angiogenesis in fetal tendon development: spatial and temporal expression of the angiogenic peptide vascular endothelial cell growth factor. *Anat. Embryol. Berl.* 205, 263–270. doi:10.1007/s00429-002-0241-1

Pillai, D. S., Dhinsa, B. S., and Khan, W. S. (2017). Tissue engineering in achilles tendon reconstruction; the role of stem cells, growth factors and scaffolds. *Curr. Stem Cell Res. Ther.* 12, 506–512. doi:10.2174/1574888x12666170523162214

Prabhath, A., Vernekar, V. N., Sanchez, E., and Laurencin, C. T. (2018). Growth factor delivery strategies for rotator cuff repair and regeneration. *Int. J. Pharm.* 544, 358–371. doi:10.1016/j.ijpharm.2018.01.006

Rickert, M. (2008). BMP-14 gene therapy increases tendon tensile strength in a rat model of achilles tendon injury. *J. Bone Jt. Surg. Am.* 90, 445–446.

Rickert, M., Jung, M., Adiyaman, M., Richter, W., and Simank, H. G. (2001). A growth and differentiation factor-5 (GDF-5)-coated suture stimulates tendon healing in an Achilles tendon model in rats. *Growth factors.* 19, 115–126. doi:10.3109/08977190109001080

Riggin, C. N., Schultz, S. M., Sehgal, C. M., and Soslowsky, L. J. (2019). Ultrasound evaluation of anti-vascular endothelial growth factor-induced changes in vascular response following tendon injury. *Ultrasound Med. Biol.* 45, 1841–1849. doi:10.1016/j.ultrasmedbio.2019.03.002

Ruan, D., Fei, Y., Qian, S., Huang, Z., Chen, W., Tang, C., et al. (2021). Early-stage primary anti-inflammatory therapy enhances the regenerative efficacy of platelet-rich plasma in a rabbit achilles tendinopathy model. *Am. J. Sports Med.* 49, 3357–3371. doi:10.1177/03635465211037354

Ruiz-Alonso, S., Lafuente-Merchan, M., Ciriza, J., Saenz-Del-Burgo, L., and Pedraz, J. L. (2021). Tendon tissue engineering: cells, growth factors, scaffolds and production techniques. *J. Control Release* 333, 448–486. doi:10.1016/j.jconrel.2021.03.040

Russo, V., El Khatib, M., Prencipe, G., Citeroni, M. R., Faydaver, M., Mauro, A., et al. (2022). Tendon immune regeneration: insights on the synergetic role of stem and immune cells during tendon regeneration. *Cells* 11, 434. doi:10.3390/cells11030434

Schepull, T., Kvist, J., Norrman, H., Trinks, M., Berlin, G., and Aspenberg, P. (2011). Autologous platelets have no effect on the healing of human achilles tendon ruptures: A randomized single-blind study. *Am. J. Sports Med.* 39, 38–47. doi:10.1177/0363546510383515

Schneider, P. R., Buhrmann, C., Mobasheri, A., Matis, U., and Shakibaei, M. (2011). Three-dimensional high-density co-culture with primary tenocytes induces tenogenic differentiation in mesenchymal stem cells. *J. Orthop. Res.* 29, 1351–1360. doi:10.1002/jor.21400

Schroer, A. K., and Merryman, W. D. (2015). Mechanobiology of myofibroblast adhesion in fibrotic cardiac disease. *J. Cell Sci.* 128, 1865–1875. doi:10.1242/jcs.162891

Seeger, J. D., West, W. A., Fife, D., Noel, G. J., Johnson, L. N., and Walker, A. M. (2006). Achilles tendon rupture and its association with fluoroquinolone antibiotics and other potential risk factors in a managed care population. *Pharmacoepidemiol Drug Saf.* 15, 784–792. doi:10.1002/pds.1214

Sharma, P., and Maffulli, N. (2006). Biology of tendon injury: healing, modeling and remodeling. *J. Musculoskelet. Neuronal Interact.* 6, 181–190.

Sharma, P., and Maffulli, N. (2005). Tendon injury and tendinopathy: healing and repair. *J. Bone Jt. Surg. Am.* 87, 187–202. doi:10.2106/jbjs.d.01850

Shen, H., Gelberman, R. H., Silva, M. J., Sakiyama-Elbert, S. E., and Thomopoulos, S. (2013). BMP12 induces tenogenic differentiation of adipose-derived stromal cells. *PLoS One* 8, e77613. doi:10.1371/journal.pone.0077613

Shojaee, A., Ejeian, F., Parham, A., and Nasr Esfahani, M. H. (2022). Optimizing tenogenic differentiation of equine adipose-derived mesenchymal stem cells (eq-ASC) using TGFB3 along with BMP antagonists. *Cell J.* 24, 370–379. doi:10.22074/cellj.2022.7892

Slane, L. C., DeWall, R., Martin, J., Lee, K., and Thelen, D. G. (2015). Middle-aged adults exhibit altered spatial variations in Achilles tendon wave speed. *Physiol. Meas.* 36, 1485–1496. doi:10.1088/0967-3334/36/7/1485

Steinmann, S., Pfeifer, C. G., Brochhausen, C., and Docheva, D. (2020). Spectrum of tendon pathologies: triggers, trails and end-state. *Int. J. Mol. Sci.* 21, 844. doi:10.3390/ijms21030844

Stuard, W. L., Titone, R., and Robertson, D. M. (2020). The IGF/Insulin-IGFBP Axis in corneal development, wound healing, and disease. *Front. Endocrinol. (Lausanne)* 11, 24. doi:10.3389/fendo.2020.00024

Sun, H., Wang, Y., He, T., He, D., Hu, Y., Fu, Z., et al. (2021). Hollow polydopamine nanoparticles loading with peptide RL-QN15: A new pro-regenerative therapeutic agent for skin wounds. *J. Nanobiotechnology* 19, 304. doi:10.1186/s12951-021-01049-2

Sun, L., Jin, H., Sun, L., Chen, S., Huang, Y., Liu, J., et al. (2015). Hydrogen sulfide alleviates myocardial collagen remodeling in association with inhibition of TGF-β/smad signaling pathway in spontaneously hypertensive rats. *Mol. Med.* 20, 503–515. doi:10.2119/molmed.2013.00096

Suwalski, A., Dabboue, H., Delalande, A., Bensamoun, S. F., Canon, F., Midoux, P., et al. (2010). Accelerated Achilles tendon healing by PDGF gene delivery with mesoporous silica nanoparticles. *Biomaterials* 31, 5237–5245. doi:10.1016/j.biomaterials.2010.02.077

Tan, Q., Lui, P. P., Rui, Y. F., and Wong, Y. M. (2012). Comparison of potentials of stem cells isolated from tendon and bone marrow for musculoskeletal tissue engineering. *Tissue Eng. Part A* 18, 840–851. doi:10.1089/ten.tea.2011.0362

Tang, J. B., Wu, Y. F., Cao, Y., Chen, C. H., Zhou, Y. L., Avanessian, B., et al. (2016). Basic FGF or VEGF gene therapy corrects insufficiency in the intrinsic healing capacity of tendons. *Sci. Rep.* 6, 20643. doi:10.1038/srep20643

Tang, Y., Leng, Q., Xiang, X., Zhang, L., Yang, Y., and Qiu, L. (2015). Use of ultrasound-targeted microbubble destruction to transfect IGF-1 cDNA to enhance the regeneration of rat wounded Achilles tendon *in vivo*. *Gene Ther.* 22, 610–618. doi:10.1038/gt.2015.32

Tauriello, D. V. F., Sancho, E., and Batlle, E. (2022). Overcoming TGFβ-mediated immune evasion in cancer. *Nat. Rev. Cancer* 22, 25–44. doi:10.1038/s41568-021-00413-6

Tempfer, H., Kaser-Eichberger, A., Lehner, C., Gehwolf, R., Korntner, S., Kunkel, N., et al. (2018). Bevacizumab improves achilles tendon repair in a rat model. *Cell Physiol. Biochem.* 46, 1148–1158. doi:10.1159/000489057

Tempfer, H., Spitzer, G., Lehner, C., Wagner, A., Gehwolf, R., Fierlbeck, J., et al. (2022). VEGF-D-mediated signaling in tendon cells is involved in degenerative processes. *FASEB J.* 36, e22126. doi:10.1096/fj.202100773rrr

Tempfer, H., and Traweger, A. (2015). Tendon vasculature in health and disease. *Front. Physiol.* 6, 330. doi:10.3389/fphys.2015.00330

Theodossiou, S. K., Tokle, J., and Schiele, N. R. (2019). TGFβ2-induced tenogenesis impacts cadherin and connexin cell-cell junction proteins in mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* 508, 889–893. doi:10.1016/j.bbrc.2018.12.023

Thermann, H., Fischer, R., Gougoulias, N., Cipollaro, L., and Maffulli, N. (2020). Endoscopic debridement for non-insertional Achilles tendinopathy with and without platelet-rich plasma. *J. Sport Health Sci.* 12, 275–280. doi:10.1016/j.jshs.2020.06.012

Titan, A. L., Foster, D. S., Chang, J., and Longaker, M. T. (2019). Flexor tendon: development, healing, adhesion formation, and contributing growth factors. *Plast. Reconstr. Surg.* 144, 639e–647e. doi:10.1097/prs.0000000000006048

Tramer, J. S., Khalil, L. S., Buckley, P., Ziedas, A., Kolowich, P. A., and Okoroha, K. R. (2021). Effect of achilles tendon rupture on player performance and longevity in women's national basketball association players. *Orthop. J. Sports Med.* 9, 232596712198998. doi:10.1177/2325967121989982

Tsiapalis, D., Kearns, S., Kelly, J. L., and Zeugolis, D. I. (2021). Growth factor and macromolecular crowding supplementation in human tenocyte culture. *Biomater. Biosyst.* 1, 100009. doi:10.1016/j.bbiosy.2021.100009

Uyar, I., Altuntas, Z., Findik, S., Yildirim, M. E. C., Yarar, S., Aktan, M., et al. (2022). The effects of a combination treatment with mesenchymal stem cell and platelet-rich plasma on tendon healing: an experimental study. *Turk J. Med. Sci.* 52, 237–247. doi:10.3906/sag-2105-145

Uysal, C. A., Tobita, M., Hyakusoku, H., and Mizuno, H. (2012). Adipose-derived stem cells enhance primary tendon repair: biomechanical and immunohistochemical evaluation. *J. Plast. Reconstr. Aesthet. Surg.* 65, 1712–1719. doi:10.1016/j.bjps.2012.06.011

Veronesi, F., Torricelli, P., Della Bella, E., Pagani, S., and Fini, M. (2015). *In vitro* mutual interaction between tenocytes and adipose-derived mesenchymal stromal cells. *Cytotherapy* 17, 215–223. doi:10.1016/j.jcyt.2014.10.006

Waggett, A. D., Ralphs, J. R., Kwan, A. P., Woodnutt, D., and Benjamin, M. (1998). Characterization of collagens and proteoglycans at the insertion of the human Achilles tendon. *Matrix Biol.* 16, 457–470. doi:10.1016/s0945-053x(98)90017-8

Walia, B., and Huang, A. H. (2019). Tendon stem progenitor cells: understanding the biology to inform therapeutic strategies for tendon repair. *J. Orthop. Res.* 37, 1270–1280. doi:10.1002/jor.24156

Wang, D., Jiang, X., Lu, A., Tu, M., Huang, W., and Huang, P. (2018a). BMP14 induces tenogenic differentiation of bone marrow mesenchymal stem cells *in vitro*. *Exp. Ther. Med.* 16, 1165–1174. doi:10.3892/etm.2018.6293

Wang, H., Yu, R., Wang, M., Wang, S., Ouyang, X., Yan, Z., et al. (2023). Insulin-like growth factor binding protein 4 loaded electrospun membrane ameliorating tendon injury by promoting retention of IGF-1. *J. Control Release* 356, 162–174. doi:10.1016/j.jconrel.2023.02.039

Wang, L., Yang, T., Ding, L., Ye, X., and Wu, L. (2022). Platelet-derived growth factor AA-modified electrospun fibers promote tendon healing. *J. Biomater. Appl.* 37 (6), 1018-1028. doi:10.1177/08853282221139274

Wang, X., Li, F., Xie, L., Crane, J., Zhen, G., Mishina, Y., et al. (2018b). Inhibition of overactive TGF-beta attenuates progression of heterotopic ossification in mice. *Nat. Commun.* 9, 551. doi:10.1038/s41467-018-02988-5

Wasniewska, A., Olewnik, L., and Polguj, M. (2022). Morphometric profile in foetuses and evolution of Achilles tendon. *Folia Morphol. Warsz.* 81, 144–149. doi:10.5603/fm.a2021.0013

Wee, J., Kim, H., Shin, S. J., Lee, T., and Lee, S. Y. (2022). Influence of mechanical and TGF-β3 stimulation on the tenogenic differentiation of tonsil-derived mesenchymal stem cells. *BMC Mol. Cell Biol.* 23, 3. doi:10.1186/s12860-021-00400-7

Wei, B., Ji, M., Lin, Y., Geng, R., Wang, Q., and Lu, J. (2023). Investigation of the medium-term effect of osteoprotegerin/bone morphogenetic protein 2 combining with collagen sponges on tendon-bone healing in a rabbit. *J. Orthop. Surg. Hong. Kong)* 31, 102255362311634. doi:10.1177/10225536231163467

Wei, X., Mao, Z., Hou, Y., Lin, L., Xue, T., Chen, L., et al. (2011). Local administration of TGFβ-1/VEGF165 gene-transduced bone mesenchymal stem cells for Achilles allograft replacement of the anterior cruciate ligament in rabbits. *Biochem. Biophys. Res. Commun.* 406, 204–210. doi:10.1016/j.bbrc.2011.02.015

Winnicki, K., Ochala-Klos, A., Rutowicz, B., Pekala, P. A., and Tomaszewski, K. A. (2020). Functional anatomy, histology and biomechanics of the human Achilles tendon - a comprehensive review. *Ann. Anat.* 229, 151461. doi:10.1016/j.aanat.2020.151461

Wolff, K. S., Wibmer, A. G., Binder, H., Grissmann, T., Heinrich, K., Schauer, S., et al. (2012). The avascular plane of the achilles tendon: A quantitative anatomic and angiographic approach and a base for a possible new treatment option after rupture. *Eur. J. Radiol.* 81, 1211–1215. doi:10.1016/j.ejrad.2011.03.015

Wu, L. M., Wang, J. K., Liu, J., Fan, C. C., Wang, Y. J., and Xiong, Y. (2021). Gait analysis combined with the expression of TGF-β1, TGF-β3 and CREB during Achilles tendon healing in rat. *Chin. J. Traumatol.* 24, 360–367. doi:10.1016/j.cjtee.2021.10.002

Xiang, X., Leng, Q., Tang, Y., Wang, L., Huang, J., Zhang, Y., et al. (2018). Ultrasound-targeted microbubble destruction delivery of insulin-like growth factor 1 cDNA and transforming growth factor beta short hairpin RNA enhances tendon regeneration and inhibits scar formation *in vivo*. *Hum. Gene Ther. Clin. Dev.* 29, 198–213. doi:10.1089/humc.2018.121

Xiao, H., Chen, Y., Li, M., Shi, Q., Xu, Y., Hu, J., et al. (2021). Cell-free book-shaped decellularized tendon matrix graft capable of controlled release of BMP-12 to improve tendon healing in a rat model. *Am. J. Sports Med.* 49, 1333–1347. doi:10.1177/0363546521994555

Xie, Y., Su, N., Yang, J., Tan, Q., Huang, S., Jin, M., et al. (2020). FGF/FGFR signaling in health and disease. *Signal Transduct. Target Ther.* 5, 181. doi:10.1038/s41392-020-00222-7

Xu, S. Y., Li, S. F., and Ni, G. X. (2016). Strenuous treadmill running induces a chondrocyte phenotype in rat achilles tendons. *Med. Sci. Monit.* 22, 3705–3712. doi:10.12659/msm.897726

Xu, Z., He, Z., Shu, L., Li, X., Ma, M., and Ye, C. (2021). Intra-Articular platelet-rich plasma combined with hyaluronic acid injection for knee osteoarthritis is superior to platelet-rich plasma or hyaluronic acid alone in inhibiting inflammation and improving pain and function. *Arthroscopy* 37, 903–915. doi:10.1016/j.arthro.2020.10.013

Yan, R., Gu, Y., Ran, J., Hu, Y., Zheng, Z., Zeng, M., et al. (2017). Intratendon delivery of leukocyte-poor platelet-rich plasma improves healing compared with leukocyte-rich platelet-rich plasma in a rabbit achilles tendinopathy model. *Am. J. Sports Med.* 45, 1909–1920. doi:10.1177/0363546517694357

Ye, T., Chen, Z., Zhang, J., Luo, L., Gao, R., Gong, L., et al. (2023). Large extracellular vesicles secreted by human iPSC-derived MSCs ameliorate tendinopathy via regulating macrophage heterogeneity. *Bioact. Mater* 21, 194–208. doi:10.1016/j.bioactmat.2022.08.007

Ye, Y. J., Zhou, Y. Q., Jing, Z. Y., Liu, Y. Y., and Yin, D. C. (2018). Electrospun heparin-loaded core-shell nanofiber sutures for achilles tendon regeneration *in vivo*. *Macromol. Biosci.* 18, e1800041. doi:10.1002/mabi.201800041

Yoshimoto, Y., Uezumi, A., Ikemoto-Uezumi, M., Tanaka, K., Yu, X., Kurosawa, T., et al. (2022). Tenogenic induction from induced pluripotent stem cells unveils the trajectory towards tenocyte differentiation. *Front. Cell Dev. Biol.* 10, 780038. doi:10.3389/fcell.2022.780038

You, T., Yuan, S., Bai, L., Zhang, X., Chen, P., and Zhang, W. (2020). Benzyl alcohol accelerates recovery from Achilles tendon injury, potentially via TGF-β1/Smad2/3 pathway. *Injury* 51, 1515–1521. doi:10.1016/j.injury.2020.03.058

Younesi Soltani, F., Javanshir, S., Dowlati, G., Parham, A., and Naderi-Meshkin, H. (2022). Differentiation of human adipose-derived mesenchymal stem cells toward tenocyte by platelet-derived growth factor-BB and growth differentiation factor-6. *Cell Tissue Bank.* 23, 237–246. doi:10.1007/s10561-021-09935-7

Yu, D., Ju, J., Xue, F., Zhao, Y., Shi, W., and Xiao, H. (2021a). Expression and significance of related genes in the early stage of post-traumatic heterotopic ossification in a rat model of Achilles tenotomy. *Acta Orthop. Traumatol. Turc* 55, 94–101. doi:10.5152/j.aott.2021.18480

Yu, T. Y., Pang, J. S., Lin, L. P., Cheng, J. W., Liu, S. J., and Tsai, W. C. (2021b). Platelet-rich plasma releasate promotes early healing in tendon after acute injury. *Orthop. J. Sports Med.* 9, 232596712199037. doi:10.1177/2325967121990377

Yu, X., Biedrzycki, A. H., Khalil, A. S., Hess, D., Umhoefer, J. M., Markel, M. D., et al. (2017). Nanostructured mineral coatings stabilize proteins for therapeutic delivery. *Adv. Mater* 29, 1701255. doi:10.1002/adma.201701255

Yuksel, S., Gulec, M. A., Gultekin, M. Z., Adanir, O., Caglar, A., Beytemur, O., et al. (2016). Comparison of the early period effects of bone marrow-derived mesenchymal stem cells and platelet-rich plasma on the Achilles tendon ruptures in rats. *Connect. Tissue Res.* 57, 360–373. doi:10.1080/03008207.2016.1189909

Zhang, B., Luo, Q., Deng, B., Morita, Y., Ju, Y., and Song, G. (2018a). Construction of tendon replacement tissue based on collagen sponge and mesenchymal stem cells by coupled mechano-chemical induction and evaluation of its tendon repair abilities. *Acta Biomater.* 74, 247–259. doi:10.1016/j.actbio.2018.04.047

Zhang, C. H., Jiang, Y. L., Ning, L. J., Li, Q., Fu, W. L., Zhang, Y. J., et al. (2018b). Evaluation of decellularized bovine tendon sheets for achilles tendon defect reconstruction in a rabbit model. *Am. J. Sports Med.* 46, 2687–2699. doi:10.1177/0363546518787515

Zhang, J., Li, F., Augi, T., Williamson, K. M., Onishi, K., Hogan, M. V., et al. (2021). Platelet HMGB1 in Platelet-Rich Plasma (PRP) promotes tendon wound healing. *PLoS One* 16, e0251166. doi:10.1371/journal.pone.0251166

Zhao, T., Qi, Y., Xiao, S., Ran, J., Wang, J., Ghamor-Amegavi, E. P., et al. (2019). Integration of mesenchymal stem cell sheet and bFGF-loaded fibrin gel in knitted PLGA scaffolds favorable for tendon repair. *J. Mater Chem. B* 7, 2201–2211. doi:10.1039/c8tb02759e

Zhou, J. J., Chang, Y. J., Chen, Y. L., Wang, X. D., Liao, Q., Shi, R. H., et al. (2021a). Comparison of myosepta development and transcriptome profiling between blunt snout bream with and Tilapia without intermuscular bones. *Biol. (Basel)* 10, 1311. doi:10.3390/biology10121311

Zhou, Y., Zhang, L., Zhao, W., Wu, Y., Zhu, C., and Yang, Y. (2013). Nanoparticle-mediated delivery of TGF-β1 miRNA plasmid for preventing flexor tendon adhesion formation. *Biomaterials* 34, 8269–8278. doi:10.1016/j.biomaterials.2013.07.072

Zhou, Y. L., Yang, Q. Q., Zhang, L., and Tang, J. B. (2021b). Nanoparticle-coated sutures providing sustained growth factor delivery to improve the healing strength of injured tendons. *Acta Biomater.* 124, 301–314. doi:10.1016/j.actbio.2021.01.008

Zou, J., Mo, X., Shi, Z., Li, T., Xue, J., Mei, G., et al. (2016). A prospective study of platelet-rich plasma as biological augmentation for acute achilles tendon rupture repair. *Biomed. Res. Int.* 2016, 1–8. doi:10.1155/2016/9364170

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Original Research
PUBLISHED 08 January 2024
DOI 10.3389/fbioe.2023.1327027

OPEN ACCESS

EDITED BY
Dai Fei Elmer Ker,
The Chinese University of Hong Kong, China

REVIEWED BY
Takashi Taguchi,
Western University of Health Sciences, United States
Wencai Liu,
Shanghai Jiao Tong University, China
Yu Zhou,
Changchun University of Chinese Medicine, China
Xiaofei Hu,
Army Medical University, China

*CORRESPONDENCE
Hong Chen,
chenhong_6612@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 24 October 2023
ACCEPTED 11 December 2023
PUBLISHED 08 January 2024

CITATION
Zhang S, Shang J, Gu Z, Gu X, Wang F, Hu X, Wu G, Zou H, Ruan J, He X, Bao C, Zhang Z, Li X and Chen H (2024), Global research trends and hotspots on tendon-derived stem cell: a bibliometric visualization study.
Front. Bioeng. Biotechnol. 11:1327027.
doi: 10.3389/fbioe.2023.1327027

Global research trends and hotspots on tendon-derived stem cell: a bibliometric visualization study

Songou Zhang[1†], Jinxiang Shang[2†], Zhiqian Gu[1†], Xiaopeng Gu[1], Fei Wang[3], Xujun Hu[3], Guoliang Wu[1], Huan Zou[4], Jian Ruan[4], Xinkun He[4], Chenzhou Bao[4], ZhenYu Zhang[5], Xin Li[5] and Hong Chen[4]*

[1]Department of Clinical Medicine, Health Science Center, Ningbo University, Ningbo, Zhejiang, China, [2]Department of Orthopedics, Affiliated Hospital of Shaoxing University, Shaoxing, Zhejiang, China, [3]Department of Orthopedics, Shaoxing People's Hospital, Shaoxing, Zhejiang, China, [4]Department of Orthopedics, Ningbo Sixth Hospital, Ningbo, Zhejiang, China, [5]Department of Clinical Medicine, School of Medicine, Shaoxing University, Shaoxing, Zhejiang, China

Purpose: This study was aimed to examine the global research status and current research hotspots in the field of tendon stem cells.

Methods: Bibliometric methods were employed to retrieve relevant data from the Web of Science Core Collection (WOSCC) database. Additionally, Citespace, Vosviewer, SCImago, and Graphad Prism were utilized to analyze the publication status in this field, identify the current research hotspots, and present a mini-review.

Results: The most active countries in this field were China and the United States. Notable authors contributing significantly to this research included Lui Pauline Po Yee, Tang Kanglai, Zhang Jianying, Yin Zi, and Chen Xiao, predominantly affiliated with institutions such as the Hong Kong Hospital Authority, Third Military Medical University, University of Pittsburgh, and Zhejiang University. The most commonly published journals in this field were Stem Cells International, Journal of Orthopedic Research, and Stem Cell Research and Therapy. Moreover, the current research hotspots primarily revolved around scaffolds, molecular mechanisms, and inflammation regulation.

Conclusion: Tendon stem cells hold significant potential as seed cells for tendon tissue engineering and offer promising avenues for further research Scaffolds, molecular mechanisms and inflammation regulation are currently research hotspots in this field.

KEYWORDS

tendon-derived stem cell, tendon injury, bibliometric analysis, scaffolds, mechanism, inflammation

1 Introduction

Tendons are fibrous connective tissues that connect muscles to bones. They are characterized by avascularity and cellular scarcity, which limits their self-repair capabilities, frequently resulting in the formation of scar tissue during the healing process, reducing tendon flexibility and increasing the risk of re-rupture

(Andarawis-Puri et al., 2015; Thomopoulos et al., 2015; Millar et al., 2021; Pearce et al., 2021). Restoring the normal structure of injured tendons poses a significant challenge in sports medicine. Tendon-derived stem cells (TDSCs) are a type of mesenchymal stem cell found within the tendon tissue. Strictly speaking, TDSCs cannot be classified as conventional stem cells due to their biological heterogeneity. It is more accurate to describe them as "stem/progenitor" cells considering their capacity to differentiate into a limited number of specific cell lineages. Besides, they possess certain stem cell features such as clonogenicity, high proliferation rate, and self-renewal ability (Bi et al., 2007). We summarize cell culture methods reported in TDSCs research in Supplementary Table S1. Simply put, the methods for culturing and isolating tendon stem cells are as follows: under aseptic conditions, tendon tissues are treated with collagenase (usually Type I or II, at a concentration of approximately 0.1%–3%) at 37°C for several hours to overnight to isolate the cells. The cells are then collected and cultured in a specific medium (such as low-glucose DMEM), with 10%–20% serum added for nutrition, and maintained at 37°C in an environment with 5% CO_2, with passaging done at appropriate intervals to maintain cell viability. TDSCs are characterized by the presence of markers such as CD44, CD146, CD105, and CD90, which are typical of mesenchymal stem cells (Zhang and Wang, 2010a; Lee et al., 2018). Due to their unique cellular microenvironment, TDSCs have a greater capability to generate tendon and joint tissue compared to bone marrow-derived mesenchymal stem cells (BMSCs) (Tan et al., 2012). The current cell sources of research on TDSCs are mainly: rat, mice, rabbit and human; a small amount of TDSC studied are from horse, pig. The main research focuses are: therapeutic targets and drug effect, disease mechanisms, tissue engineering, and cell properties. (Supplementary Table S1)

After a tendon injury, successful restoration of tendon integrity involves three stages: the inflammatory phase, cellular proliferation phase, and extracellular matrix (ECM) reconstruction phase. In the inflammatory phase, it involves the infiltration of inflammatory cells, secretion of inflammatory factors, and recruitment and activation of TDSCs (Vinhas et al., 2018; Ackerman et al., 2021). The cellular proliferation phase is characterized by the generation of new tendon cells, while the ECM reconstruction phase involves the formation of new ECM and tendon structure. TDSCs play a crucial role in tendon repair by secreting ECM specific to tendons and differentiating into tendon cells (Zhang et al., 2019a). Activating endogenous tendon stem cells or transplanting TDSCs using appropriate techniques has emerged as an innovative approach to promote tendon injury repair (Lee et al., 2015). Therefore, TDSCs hold significant potential in enhancing the healing of tendons and tendon-bone junctions (Chen et al., 2013).

The importance of TDSCs in orthopedic research has led to a considerable amount of research in recent years (Leong et al., 2020). However, most studies have focused on specific aspects of TDSCs research, resulting in a lack of comprehensive analysis of the literature in this area. A particular article claims to employ bibliometric methods to study TDSCs (Long et al., 2022); However, its literature search content was inaccurate. Although the discovery of TDSCs dates back to 2003, the selected literature in that study included a substantial number of publications prior to 2003. A thorough examination of its search methodology revealed that the chosen literature mainly concerned adipose-derived stem cells (ADSCs) and BMSCs in the context of tendon injury research, with limited relevance to TDSCs. As a result, their research primarily reflects the involvement of stem cells in tendon injuries (Long et al., 2022). Therefore, it is essential to utilize appropriate methods to investigate the global knowledge framework, research frontiers, and hotspots in the field of TDSCs research.

Bibliometrics is a research methodology that employs mathematical and statistical techniques to explore the fundamental aspects of scientific research (Shang et al., 2023). Recently, this method has gained widespread usage in scientific research to identify research hotspots and future directions in specific fields. However, literature searches have not produced any accurate bibliometric studies reflecting the current status of TDSCs research. Consequently, a new bibliometric study is necessary to unveil the authentic state of research in this field. This study utilizes software tools such as VOSviewer and CiteSpace to analyze trends and hotspots in TDSCs-related research.

2 Methods

2.1 Search strategy

A literature search focused on TDSCs was conducted using the Web of Science Core Collection (WOSCC) database

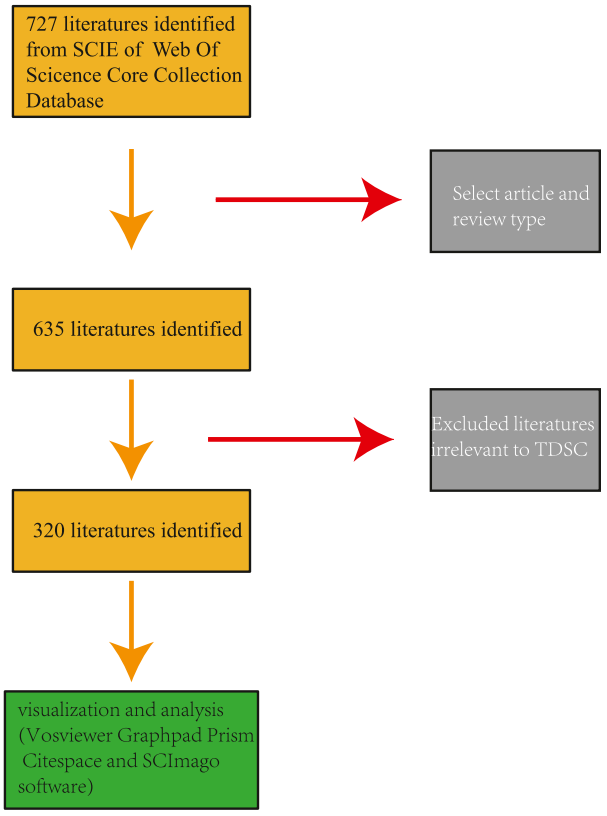


FIGURE 1
Publication screening flowchart.

FIGURE 2
Annual output of TDSCs.

(https://www.webofscience.com/wos/woscc/basic-search). The search strategy incorporated various title search terms, including "tendon stem cell*", "tendon derived stem cell*," "tendon progenitor cell*," "tendon stem/progenitor cell*," "tendon derived stem/progenitor cell*," and "tendon progenitor/stem cell*." The search was limited to articles from the Science Citation Index Expanded (SCIE) database, written in English, and comprising original articles and reviews. The retrieved content included article titles, authors, abstracts, keywords, and all citations, which were downloaded in plain text format. The search was concluded on 12 July 2023.

2.2 Data extraction and bibliometric analysis

Following the literature search, abstract readings were performed by SongOu Zhang to select relevant articles on TDSCs while excluding studies on mesenchymal stem cells like BMSCs and ADSCs in the context of tendon injuries, as well as any TDSCs research unrelated to the study. Subsequently, all plain text data were downloaded, and the number of articles and the annual publication rate were recorded. To conduct literature analysis and visualization, four software applications were employed: Citespace (Ver 6.1.6), VOSviewer (Ver. 1.6.18), SCImago (Ver. 1.0.35), and GraphPad Prism (Ver. 9.0.2). (Figure 1).

3 Results

3.1 Annal publications

During the literature screening concerning TDSCs, we rigorously selected articles directly relevant to TDSCs and excluded those unrelated ones. Based on Citespace software, we analyzed the annual publication count and identified the earliest literature on TDSCs that published in 2003, followed by scattered articles in 2006, 2007, and 2009, and a subsequent rapid increase of articles from 2010 to 2017. From 2018 to 2023, there has been a plateau period in TDSCs research. Thus, the field of TDSCs research is relatively new and has been experiencing a growing trend in the number of studies (Figure 2).

3.2 Country and organization analysis

Authors involved in TDSCs research came from 25 different countries. Among them, 10 countries had each published more than 5 articles in this field, including China, the United States, Germany, Italy, England, Japan, South Korea, Switzerland, and Australia. Supplementary Table S2 presents the top ten countries based on their publication numbers and corresponding citation counts. Notably, China showed the highest publication count, reaching 211, significantly surpassing other countries. China also led in citation count with 5356 citations, although the average citation per article was relatively low. On the other hand, the United States has the highest average citation per article, reaching 60.1 times per article. These findings suggest that research from China is most active, while research from the United States attracts substantial attention from authors in this field. Additionally, an analysis of country collaborations revealed that cooperation between China and the United States was the most prominent (Figure 3A).

Within this field, a total of 340 institutions have contributed to article publications. Table 1 showcases the top ten institutions based on their publication volume. Notably, nine out of these top institutions were from China, indicating that Chinese scientists are the most prolific contributors in this area. For visualization analysis, institutions that have published more than 5 articles were included, totaling 26 institutions. The visualization graph illustrates the collaborations between these institutions. (Figure 3B).

3.3 Author analysis

In the author analysis, a total of 1371 authors participated in publications related to TDSCs. Authors who appeared in five or more articles were included for analysis, resulting in 60 authors meeting this criterion. Table 2 presents the top ten authors based on the number of publications. It was evident that Lui Pauline Po Yee from Hong Kong Hospital Authority was the most prolific author, with a total of 24 published papers on TDSCs and 1576 citations for those papers. Both the publication counts and citation counts ranked first, indicating that Lui Pauline Po

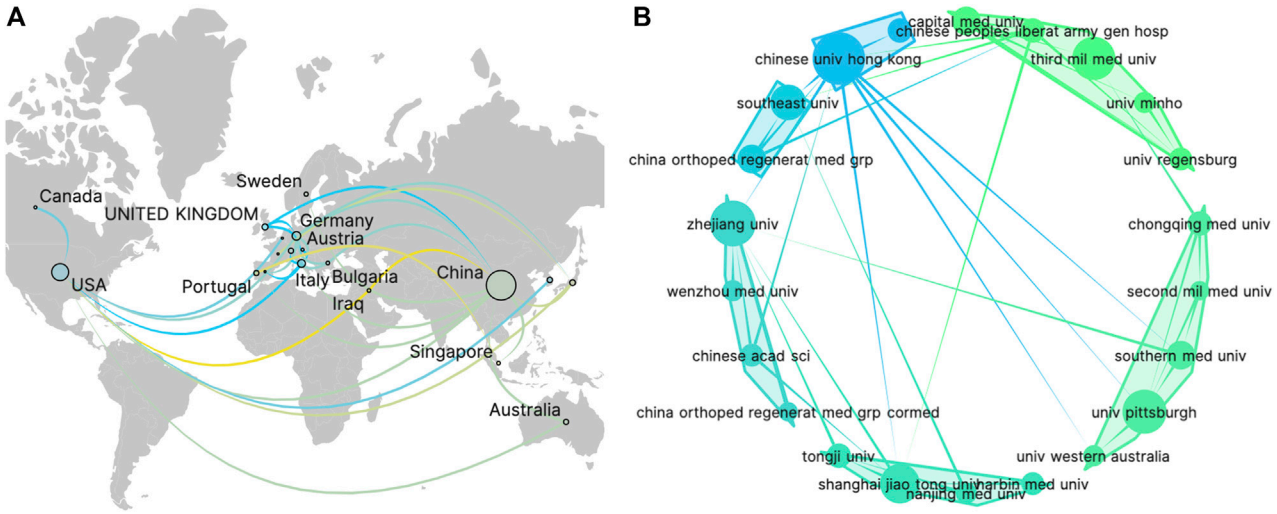


FIGURE 3
The visualization of countries **(A)** and organizations **(B)** on research of TDSCs.

TABLE 1 Top 10 organizations with most publications.

Organization	Publications	Total citations	Citations/Publications	From
Chinese University Hong Kong	37	1918	51.8	China
Zhejiang University	28	1135	40.5	China
University Pittsburgh	26	1452	55.8	United States
Third Mil Med University	25	618	24.7	China
Shanghai Jiao Tong university	21	412	19.6	China
Southeast University	17	364	21.4	China
China Orthoped Regenerat Med grp	11	178	16.2	China
Capital Med University	11	150	13.6	China
Southern Med University	11	233	21.2	China
Chinese Peoples Liberat Army Gen Hospital	8	102	12.8	China

Yee is the most active author in the TDSCs field. Among the top ten authors, 8 are from China, 1 is from the United States, and 1 is from Germany.

Figure 4A illustrates the situation of the top 20 authors based on the number of citations they received. Among them, 4 authors are affiliated with Third Military Medical University, and 2 authors are affiliated with Zhejiang University. Figure 4B displays the number of publications by each author and their collaborative relationships. The size of the circles represents the number of publications, while the colors and convex hulls indicate different clusters based on collaboration relationships. The lines connecting the circles indicate the extent of collaboration (Figure 4B). This visualization allowed us to observe the collaborative patterns among the authors.

3.4 Journal analysis

Regarding journal publications, only journals that have published more than 5 articles related to TDSCs were included in the analysis, resulting in 16 articles being included in the study. Supplementary Table S3 displays the top 10 journals based on the number of publications. Among these journals, *Stem Cells* International had the highest publication count, with a total of 19 articles. Figure 5A illustrates the status of journals focused on the field of TDSCs, where the size of circles indicates the publication volume and the colors represent the publication years. Figure 5B presents the citation counts for the relevant journals. Notably, the Journal of Orthopaedic Research has the highest number of citations, with a total of 1000. Among these ten journals, Biomaterials has the highest impact factor (IF), reaching 14.

TABLE 2 Top 10 authors with most publications.

Author	Publications	Citations	Citations/Publications	Organization	From
Lui Pauline Po Yee	24	1576	65.7	Hong Kong Hospital Authority	China
Tang Kanglai	21	393	18.7	Third Military Medical University	China
Zhang Jianying	19	1266	66.6	University of Pittsburgh	United States of America
Yin Zi	15	937	62.5	Zhejiang University	China
Chen Xiao	15	961	64.1	Zhejiang University	China
Zhou Mei	13	161	12.4	Third Military Medical University	China
Li Gang	13	704	54.2	The Chinese University of Hong Kong	China
Tang Hong	12	160	13.3	Third Military Medical University	China
Chen Lei	12	367	30.6	Third Military Medical University	China
Docheva Denitsa	12	556	46.3	University Medical Centre Regensburg	Germany

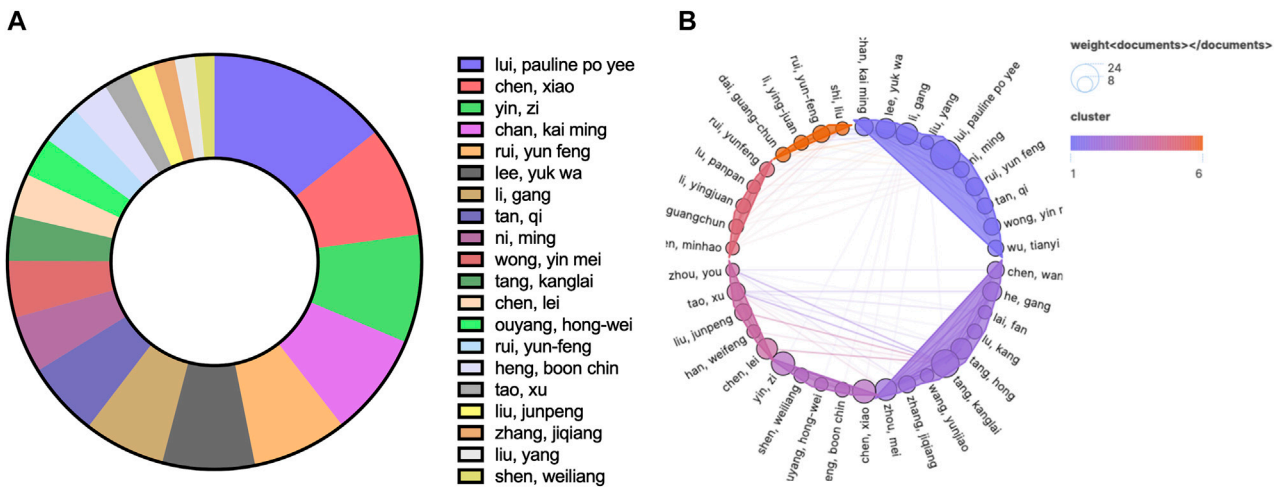


FIGURE 4
The top 20 authors by number of published articles **(A)** and their collaborative relationships **(B)**.

3.5 Publications analysis

The analysis of publication can reflect the status of published papers in the field, including the papers that receive the most attention, the papers that appear most in the references. Out of 320 articles, there were 19 articles that have been cited more than 100 times. Table 3 presents the top ten articles based on citation count. The most cited article was "*Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche*," authored by Yanming Bi, with 970 citations. It reported the first identification of TDSCs from both animal and human tendons. Among the top ten cited articles, the earliest one was authored by R Salingcarnboriboon. It was cited for 194 times, focusing on the demonstration of mesenchymal stem cell characteristics in cell lines derived from tendons.

In the field of this research, a total of 8752 articles have been cited. Among them, 51 articles have been cited more than 20 times. Table 4 displays the top ten articles based on the number of co-citations. Notably, the most co-cited article was "*Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche*," authored by Yanming Bi. It indicates that this article holds significant influence in the field.

3.6 Keyword analysis

Keyword analysis through VOSviewer and Citespace software can display the keywords with the highest frequency and the specific time when keywords appear together, and can also reflect changes in research hotspots. Regarding keyword analysis, a total of 1248 keywords were identified after merging synonyms. Among

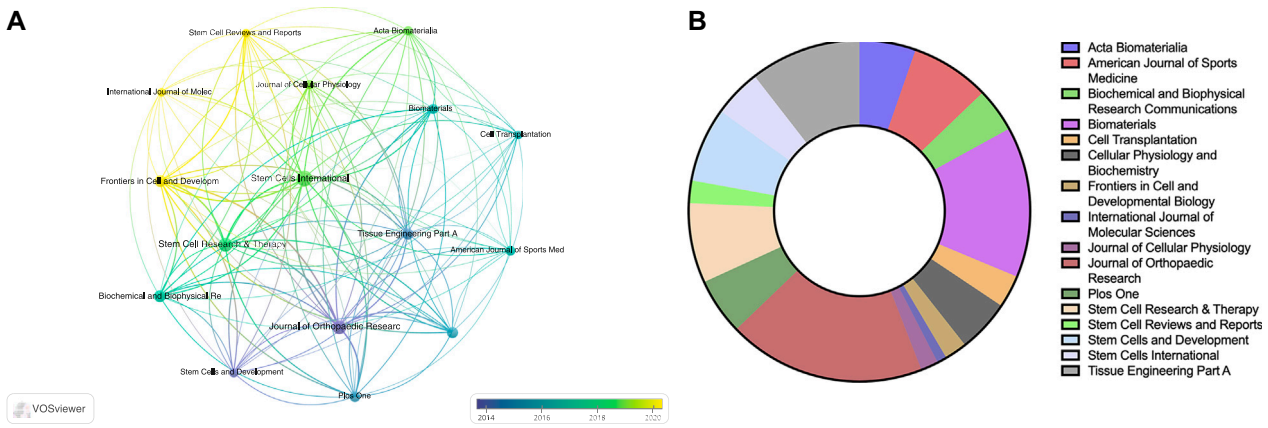


FIGURE 5
The visualization of journals **(A, B)** on research of TDSCs.

TABLE 3 Top 10 citations of publication.

Rank	Title	Author	Citations	Published year	DOI
1	Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche	Yanming Bi	970	2007	10.1038/nm1630
2	The regulation of tendon stem cell differentiation by the alignment of nanofibers	Zi Yin	471	2010	10.1016/j.biomaterials.2009.11.083
3	Characterization of differential properties of rabbit tendon stem cells and tenocytes	Jianying Zhang	260	2010	10.1186/1471-2474-11-10
4	Isolation and Characterization of Multipotent Rat Tendon-Derived Stem Cells	Yun-Feng Rui	225	2010	10.1089/ten.TEA.2009.0529
5	Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property	R Salingcarnboriboon	194	2003	10.1016/s0014-4827 (03) 00107-1
6	Platelet-Rich Plasma Releasate Promotes Differentiation of Tendon Stem Cells into Active Tenocytes	Jianying Zhang	161	2010	10.1177/0363546510376750
7	Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defect model	Ming Ni	156	2012	10.1002/jor.21559
8	Mechanobiological response of tendon stem cells: Implications of tendon homeostasis and pathogenesis of tendinopathy	Jianying Zhang	156	2010	10.1002/jor.21046
9	Harnessing endogenous stem/progenitor cells for tendon regeneration	Chang H. Lee	153	2015	10.1172/JCI81589
10	Comparison of Potentials of Stem Cells Isolated from Tendon and Bone Marrow for Musculoskeletal Tissue Engineering	Qi Tan	144	2012	10.1089/ten.TEA.2011.0362

these, 51 keywords appeared more than 10 times. Supplementary Table S4 presents the top 20 keywords based on the number of occurrences. Figure 6A displays a keyword density map, where larger circles indicate a higher frequency of appearance. Figure 6B shows the keyword overlay map, with different colors representing the appearance time of each keyword. Figure 6C presents the keyword network map, where the 51 keywords are divided into 3 clusters. Cluster 1 contains 18 keywords, including activation, basic science, bone, collagen, expression, hypoxia, inflammation, injury, matrix, mechanisms, model, osteogenic differentiation, pathogenesis, proliferation, tendinopathy, tendon stem cells, tenocytes, and therapy. Cluster 2 includes 17 keywords, such as bone-marrow, growth-factors, *in-vitro*, *in-vivo*, mesenchymal stem cell, platelet-rich plasma, regeneration, rotator cuff, rotator cuff repair, scaffolds, stem cell, tears, tendon, tendon regeneration, tenogenic differentiation, tissue, and tissue engineering. Cluster 3 comprises 16 keywords, including Achilles tendon, age, degeneration, differentiation, extracellular matrix, fibroblasts, gene, growth, identification, mechanical-properties, patellar tendon, scleraxis, self-renewal, senescence, tenomodulin, and Transforming growth factor beta (TGF-beta). CiteSpace was also utilized to detect keyword bursts. Figure 6D displays the top 25 keywords with the highest burst strength. The earliest keyword burst was "human bone marrow," the longest-lasting burst was "scaffolds," and the most recent burst was "inflammation." Through keyword analysis, we can identify the recent hotspots in TDSCs research.

TABLE 4 Top 10 Co-cited publications.

Rank	Title	Author	Publised year	Doi
1	Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche	Yanming Bi	2007	doi 10.1038/nm1630
2	Isolation and characterization of multipotent rat tendon-derived stem cells	Yun-Feng Rui	2010	10.1089/ten.tea. 2009.0529
3	Characterization of differential properties of rabbit tendon stem cells and tenocytes	Jianying Zhang	2010	10.1186/1471-2474-11-10
4	Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defect model	Ming Ni	2012	10.1002/jor.21559
5	Comparison of potentials of stem cells isolated from tendon and bone marrow for musculoskeletal tissue engineering	Qi Tan	2012	10.1089/ten.tea. 2011.0362
6	Tendon-derived stem/progenitor cell aging: defective self-renewal and altered fate	Zuping Zhou	2010	10.1111/j.1474-9726.2010.00598.x
7	Mechanobiological response of tendon stem cells: implications of tendon homeostasis and pathogenesis of tendinopathy	Jianying Zhang	2010	10.1002/jor.21046
8	Uncovering the cellular and molecular changes in tendon stem/progenitor cells attributed to tendon aging and degeneration	Julia Kohler	2013	10.1111/acel.12124
9	The regulation of tendon stem cell differentiation by the alignment of nanofibers	Zi Yin	2010	10.1016/j.biomaterials. 2009.11.083
10	Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments	R Schweitzer	2001	10.1242/dev.128.19.3855

4 Discussion

4.1 General information

The traditional viewpoint asserts that tendons only consist of tenocytes. However, in 2003, R Salingcarnboriboon et al. from Tokyo Medical and Dental University in Japan reported the presence of mesenchymal stem cells in 3 cell lines (TT-E4, TT-G11, and TT-D6) cultured from transgenic mice. These cell lines express tendon-specific genes such as scleraxis, SIX homeobox 1(Six1), EPH receptor A4 (EphA4), cartilage oligomeric matrix protein (COMP), and type I collagen. They can differentiate into tenocytes, fibrocartilage, osteoblasts, and adipocytes under specific conditions (Salingcarnboriboon et al., 2003). Subsequently, in 2007, Bi et al., 2007 extracted TDSCs with multidirectional differentiation and self-renewal ability from human and mouse tendon tissue. In 2010, similar stem cell characteristics were successfully extracted from species such as rats and rabbits (Zhang and Wang, 2010a; Rui et al., 2010). Since then, scientists have begun to focus on tendon stem cells. Over the past 15 years, extensive research has been conducted on TDSCs, which are widely recognized for their role in musculoskeletal system diseases. Due to their convenience of extraction, strong differentiation capacity, and abundant cell sources, TDSCs hold great promise for the repair of musculoskeletal system diseases (Liu et al., 2023a; Zhang et al., 2023a; Zhang et al., 2023b). This article systematically presented the current research status of the TDSCs field. We employ VOSviewer, CiteSpace, and SCImago software to analyze TDSCs literature downloaded from the WOSCC database. Our analysis included 260 articles authored by 1,052 individuals affiliated with 241 organizations from 18 countries. These articles were published in 25 journals and have received a total of 6,752 citations. Approximately 95% of the articles were original research.

Over the past 15 years, there has been a consistent increase in the number of publications in the field of TDSCs. Among the 18 countries contributing to this research, China (181%, 69.62%) ranked as the leading contributor, followed by the United States (58%, 22.31%), Germany (15%, 5.77%), Italy (8%, 3.08%), and Japan (7%, 2.69%). Notably, China had the largest number of publications and citations, with 4,460 citations for 181 articles, while the United States received 3,125 citations for 58 articles. On average, each article from China received 24.64 citations, whereas each article from the United States received 53.88 citations. Although China conducted extensive research in this field, its average attention per article was significantly lower compared to the United States.

Among the institutions involved in TDSCs research, five have published more than 20 articles. The top ten institutions with the most published articles included Chinese University Hong Kong, Zhejiang University, University Pittsburgh, Third Military Medical University, Shanghai Jiao Tong University, Southeast University, China Orthopedic Regenerative Medicine Group, Capital Medical University, Southern Medical University, and The Chinese People's Liberation Army General Hospital. Nine out of these ten institutions are based in China, with only one from the United States. It is noteworthy that the average number of citations per article was the highest at University Pittsburgh, reaching 55.8 times.

In terms of foundational research, the most influential article in the TDSCs field is "*Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche*" published by Bi et al. in the journal Nature Medicine. This article introduced the concept of TDSCs and provided methods for culturing and identifying them. It is also the most cited article in the field. The second and third most cited articles are "*The regulation of tendon stem cell differentiation by the alignment of*

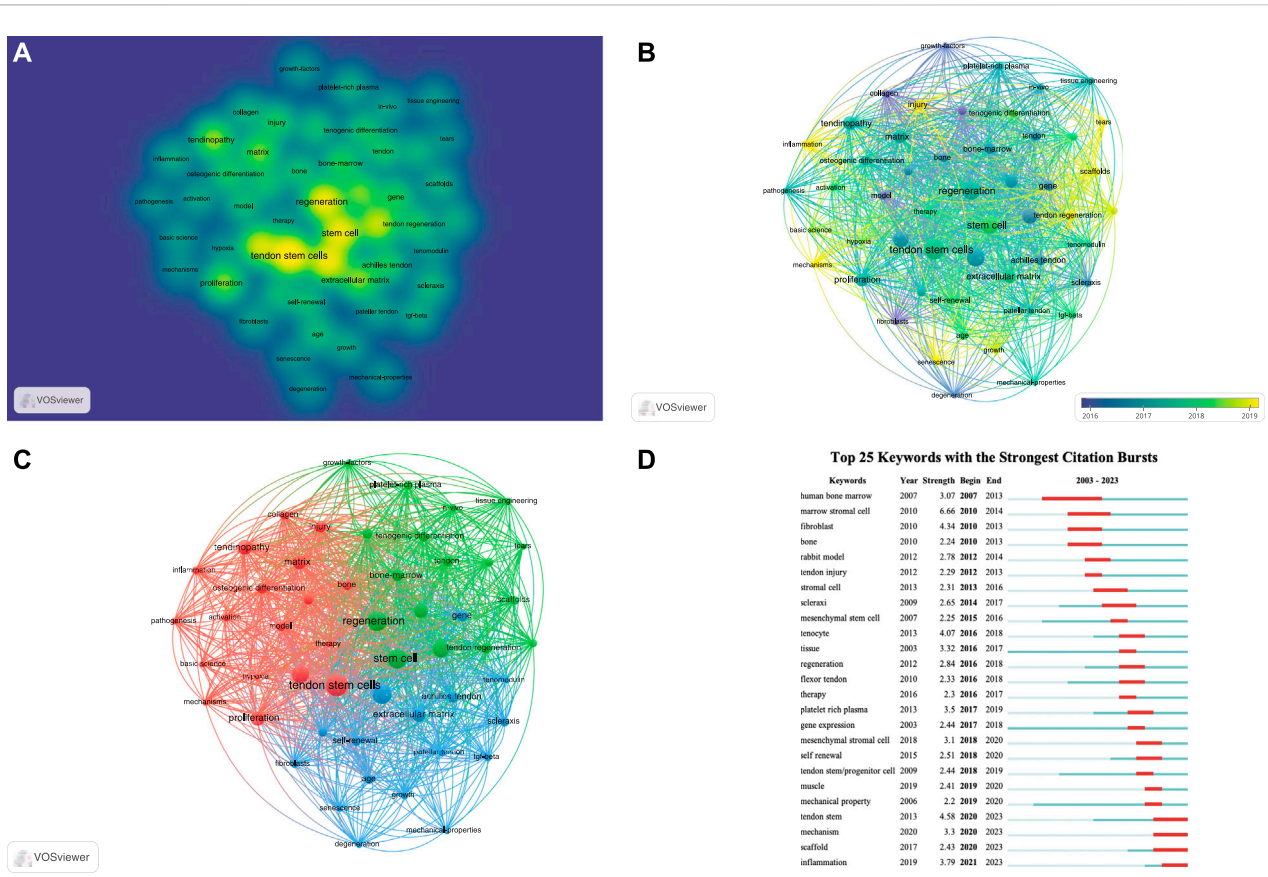


FIGURE 6
The visualization of keywords **(A–C)** and keywords bursts **(D)** on research of TDSCs.

nanofibers" published by Yin Zi from Zhejiang University (Yin et al., 2010). This article used human-derived TDSCs to inoculate aligned nanofibers, which can promote the differentiation of TDSCs toward tendons. It introduced a very clever physical method to induce directional differentiation of TDSCs, which provided a broad idea for the application of TDSCs. The third most cited article is "*Characterization of differential properties of rabbit tendon stem cells and tenocytes*" published by Jianying Zhang et al. (Zhang and Wang, 2010a) The fourth and fifth ranked articles were "*Isolation and characterization of multipotent rat tendon-derived stem cells*" published by Yun-Feng Rui et al. (Rui et al., 2010) and "*Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property*" published by R Salingcarnboriboon et al., 2003. These three articles all introduce methods for the acquiration and isolation of tendon stem cells from tendon tissue, as well as their characteristic features. The sixth (5th) (Zhang and Wang, 2010b) and eighth (6th) (Zhang and Wang, 2010c) most cited articles were also authored by Jianying Zhang et al. The seventh most cited article is "*Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defect model*" published by Ming Ni et al. (Lee et al., 2018). The ninth most cited article is "*Harnessing endogenous stem/progenitor cells for tendon regeneration*" by Chang H. Lee et al. (Tan et al., 2012). The four articles explored the application of TDSCs in tendon-related diseases. The 10th most cited article was "*Comparison of Potentials of Stem Cells Isolated from Tendon and Bone Marrow for Musculoskeletal Tissue Engineering*" by Qi Tan et al. (Tan et al., 2012), which compared the advantages and disadvantages of BMSCs and TDSCs as seed cells for musculoskeletal diseases. It concluded that TDSCs exhibited stronger multi-lineage differentiation ability than BMSCs and possessed significant therapeutic potential.

4.2 Hotspot and frontiers

Keyword analysis is an important bibliometric method for identifying research frontiers. Based on the citation burst of keywords, we can identify the current research frontiers in the TDSCs field. Using the keyword burst function of CiteSpace software, Figure 6D presents the keyword burst of the top 25 TDSCs keywords over the past 20 years. The blue line indicates the continuous appearance of keywords, while the red line indicates intensive appearances. It is evident that the earliest intensively appearing keywords were human bone marrow, marrow stromal cell, fibroblast, and bone. Based on the co-occurrence map of keywords, the current research hotspots have shifted towards scaffolds, mechanisms, and inflammation (Figure 7).

4.2.1 Scaffolds

The two most crucial considerations in tendon tissue engineering were seed cells and biological scaffolds. The topography of the cellular microenvironment plays a vital role in

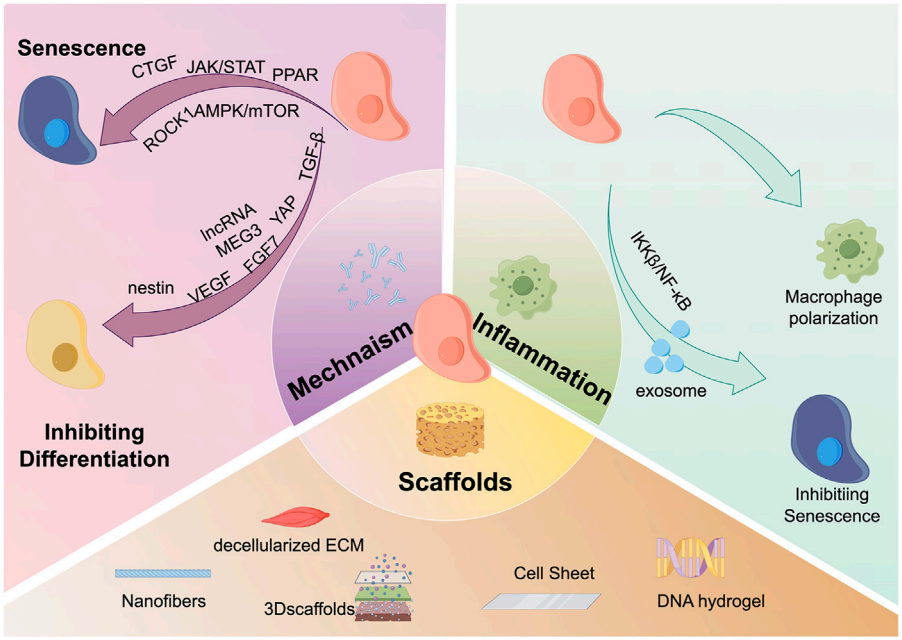


FIGURE 7
Hot spots and frontiers in the field of TDSCs research in recent years. There are three main research hotspots and frontiers in the field of TDSCs currently. The first is scaffold materials, which mainly include: nanofibers, decellularized ECM, 3D scaffolds, cell sheets, DNA scaffolds, etc.; the second hotspot is inflammation, and TDSCs can directly regulate macrophage polarization. TDSCs can also regulate inflammation-induced cell senescence through the release of exosomes and the IKKβ/NFκB signaling pathway; the third hotspot is the molecular mechanism. TDSCs mainly affects CTGF, JAK/STAT, PPAR, ROCK1, and AMPK./mTOR and other signaling pathways affect cell aging; the cell differentiation ability is regulated through molecular mechanisms such as TGF-β, YAP, FGF7, lncRNA MEG3, VEGF, and nestin.

cell activities, guiding cell attachment, morphology, proliferation, and differentiation. Furthermore, it influences cell signaling and morphology Cells can sense morphological changes in the extracellular matrix of cells and convert this information into morphological changes, thereby affecting cell differentiation (Sanie-Jahromi et al., 2023). In 2010, Yin Zi's team introduced bioengineered scaffolds into the TDSCs research field by planting human-derived TDSCs on electrospun nanofiber scaffolds (Yin et al., 2010). Their findings revealed that aligned electrospun nanofibers were more favorable for TDSCs differentiation towards tendons compared to disorganized scaffolds. Moreover, apart from synthetic scaffolds, natural tissue-derived scaffolds are also employed in various tissue engineering studies (Capella-Monsonis et al., 2023). Three years later, Yin Zi's team incorporated decellularized biological scaffolds into TDSCs research. By comparing the effects of three decellularized ECMs from tendon tissue, bone tissue, and dermal tissue on seeded TDSCs, they discovered that all three ECMs promoted TDSCs adhesion and proliferation. Notably, the decellularized ECM of bone facilitated TDSCs differentiation towards osteogenesis, while the decellularized ECM of tendon tissue promoted TDSC differentiation towards tendon formation. Based on this characteristic, they studied a unique scaffold composed of tendon decellularized ECM and TDSCs, which, in an *in vivo* study, demonstrated enhanced tendon maturation and biomechanical properties (Yin et al., 2013). Two-dimensional scaffolds fail to replicate the three-dimensional conditions of the extracellular matrix within the body. As an alternative to two-dimensional scaffolds, researchers have explored the application of TDSCs to three-dimensional scaffolds. Compared to two-dimensional scaffolds, three-dimensional scaffolds are more conducive to the tendon differentiation of TDSCs. Sihao Li et al. observed that TDSCs cultured in three-dimensional scaffolds exhibited stronger tenogenic differentiation through the phosphatidylinositol 3-kinase (PI3K)/Akt kinase (AKT) signaling pathway, along with reduced inflammatory phenotypes (Li et al., 2023). *In vivo* studies employing three-dimensional scaffolds revealed a reduction in ectopic calcification complications. Considering the biocompatibility concerns of cell scaffolds, cell sheet technology has rapidly developed Connective tissue growth factor and ascorbic acid can promote TDSCs to secrete extracellular matrix, forming a cell sheet resistant to trypsin breakdown. In addition to TDSCs, the cell sheet contains a substantial number of active substances secreted by TDSCs, including factors inducing tendonogenesis, osteogenesis, and chondrogenesis differentiation (Mifune et al., 2012). Pauline Po Yee Lui et al. (Long et al., 2022) utilized rat TDSCs to culture a cell sheet *in vitro*, wrapping it around tendons for anterior cruciate ligament reconstruction. They observed improved morphology, imaging, biomechanics, and early tendon healing in the cell sheet group (Lui et al., 2014). Early functional exercise post-surgery is crucial for patient functional recovery, indicating that the cell sheet may serve as a valuable graft improvement method. To mitigate immunogenicity issues, which affect post-transplant healing. Removing cellular immunogenicity from the cell sheet may be a

better option, indicating that the cell sheet can be applied to large-scale applications rather than personalized customization. In further research, Pauline Po Yee Lui et al. (Shang et al., 2023) chose to decellularize the TDSCs-formed cell sheet, and found that the decellularized cell sheet also promoted graft healing, tunnel bone formation, and angiogenesis (Yao et al., 2023). Hence, the extracellular matrix in the cell sheet may play a pivotal role in promoting healing.

Injecting TDSCs into injured tendon areas has been reported to facilitate injury repair (Gomez-Florit et al., 2022). However, due to tendon tissue sliding, effective attachment of TDSCs to the tendinopathy site is challenging, potentially explaining the limited efficacy of transplanted TDSCs *in vivo* (Gomez-Florit et al., 2022). To address this issue, Guanglin Wang et al. developed a DNA hydrogel scaffold encapsulating TDSCs. This DNA hydrogel scaffold extends the retention time of TDSCs on the tendon, providing an artificial microenvironment conducive to better nutrition, thereby promoting tendon injury repair (Ge et al., 2023).

4.2.2 Mechanism

Tendon aging is a significant contributor to tendon injuries. It leads to structural and functional changes, rendering tendons more susceptible to degeneration and damage. Tendon aging is related to functional changes in TDSCs. TDSCs undergo senescence during the aging process of tendons. Aging TDSCs exhibit reduced proliferation, migration, and multi-lineage differentiation capabilities compared to their younger counterparts. These changes hinder the repair potential of aging tendons. Numerous studies have investigated the mechanisms underlying TDSCs aging. For example, the downregulation of Pin1 and FoxP1 expression has been observed during TDSCs aging (Chen et al., 2015a; Xu and Liu, 2018), affecting peroxisome proliferator-activated receptor (PPAR) (Han et al., 2023), Janus kinase (JAK)/signal transducer and activator of transcription (STAT) (Chen et al., 2021a), protein kinase AMP-activated catalytic subunit alpha (AMPK)/mechanistic target of rapamycin kinase (mTOR) (Dai et al., 2023), non-canonical Wnt signaling pathway (Chen et al., 2021b) and autophagy (Nie et al., 2021a). These pathways are believed to be effective in improving TDSCs aging.

During tendon aging, specific proteins are of interest during tendon aging. Tenomodulin serves as a marker protein for tendon cells and plays a crucial role in TDSCs proliferation, multi-lineage differentiation, and other cellular functions. Research indicates that Tenomodulin is an important gene influencing TDSCs aging. Knocking out Tenomodulin can significantly accelerates TDSCs aging (Alberton et al., 2015). Connective TGF (CTGF) is a cysteine-rich secreted protein expressed widely across various tissues and organs (Yan et al., 2022). Bone morphogenetic proteins (BMP) play a pivotal role in bone development, but their overexpression in aging tendons can result in ectopic calcification (Dai et al., 2020). In aging TDSCs, CTGF expression decreases significantly and correlates with the expression of the aging marker p16. The application of recombinant CTGF protein has been shown to significantly enhance the self-renewal and differentiation capabilities of aging TDSCs (Rui et al., 2019).

Fluid loss is a common occurrence in various tissues during the aging process, and it is closely associated with the function of aquaporins (Kim et al., 2023). YunFeng Rui et al. discovered that aquaporin expression decreased during tendon aging and interfering with its expression can alleviate age-related decline in cell function. The JAK/STAT signaling pathway mediates the impact of aquaporins on aging (Chen et al., 2020). Noncoding RNAs play crucial roles in tendon-related diseases. Chen Lei et al. found that circular RNA (circRNA) PVT1 (circPVT1) regulated TDSCs aging by targeting microRNA (miR)-199a-5p, which further downregulated sirtuin 1 (Han et al., 2022). Additionally, miR-135a inhibits Rho-associated coiled-coil containing protein kinase 1 (ROCK1), thereby improving TDSCs aging (Chen et al., 2015b). Cellular senescence affects the stiffness and size of TDSCs cells, consequently influencing their physical properties and cellular functions (Dulińska-Molak et al., 2014). As cells age, TDSCs stiffness and size decrease; however, inhibiting ROCK1 can restore the stiffness of aging TDSCs (Kiderlen et al., 2019).

TDSCs express numerous markers specific to tendon-related cells, including scleraxis and tenomodulin, and possess the ability to differentiate into tendon tissue. TDSCs play a pivotal role in tendon regeneration and maintenance. Apart from understanding the mechanisms underlying aging, targeting TDSCs differentiation is crucial for maximizing their potential applications. Researchers have discovered that Irisin and activated platelet-rich plasma promote TDSCs differentiation towards tendons, resulting in greater expression of tendon markers and exhibiting potential for tendon treatment (Zhang et al., 2019b; Xu et al., 2022). Weifeng Han et al. reported that p16/miR-217/EGR1 can restore the tenogenic potential of aging TDSCs (Han et al., 2017). Hongwei Ouyang's team conducted microarray screening on newborn rats and found that the expression of transcription factor Fos decreased over time. Fos is a vital gene in early tendon development and significantly promotes the tenogenic differentiation of TDSCs (Chen et al., 2017). Using single-cell sequencing technology, Hongwei Ouyang's team observed significant activation of nestin during a specific period of tendon development. $Nestin^{+}$ TDSCs exhibited superior tendon differentiation capabilities compared to $nestin^{-}$ TDSCs, highlighting their importance during tendon development (Yin et al., 2016). In addition to promoting tendon differentiation, inhibiting TDSCs differentiation towards osteogenesis and adipogenesis can reduce tendon complications. Long noncoding RNA MEG3 inhibits the osteogenic differentiation potential of TDSCs through the miR-129-5p/transcription factor 4 (TCF4)/β-Catenin axis, thus reducing ectopic ossification caused by trauma in tendon tissue (Liu et al., 2023b). Vascular endothelial growth factor (VEGF) and PPAR gamma can also suppress adipogenic differentiation of TDSCs, presenting them as promising therapeutic targets for tendon degeneration (Lai et al., 2021; Lai et al., 2022). The recruitment and accumulation of endogenous TDSCs at the site of tendon injury initiate their participation in the repair process. TGF-beta, secreted by TDSCs themselves, is considered an inducing factor for TDSCs recruitment (Tan et al., 2021). Over-activating specific cellular molecular signals or employing recombinant cytokines are commonly used methods to study the repair effects of TDSCs. Periostin (Wang et al., 2021), Yes-

associated protein (YAP) signaling pathway (Lu et al., 2023; Wang et al., 2023), mTOR signaling pathway (Nie et al., 2021b), and Fibroblast growth factor 7 (FGF7) (Zhang et al., 2022) have been reported to enhance cellular functions of TDSCs, thereby promoting tendon repair.

4.2.3 Inflammation

Inflammation plays a pivotal role in the context of tendon injuries, particularly regarding the involvement of TDSCs. Following an injury, the body initiates an inflammatory response as part of the natural healing process. This inflammation not only aids in clearing damaged tissue but also regulates the activation and behavior of TDSCs. Inflammatory factors released by inflammatory cells during this process can reduce collagen secretion, leading to vasodilation and decreased blood vessel density, which subsequently affects TDSCs. In the presence of inflammation, TDSCs become activated, contributing to the repair and regeneration of injured tendon tissue. Activated TDSCs improve the inflammatory response in tendon tissue, increase the secretion of IL10, inhibit the polarization of macrophages towards M1 (Tarafder et al., 2017), and promote the M2 polarization of macrophages (Mao et al., 2022). Annexin A1 and CD200 serves as an important marker and drug target in tendon inflammatory diseases (Liu et al., 2018; Giancola et al., 2022). Excessive inflammation may induce senescence in TDSCs. Studies have found that nonsteroidal anti-inflammatory drugs can alleviate inflammation-induced TDSCs aging and promote the progression of tendon degeneration (Cai et al., 2022). I-kappaB kinase beta (IKKβ)/nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) is activated in degenerated tendon tissue, and targeted inhibition of IKKβ/NF-κB improves inflammation-induced TDSCs senescence. Furthermore, in conditions of diabetes, the differentiation ability of TDSCs decreases, which is closely related to inflammatory cytokines. Du-Hwan Kim et al. found that using Migration Inhibitory Factor can directly enhance the differentiation ability of TDSCs under high blood sugar conditions (Kim et al., 2021). Exosomes, small membrane-enclosed sacs involved in cell-to-cell communication and molecule transportation within the body, have gained significant attention in the fields of biology and medicine (He et al., 2018; Kalluri and LeBleu, 2020). They play a crucial role in various physiological and pathological processes, including inflammation-related diseases (Noonin and Thongboonkerd, 2021), and TDSCs-derived exosomes have shown therapeutic effects on tendon inflammation and apoptosis after Achilles tendon injury (Zhang et al., 2020). Additionally, drug monomers like tectorigenin have been found to improve the inflammatory response of TDSCs through the inflammatory signaling pathway NF-κB and MAPK (Moqbel et al., 2020). Aspirin is a classic anti-inflammatory drug. Wang (Wang et al., 2019) reported that aspirin can reverse the inflammatory response induced by IL-1β in TDSCs.

4.3 Strengths and limitations

This study possessed several strengths. Firstly, it corrected and updated similar articles in the original publication. Although an earlier article has employed bibliometrics to study TDSCs, their data was not filtered and it included numerous irrelevant papers. Consequently, the results failed to accurately represent the current status and hotspots in the field of TDSCs research. Our study provided accurate literature data that truly reflected the correct research status. Secondly, we conducted a comprehensive literature review guided by research hotspots. Thirdly, we summarize the research focus, cell sources, intervention factors, experimental methods, experimental indicators and main research findings of the current original research on TDSCs, and provide a detailed summary table for scientific researchers to find. However, our study only included data from one database, potentially resulting in the omission of some relevant research.

5 Conclusion

Tendon stem cells are crucial seed cells for tendon tissue engineering and hold significant research prospects. Currently, scaffolds, molecular mechanisms, and inflammation regulation have been prominent research areas in this field.

Data availability statement

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

Author contributions

SZ: Conceptualization, Writing–original draft, Writing–review and editing. JS: Writing–original draft. ZG: Writing–original draft. XG: Methodology, Writing–original draft. FW: Data curation, Methodology, Writing–review and editing. XHu: Investigation, Software, Writing–review and editing. GW: Investigation, Software, Writing–review and editing. HZ: Formal Analysis, Methodology, Writing–review and editing. JR: Software, Writing–review and editing. XHe: Investigation, Software, Writing–review and editing. CB: Investigation, Software, Writing–review and editing. ZZ: Investigation, Software, Writing–review and editing. XL: Investigation, Software, Writing–review and editing. HC: Conceptualization, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Zhejiang Provincial Department of Health Project (2022KY1313 and 2022KY1288).

Acknowledgments

Thanks to figdraw software for providing a picture painting platform.

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2023.1327027/full#supplementary-material

References

Ackerman, J. E., Best, K. T., Muscat, S. N., and Loiselle, A. E. (2021). Metabolic regulation of tendon inflammation and healing following injury. *Curr. Rheumatol. Rep.* 23 (3), 15. Epub 20210210. doi:10.1007/s11926-021-00981-4

Alberton, P., Dex, S., Popov, C., Shukunami, C., Schieker, M., and Docheva, D. (2015). Loss of tenomodulin results in reduced self-renewal and augmented senescence of tendon stem/progenitor cells. *Stem Cells Dev.* 24 (5), 597–609. Epub 20141210. doi:10.1089/scd.2014.0314

Andarawis-Puri, N., Flatow, E. L., and Soslowsky, L. J. (2015). Tendon basic science: development, repair, regeneration, and healing. *J. Orthop. Res.* 33 (6), 780–784. Epub 20150424. doi:10.1002/jor.22869

Bi, Y., Ehirchiou, D., Kilts, T. M., Inkson, C. A., Embree, M. C., Sonoyama, W., et al. (2007). Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat. Med.* 13 (10), 1219–1227. Epub 20070909. doi:10.1038/nm1630

Cai, Z., Zhang, Y., Liu, S., and Liu, X. (2022). Celecoxib, beyond anti-inflammation, alleviates tendon-derived stem cell senescence in degenerative rotator cuff tendinopathy. *Am. J. Sports Med.* 50 (9), 2488–2496. Epub 20220606. doi:10.1177/03635465221098133

Capella-Monsonis, H., Cramer, M., Turner, N., Reing, J., Zhang, L., Kronengold, R., et al. (2023). The composition and mechanical properties of porcine placental ecm from three different breeds. *Biomed. Phys. Eng. Express* 9, 065012. Epub 20230919. doi:10.1088/2057-1976/acfb05

Chen, H. S., Chen, Y. L., Harn, H. J., Lin, J. S., and Lin, S. Z. (2013). Stem cell Therapy for tendon injury. *Cell Transpl.* 22 (4), 677–684. Epub 20121008. doi:10.3727/096368912x655118

Chen, J., Zhang, E., Zhang, W., Liu, Z., Lu, P., Zhu, T., et al. (2017). Fos promotes early stage teno-lineage differentiation of tendon stem/progenitor cells in tendon. *Stem Cells Transl. Med.* 6 (11), 2009–2019. Epub 20171010. doi:10.1002/sctm.15-0146

Chen, L., Liu, J., Tao, X., Wang, G., Wang, Q., and Liu, X. (2015a). The role of Pin1 protein in aging of human tendon stem/progenitor cells. *Biochem. Biophys. Res. Commun.* 464 (2), 487–492. Epub 20150703. doi:10.1016/j.bbrc.2015.06.163

Chen, L., Wang, G. D., Liu, J. P., Wang, H. S., Liu, X. M., Wang, Q., et al. (2015b). Mir-135a modulates tendon stem/progenitor cell senescence via suppressing Rock1. *Bone* 71, 210–216. Epub 20141108. doi:10.1016/j.bone.2014.11.001

Chen, M., Li, Y., Xiao, L., Dai, G., Lu, P., and Rui, Y. (2021b). Noncanonical Wnt5a signaling regulates tendon stem/progenitor cells senescence. *Stem Cell Res. Ther.* 12 (1), 544. Epub 20211018. doi:10.1186/s13287-021-02605-1

Chen, M., Li, Y., Xiao, L., Dai, G., Lu, P., Wang, Y., et al. (2020). Aqp1 modulates tendon stem/progenitor cells senescence during tendon aging. *Cell Death Dis.* 11 (3), 193. Epub 20200318. doi:10.1038/s41419-020-2386-3

Chen, M., Xiao, L., Dai, G., Lu, P., Zhang, Y., Li, Y., et al. (2021a). Inhibition of jak-stat signaling pathway alleviates age-related phenotypes in tendon stem/progenitor cells. *Front. Cell Dev. Biol.* 9, 650250. Epub 20210329. doi:10.3389/fcell.2021.650250

Dai, G., Li, Y., Liu, J., Zhang, C., Chen, M., Lu, P., et al. (2020). Higher bmp expression in tendon stem/progenitor cells contributes to the increased heterotopic ossification in Achilles tendon with aging. *Front. Cell Dev. Biol.* 8, 570605. Epub 20200925. doi:10.3389/fcell.2020.570605

Dai, G., Li, Y., Zhang, M., Lu, P., Zhang, Y., Wang, H., et al. (2023). The regulation of the ampk/mtor Axis mitigates tendon stem/progenitor cell senescence and delays tendon aging. *Stem Cell Rev. Rep.* 19 (5), 1492–1506. Epub 20230314. doi:10.1007/s12015-023-10526-0

Dulińska-Molak, I., Pasikowska, M., Pogoda, K., Lewandowska, M., Eris, I., and Lekka, M. (2014). Age-related changes in the mechanical properties of human fibroblasts and its prospective reversal after anti-wrinkle tripeptide treatment. *Int. J. Pept. Res. Ther.* 20 (1), 77–85. Epub 20130918. doi:10.1007/s10989-013-9370-z

Ge, Z., Li, W., Zhao, R., Xiong, W., Wang, D., Tang, Y., et al. (2023). Programmable DNA hydrogel provides suitable microenvironment for enhancing tspcs Therapy in healing of tendinopathy. *Small* 19 (32), e2207231. Epub 20230417. doi:10.1002/smll.202207231

Giancola, R., Oliva, F., Gallorini, M., Michetti, N., Gissi, C., Moussa, F., et al. (2022). CD200 as a potential new player in inflammation during rotator cuff tendon injury/repair: an *in vitro* model. *Int. J. Mol. Sci.* 23 (23), 15165. Published 2022 Dec 2. doi:10.3390/ijms232315165

Gomez-Florit, M., Labrador-Rached, C. J., Domingues, R. M. A., and Gomes, M. E. (2022). The tendon microenvironment: engineered *in vitro* models to study cellular crosstalk. *Adv. Drug Deliv. Rev.* 185, 114299. Epub 20220415. doi:10.1016/j.addr.2022.114299

Han, W., Gu, D., Chen, H., Tao, X., and Chen, L. (2023). Hpf1 regulates tendon stem/progenitor cell senescence and tendon repair via parp1-mediated poly-adp ribosylation of hur. *Genes Genomics*. Epub 20230915. doi:10.1007/s13258-023-01447-w

Han, W., Tao, X., Weng, T., and Chen, L. (2022). Circular rna Pvt1 inhibits tendon stem/progenitor cell senescence by sponging microrna-199a-5p. *Toxicol Vitro* 79, 105297. Epub 20211209. doi:10.1016/j.tiv.2021.105297

Han, W., Wang, B., Liu, J., and Chen, L. (2017). The P16/mir-217/egr1 pathway modulates age-related tenogenic differentiation in tendon stem/progenitor cells. *Acta Biochim. Biophys. Sin. (Shanghai)* 49 (11), 1015–1021. doi:10.1093/abbs/gmx104

He, C., Zheng, S., Luo, Y., and Wang, B. (2018). Exosome theranostics: biology and translational medicine. *Theranostics* 8 (1), 237–255. Epub 20180101. doi:10.7150/thno.21945

Kalluri, R., and LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science* 367 (6478), eaau6977. doi:10.1126/science.aau6977

Kiderlen, S., Polzer, C., Rädler, J. O., Docheva, D., Clausen-Schaumann, H., and Sudhop, S. (2019). Age related changes in cell stiffness of tendon stem/progenitor cells and a rejuvenating effect of rock-inhibition. *Biochem. Biophys. Res. Commun.* 509 (3), 839–844. Epub 20190111. doi:10.1016/j.bbrc.2019.01.027

Kim, D. H., Noh, S. U., Chae, S. W., Kim, S. J., and Lee, Y. T. (2021). Altered differentiation of tendon-derived stem cells in diabetic conditions mediated by macrophage migration inhibitory factor. *Int. J. Mol. Sci.* 22 (16), 8983. Epub 20210820. doi:10.3390/ijms22168983

Kim, S. J., Baek, K. W., Jung, Y. K., Kim, J. S., Kim, B. G., Yu, H. S., et al. (2023). Changes in aquaporins expression due to acute water restriction in naturally aging mice. *J. Physiol. Biochem.* 79 (1), 71–81. Epub 20220921. doi:10.1007/s13105-022-00921-5

Lai, F., Wang, J., Tang, H., Bian, X., Lu, K., He, G., et al. (2021). Adipogenic differentiation was inhibited by downregulation of ppary signaling pathway in aging tendon stem/progenitor cells. *J. Orthop. Surg. Res.* 16 (1), 614. Epub 20211018. doi:10.1186/s13018-021-02720-y

Lai, F., Wang, J., Tang, H., Huang, P., Liu, J., He, G., et al. (2022). Vegf promotes tendon regeneration of aged rats by inhibiting adipogenic differentiation of tendon stem/progenitor cells and promoting vascularization. *Faseb J.* 36 (8), e22433. doi:10.1096/fj.202200213R

Lee, C. H., Lee, F. Y., Tarafder, S., Kao, K., Jun, Y., Yang, G., et al. (2015). Harnessing endogenous stem/progenitor cells for tendon regeneration. *J. Clin. Invest.* 125 (7), 2690–2701. Epub 20150608. doi:10.1172/jci81589

Lee, K. J., Clegg, P. D., Comerford, E. J., and Canty-Laird, E. G. (2018). A comparison of the stem cell characteristics of murine tenocytes and tendon-derived stem cells. *BMC Musculoskelet. Disord.* 19 (1), 116. Epub 20180412. doi:10.1186/s12891-018-2038-2

Leong, N. L., Kator, J. L., Clemens, T. L., James, A., Enamoto-Iwamoto, M., and Jiang, J. (2020). Tendon and ligament healing and current approaches to tendon and ligament regeneration. *J. Orthop. Res.* 38 (1), 7–12. Epub 20190930. doi:10.1002/jor.24475

Li, S., Sun, Y., Chen, Y., Lu, J., Jiang, G., Yu, K., et al. (2023). Sandwich biomimetic scaffold based tendon stem/progenitor cell alignment in a 3d microenvironment for

functional tendon regeneration. *ACS Appl. Mater Interfaces* 15 (3), 4652–4667. Epub 20230112. doi:10.1021/acsami.2c16584

Liu, C., Li, T. Y., Chen, Y., Yang, H. H., and Sun, Y. L. (2023a). Tendon microstructural disruption promotes tendon-derived stem cells to express chondrogenic genes by activating endoplasmic reticulum stress. *J. Orthop. Res.* 41 (2), 290–299. Epub 20220528. doi:10.1002/jor.25362

Liu, H., Sun, Z., Luo, G., Hu, Y., Ruan, H., Tu, B., et al. (2023b). Lncrna Meg3 promotes osteogenic differentiation of tendon stem cells via the mir-129-5p/tcf4/B-catenin Axis and thus contributes to trauma-induced heterotopic ossification. *Stem Cell Rev. Rep.* 19, 2311–2328. Epub 20230607. doi:10.1007/s12015-023-10562-w

Liu, Y., Feng, L., Wang, H., Wang, Y. J., Chan, H. C., Jiang, X. H., et al. (2018). Identification of an anti-inflammation protein, annexin A1, in tendon derived stem cells (tdscs) of cystic fibrosis mice: a comparative proteomic analysis. *Proteomics Clin. Appl.* 12 (6), e1700162. Epub 20180705. doi:10.1002/prca.201700162

Long, H., Yuan, Z., Yin, H., Yang, B., and Guo, A. (2022). Global research trends in tendon stem cells from 1991 to 2020: a bibliometric and visualized study. *Stem Cells Int.* 2022, 1–14. Epub 20220618. doi:10.1155/2022/7937765

Lu, J., Yang, X., He, C., Chen, Y., Li, C., Li, S., et al. (2023). Rejuvenation of tendon stem/progenitor cells for functional tendon regeneration through platelet-derived exosomes loaded with recombinant Yap1. *Acta Biomater.* 161, 80–99. Epub 20230217. doi:10.1016/j.actbio.2023.02.018

Lui, P. P., Wong, O. T., and Lee, Y. W. (2014). Application of tendon-derived stem cell sheet for the promotion of graft healing in anterior cruciate ligament reconstruction. *Am. J. Sports Med.* 42 (3), 681–689. Epub 20140122. doi:10.1177/0363546513517539

Mao, X., Yao, L., Li, M., Zhang, X., Weng, B., Zhu, W., et al. (2022). Enhancement of tendon repair using tendon-derived stem cells in small intestinal submucosa via M2 macrophage polarization. *Cells* 11 (17), 2770. Epub 20220905. doi:10.3390/cells11172770

Mifune, Y., Matsumoto, T., Ota, S., Nishimori, M., Usas, A., Kopf, S., et al. (2012). Therapeutic potential of anterior cruciate ligament-derived stem cells for anterior cruciate ligament reconstruction. *Cell Transpl.* 21 (8), 1651–1665. Epub 20120620. doi:10.3727/096368912x647234

Millar, N. L., Silbernagel, K. G., Thorborg, K., Kirwan, P. D., Galatz, L. M., Abrams, G. D., et al. (2021). Tendinopathy. *Nat. Rev. Dis. Prim.* 7 (1), 1. Epub 20210107. doi:10.1038/s41572-020-00234-1

Moqbel, S. A. A., Xu, K., Chen, Z., Xu, L., He, Y., Wu, Z., et al. (2020). Tectorigenin alleviates inflammation, apoptosis, and ossification in rat tendon-derived stem cells via modulating nf-kappa B and mapk pathways. *Front. Cell Dev. Biol.* 8, 568894. Epub 20201022. doi:10.3389/fcell.2020.568894

Nie, D., Zhang, J., Zhou, Y., Sun, J., Wang, W., and Wang, J. H. (2021a). Rapamycin treatment of tendon stem/progenitor cells reduces cellular senescence by upregulating autophagy. *Stem Cells Int.* 2021, 1–10. Epub 20210201. doi:10.1155/2021/6638249

Nie, D., Zhou, Y., Wang, W., Zhang, J., and Wang, J. H. (2021b). Mechanical overloading induced-activation of mtor signaling in tendon stem/progenitor cells contributes to tendinopathy development. *Front. Cell Dev. Biol.* 9, 687856. Epub 20210712. doi:10.3389/fcell.2021.687856

Noonin, C., and Thongboonkerd, V. (2021). Exosome-inflammasome crosstalk and their roles in inflammatory responses. *Theranostics* 11 (9), 4436–4451. Epub 20210304. doi:10.7150/thno.54004

Pearce, O., Brown, M. T., Fraser, K., and Lancerotto, L. (2021). Flexor tendon injuries: repair and rehabilitation. *Injury* 52 (8), 2053–2067. doi:10.1016/j.injury.2021.07.036

Rui, Y. F., Chen, M. H., Li, Y. J., Xiao, L. F., Geng, P., Wang, P., et al. (2019). Ctgf attenuates tendon-derived stem/progenitor cell aging. *Stem Cells Int.* 2019, 1–12. Epub 20191111. doi:10.1155/2019/6257537

Rui, Y. F., Lui, P. P., Li, G., Fu, S. C., Lee, Y. W., and Chan, K. M. (2010). Isolation and characterization of multipotent rat tendon-derived stem cells. *Tissue Eng. Part A* 16 (5), 1549–1558. doi:10.1089/ten.TEA.2009.0529

Salingcarnboriboon, R., Yoshitake, H., Tsuji, K., Obinata, M., Amagasa, T., Nifuji, A., et al. (2003). Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property. *Exp. Cell Res.* 287 (2), 289–300. doi:10.1016/s0014-4827(03)00107-1

Sanie-Jahromi, F., Nowroozzadeh, M. H., Shaabanian, M., Khademi, B., Owji, N., and Mehrabani, D. (2023). Characterization of central and nasal orbital adipose stem cells and their neural differentiation footprints. *Curr. Stem Cell Res. Ther.* 19. Epub 20230905. doi:10.2174/1574888x19666230905114246

Shang, J., Jiang, C., Cai, J., Chen, Z., Jin, S., Wang, F., et al. (2023). Knowledge mapping of macrophage in spinal cord injury: a bibliometric analysis. *World Neurosurg.* Epub 20230913. doi:10.1016/j.wneu.2023.09.022

Tan, G. K., Pryce, B. A., Stabio, A., Keene, D. R., Tufa, S. F., and Schweitzer, R. (2021). Cell autonomous tgfβ signaling is essential for stem/progenitor cell recruitment into degenerative tendons. *Stem Cell Rep.* 16 (12), 2942–2957. Epub 20211124. doi:10.1016/j.stemcr.2021.10.018

Tan, Q., Lui, P. P., Rui, Y. F., and Wong, Y. M. (2012). Comparison of potentials of stem cells isolated from tendon and bone marrow for musculoskeletal tissue engineering. *Tissue Eng. Part A* 18 (7-8), 840–851. Epub 20111213. doi:10.1089/ten.TEA.2011.0362

Tarafder, S., Chen, E., Jun, Y., Kao, K., Sim, K. H., Back, J., et al. (2017). Tendon stem/progenitor cells regulate inflammation in tendon healing via jnk and Stat3 signaling. *Faseb J.* 31 (9), 3991–3998. Epub 20170522. doi:10.1096/fj.201700071R

Thomopoulos, S., Parks, W. C., Rifkin, D. B., and Derwin, K. A. (2015). Mechanisms of tendon injury and repair. *J. Orthop. Res.* 33 (6), 832–839. Epub 20150302. doi:10.1002/jor.22806

Vinhas, A., Rodrigues, M. T., and Gomes, M. E. (2018). Exploring stem cells and inflammation in tendon repair and regeneration. *Adv. Exp. Med. Biol.* 1089, 37–46. doi:10.1007/5584_2018_258

Wang, G., Wang, S., Ouyang, X., Wang, H., Li, X., Yao, Z., et al. (2023). Glycolipotoxicity conferred tendinopathy through ferroptosis dictation of tendon-derived stem cells by yap activation. *IUBMB Life* 75, 1003–1016. Epub 20230728. doi:10.1002/iub.2771

Wang, Y., He, G., Tang, H., Shi, Y., Kang, X., Lyu, J., et al. (2019). Aspirin inhibits inflammation and scar formation in the injury tendon healing through regulating JNK/STAT-3 signalling pathway. *Cell Prolif.* 52 (4), e12650. doi:10.1111/cpr.12650

Wang, Y., Jin, S., Luo, D., He, D., Shi, C., Zhu, L., et al. (2021). Functional regeneration and repair of tendons using biomimetic scaffolds loaded with recombinant periostin. *Nat. Commun.* 12 (1), 1293. Epub 20210226. doi:10.1038/s41467-021-21545-1

Xu, H., and Liu, F. (2018). Downregulation of Foxp1 correlates with tendon stem/progenitor cells aging. *Biochem. Biophys. Res. Commun.* 504 (1), 96–102. Epub 20180829. doi:10.1016/j.bbrc.2018.08.136

Xu, L., Chen, Z., Geng, T., Ru, B., Wan, Q., Zhang, J., et al. (2022). Irisin promotes the proliferation and tenogenic differentiation of rat tendon-derived stem/progenitor cells via activating YAP/TAZ. *Vitro Cell Dev Biol Anim* 58 (8), 658–668. Epub 20220920. doi:10.1007/s11626-022-00699-2

Yan, S., Zhang, M., Yang, G., Sun, Y., and Ai, D. (2022). Ctgf promotes the osteoblast differentiation of human periodontal ligament stem cells by positively regulating bmp2/smad signal transduction. *Biomed. Res. Int.* 2022, 1–10. Epub 20220915. doi:10.1155/2022/2938015

Yao, S., Liang, Z., Lee, Y. W., Yung, P. S. H., and Lui, P. P. Y. (2023). Bioactive decellularized tendon-derived stem cell sheet for promoting graft healing after anterior cruciate ligament reconstruction. *Am. J. Sports Med.* 51 (1), 66–80. doi:10.1177/03635465221135770

Yin, Z., Chen, X., Chen, J. L., Shen, W. L., Hieu Nguyen, T. M., Gao, L., et al. (2010). The regulation of tendon stem cell differentiation by the alignment of nanofibers. *Biomaterials* 31 (8), 2163–2175. Epub 20091207. doi:10.1016/j.biomaterials.2009.11.083

Yin, Z., Chen, X., Zhu, T., Hu, J. J., Song, H. X., Shen, W. L., et al. (2013). The effect of decellularized matrices on human tendon stem/progenitor cell differentiation and tendon repair. *Acta Biomater.* 9 (12), 9317–9329. Epub 20130726. doi:10.1016/j.actbio.2013.07.022

Yin, Z., Hu, J. J., Yang, L., Zheng, Z. F., An, C. R., Wu, B. B., et al. (2016). Single-cell analysis reveals a Nestin(+) tendon stem/progenitor cell population with strong tenogenic potentiality. *Sci. Adv.* 2 (11), e1600874. Epub 20161118. doi:10.1126/sciadv.1600874

Zhang, C., Zhu, J., Zhou, Y., Thampatty, B. P., and Wang, J. H. (2019a). Tendon stem/progenitor cells and their interactions with extracellular matrix and mechanical loading. *Stem Cells Int.* 2019, 1–10. Epub 20191013. doi:10.1155/2019/3674647

Zhang, H., Chen, Y., Fan, C., Liu, R., Huang, J., Zhang, Y., et al. (2022). Cell-subpopulation alteration and Fgf7 activation regulate the function of tendon stem/progenitor cells in 3d microenvironment revealed by single-cell analysis. *Biomaterials* 280, 121238. Epub 20211105. doi:10.1016/j.biomaterials.2021.121238

Zhang, H., Dai, Y., Long, H., Cao, R., Shi, L., Zhao, J., et al. (2023a). Tendon stem/progenitor cell-laden nanofiber hydrogel enhanced functional repair of patellar tendon. *Tissue Eng. Part A* 29 (5-6), 150–160. Epub 20230125. doi:10.1089/ten.TEA.2022.0183

Zhang, J., Nie, D., Williamson, K., Rocha, J. L., Hogan, M. V., and Wang, J. H. (2019b). Selectively activated prp exerts differential effects on tendon stem/progenitor cells and tendon healing. *J. Tissue Eng.* 10, 204173141882003. Epub 20190116. doi:10.1177/2041731418820034

Zhang, J., and Wang, J. H. (2010a). Characterization of differential properties of rabbit tendon stem cells and tenocytes. *BMC Musculoskelet. Disord.* 11, 10. Epub 20100118. doi:10.1186/1471-2474-11-10

Zhang, J., and Wang, J. H. (2010b). Platelet-rich plasma releasate promotes differentiation of tendon stem cells into active tenocytes. *Am. J. Sports Med.* 38 (12), 2477–2486. Epub 20100827. doi:10.1177/0363546510376750

Zhang, J., and Wang, J. H. (2010c). Mechanobiological response of tendon stem cells: implications of tendon homeostasis and pathogenesis of tendinopathy. *J. Orthop. Res.* 28 (5), 639–643. doi:10.1002/jor.21046

Zhang, M., Liu, H., Cui, Q., Han, P., Yang, S., Shi, M., et al. (2020). Tendon stem cell-derived exosomes regulate inflammation and promote the high-quality healing of injured tendon. *Stem Cell Res. Ther.* 11 (1), 402. Epub 20200917. doi:10.1186/s13287-020-01918-x

Zhang, Y., Zhang, E., Qin, T., Liu, M., Zhou, S., Lin, R., et al. (2023b). Matrix stiffness-mediated tenogenesis of tendon stem/progenitor cells via integrin-αm for tendon regeneration. *Biochem. Biophys. Res. Commun.* 678, 90–96. Epub 20230817. doi:10.1016/j.bbrc.2023.08.007

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Original Research
PUBLISHED 08 January 2024
DOI 10.3389/fbioe.2023.1290693

OPEN ACCESS

EDITED BY
Cheemeng Tan,
University of California, Davis,
United States

REVIEWED BY
Roland Manfred Klar,
University of Missouri–Kansas City,
United States
Linda A. Dahlgren,
Virginia Tech, United States

*CORRESPONDENCE
Mandi Lopez,
mlopez@lsu.edu

†These authors share first authorship

RECEIVED 07 September 2023
ACCEPTED 11 December 2023
PUBLISHED 08 January 2024

CITATION
Taguchi T, Lopez M and Takawira C (2024), Viable tendon neotissue from adult adipose-derived multipotent stromal cells.
Front. Bioeng. Biotechnol. 11:1290693.
doi: 10.3389/fbioe.2023.1290693

Viable tendon neotissue from adult adipose-derived multipotent stromal cells

Takashi Taguchi†, Mandi Lopez*† and Catherine Takawira

Laboratory for Equine and Comparative Orthopedic Research, School of Veterinary Medicine, Veterinary Clinical Sciences Department, Louisiana State University, Baton Rouge, LA, United States

Background: Tendon healing is frequently prolonged, unpredictable, and results in poor tissue quality. Neotissue formed by adult multipotent stromal cells has the potential to guide healthy tendon tissue formation.

Objectives: The objective of this study was to characterize tendon neotissue generated by equine adult adipose-derived multipotent stromal cells (ASCs) on collagen type I (COLI) templates under 10% strain in a novel bioreactor. The tested hypothesis was that ASCs assume a tendon progenitor cell-like morphology, express tendon-related genes, and produce more organized extracellular matrix (ECM) in tenogenic versus stromal medium with perfusion and centrifugal fluid motion.

Methods: Equine ASCs on COLI sponge cylinders were cultured in stromal or tenogenic medium within bioreactors during combined perfusion and centrifugal fluid motion for 7, 14, or 21 days under 10% strain. Viable cell distribution and number, tendon-related gene expression, and micro- and ultra-structure were evaluated with calcein-AM/EthD-1 staining, resazurin reduction, RT-PCR, and light, transmission, and scanning electron microscopy. Fibromodulin was localized with immunohistochemistry. Cell number and gene expression were compared between culture media and among culture periods ($p < 0.05$).

Results: Viable cells were distributed throughout constructs for up to 21 days of culture, and cell numbers were higher in tenogenic medium. Individual cells had a round or rhomboid shape with scant ECM in stromal medium in contrast to clusters of parallel, elongated cells surrounded by highly organized ECM in tenogenic medium after 21 days of culture. Transcription factor, extracellular matrix, and mature tendon gene expression profiles confirmed ASC differentiation to a tendon progenitor-like cell in tenogenic medium. Construct micro- and ultra-structure were consistent with tendon neotissue and fibromodulin was present in the ECM after culture in tenogenic medium.

Conclusion: Long-term culture in custom bioreactors with combined perfusion and centrifugal tenogenic medium circulation supports differentiation of equine adult ASCs into tendon progenitor-like cells capable of neotissue formation.

KEYWORDS

bioengineering, ligament, stem cells, bioreactor, tissue regeneration, *de novo* tissue generation, equine

Introduction

Tendinopathy and desmitis comprise a large majority of musculoskeletal injuries that are responsible for up to 72% of lost training days and 14% of early retirements by equine athletes (Rossdale et al., 1985; Olivier et al., 1997; Lam et al., 2007). Superficial digital flexor tendinopathy and suspensory ligament desmitis are the most common, comprising 46% of all limb injuries (Williams et al., 2001; Bertuglia et al., 2014). The predominant type of tendon and ligament injury varies among disciplines, but all equine companions can be impacted. Strain induced injuries are common in the equine suspensory apparatus including the suspensory ligament, superficial digital flexor tendon, and deep digital flexor tendon (O'Sullivan, 2007). Many acute and chronic tendon and ligament lesions are thought to result from focal accumulation of microtrauma and poorly organized repair tissue that can coalesce into large lesions and predispose to spontaneous rupture in numerous species (Kannus and Jozsa, 1991).

Diagnosis is usually a combination of physical examination and ultrasound imaging (Dyson et al., 2018). Treatments vary widely and can range from rest with anti-inflammatory drugs, cold therapy, and pressure bandaging to intralesional therapies and extracorporeal shock wave treatment (Bostrom et al., 2022; Giunta et al., 2019; S; Witte et al., 2016). Intralesional regenerative treatments such as platelet rich plasma, stem cells, and genetic material have been applied with variable success (Aimaletdinov et al., 2020; Geburek et al., 2017; Kovac et al., 2018; Witte et al., 2011). Short-term outcomes of these treatments are favorable. However, poor, or abnormal tissue repair contributes to a reinjury rate in horses as high as 67% within 2 years (Dyson, 2004; Marr et al., 1993). To date, there is no single gold standard to promote healing of ligament and tendon lesions.

There are four recognized stages of tendon and ligament healing: an acute inflammatory phase, a subacute reparative phase, a collagen phase, and a chronic remodeling phase. Low cell numbers and metabolic activity, limited blood supply, and failure of endogenous tenocytes and ligamentocytes to migrate to the injury site affect all stages of healing and contribute to poor tissue healing capacity in adult animals (Longo et al., 2007; Sakabe and Sakai, 2011). Research confirms enhanced healing capacity of neonatal tendon over that of adults since early fibrous scar tissue is replaced with normal tendon by endogenous tenocytes recruited by TGF-β signaling (Howell et al., 2017; Kaji et al., 2020).

Autologous tenocyte implantation is one mechanism to deliver endogenous cells to the site of tendon or ligament injury in adult animals and humans (Chen et al., 2011; Wang et al., 2013; Wang et al., 2005); however, the therapy is limited by few harvest sites and harvest morbidity, and it is not practical in horses. Administration of exogenous adult multipotent stromal cells (MSCs) is reported to augment natural healing in naturally-occurring and experimentally-induced equine tendon and ligament injuries (Smith et al., 2013; Van Loon et al., 2014; Geburek et al., 2017; Romero et al., 2017; Carlier et al., 2023). Results are mixed, in part due to differences among cell isolates, lesions, individual healing capacity, and low engraftment of exogenous cells (<0.001%) (Reed and Leahy, 2013; Geburek et al., 2017). Further, there is evidence that an inflammatory environment may impede differentiation of MSCs, and the cells may assume an abnormal phenotype leading to unwanted side effects (Harris et al., 2004; Fahy et al., 2014).

The delivery of cells on scaffold matrix, often made of natural and/or synthetic polymers, is reported to improve cellular retention at the site of implantation (Pillai et al., 2017; Rashedi et al., 2017; Vadaye Kheiry et al., 2021; Zhao et al., 2010). Collagen is the most abundant natural polymer in the body and a common material for tissue engineering templates due to inherent biocompatibility (Xie et al., 2013). Collagen type I (COLI) comprises 60%–80% of tendon and ligament structure, and there are numerous commercially available, FDA-approved formulations (Cockerham and Hsu, 2009; Longo et al., 2010; Snedeker and Foolen, 2017; Meyer, 2019). Scaffolds composed of COLI are routinely used for delivery and retention of stem cells in tendon and ligament tissue (Ma et al., 2019). Published information confirms that COLI matrix supports differentiation of equine MSCs into diverse tissue lineages (Xie et al., 2013; Muller et al., 2016; Duan et al., 2017). Additionally, evidence suggests differentiation of MSCs into lineages native to the site of injection prior to implantation is essential (Shojaee, Parham, 2019). Pre-implantation differentiation of cells into tenocytes is reported to minimize the risk of ectopic bone and cartilage formation as well as tumor formation at the site of injection (Lui et al., 2011). Recently, injection of tenogenically differentiated allogeneic equine MSCs into naturally occurring tendon lesions resulted in a lower reinjury rate (18%) than conventional treatments (44%) 24 months post-treatment (Beerts et al., 2017). Regardless of the specific target tissue, current knowledge supports that MSCs that are induced to assume characteristics of native tissue lineage and embedded in scaffold matrix prior to implantation have better engraftment and promote more robust tissue healing than undifferentiated primary cell isolates (Aurich et al., 2009).

In the study reported here, tendon neotissue was created by culturing equine adult adipose-derived MSCs (ASCs) on COLI templates. The templates were maintained under 10% strain with combined perfusion and centrifugal culture medium motion within custom perfusion bioreactors. The hypothesis was that ASCs assume a tendon progenitor cell-like morphology, express tendon-related genes, and produce more organized extracellular matrix in tenogenic versus stromal medium with perfusion and centrifugal fluid motion for 7, 14, or 21 days. Generation of viable tendon neotissue implants has the potential to augment contemporary therapies for equine tendinopathy. Longitudinal evaluation of constructs from shortly after fabrication to up to 21 days of culture is vital to establishing the process and time course of *de novo* equine tendon tissue generation for preclinical testing and clinical translation.

Materials and methods

Study design

Equine ASC-COLI constructs were created by addition of 1.0×10^6 cells/cm^3 (P2) from individual donors (4 geldings, 4 mares, body condition score 4–7, 5–21 years, 425–500 kg) to culture medium in separate, custom-designed perfusion bioreactor chambers that each contained a cylinder of commercially available bovine COLI (Avitene™ Ultrafoam™ Collagen Sponge, Davol Inc., Warwick, RI) (n = 48 constructs, 3 construct pairs/donor). One-half of the constructs from each donor were cultured in stromal or tenogenic medium (n = 3 medium/donor) within individual perfusion

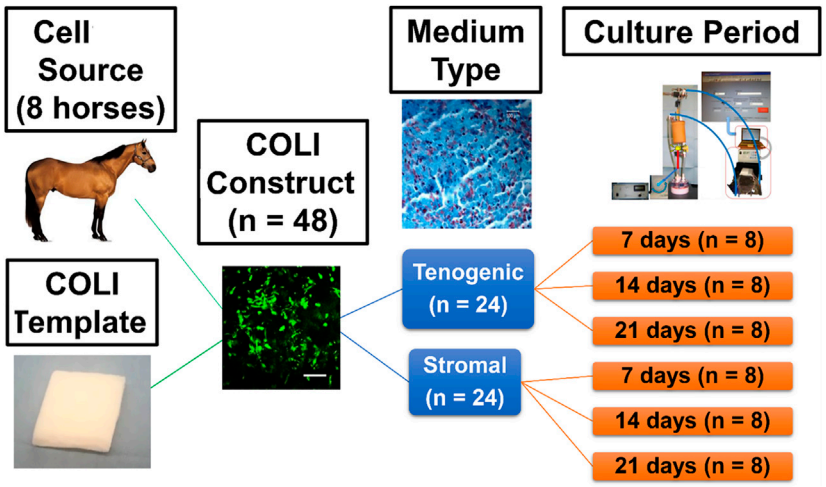


FIGURE 1
Study design schematic.

bioreactors for 7, 14, or 21 days (n = 1 donor/medium/time point) under 10% static strain (Figure 1). Construct gross appearance was documented with digital imaging prior to sample collection from the upper, middle, and lower regions of the construct relative to bioreactor orientation for outcome measures. Viable cell distribution and number, tendon-related gene expression, and micro- and ultra-structure were evaluated with calcein-AM/EthD-1 staining, resazurin reduction, RT-PCR, and light-as well as transmission and scanning electron microscopy. The deposition of fibromodulin within constructs was localized with immunohistochemistry. Fibromodulin is a key regulator of tendon fibril maturation and it is most prevalent in the later stages of embryonic fibrillogenesis (Banos et al., 2008; Zhao et al., 2023).

Primary cell isolates

No horses were euthanized for this study. All tissues for primary cell isolation were collected immediately post-mortem from horses euthanized as part of an approved study protocol (IACUCAM-21–141) that was unrelated to this study. About 45 mL of subcutaneous adipose tissue was aseptically harvested via sharp dissection from the supragluteal region of 4 adult geldings and 4 mares (Vidal et al., 2007). Harvested tissues were placed in sterile 50 mL conical tubes (Nunc™, Thermo Fisher Scientific, Waltham, MA) for transport. Donor inclusion criteria were: 1) 425–500 kg; 2) 5–21 years; 3) no acute or chronic systemic illness; 4) mare or gelding; 5) body condition score 4–7. The stromal vascular fraction was isolated as previously described within 2 h of harvest (Vidal et al., 2007). Briefly, adipose tissue was minced and mixed with an equal volume of phosphate buffered saline (PBS, PBS 1X, Thermo Fisher Scientific). The mixture was allowed to separate into two phases, and the infranatant was digested for 2 h at 37°C in an equal volume of PBS with 1% bovine serum albumin (BSA, Sigma Aldrich, Co, Saint Louis, MO) and 0.1% type I collagenase (Worthington Biochemical, Lakewood, NJ). After addition of 1% BSA, the mixture was centrifuged (2.6×10^2 g, 5 min, 4°C). The resulting stromal vascular fraction pellet was resuspended in PBS and centrifuged (2.6×10^2 g, 5 min). The pellet was resuspended in stromal medium (Dulbecco's modified eagle medium (DMEM)-Ham F12 (HyClone Laboratories, LLC, Logan, UT), 10% fetal bovine serum (FBS, HyClone Laboratories), 1% antibiotic/antimycotic solution (HyClone Laboratories). Viable cell numbers were quantified with methylene blue (methylene blue hydrate, Sigma Aldrich) staining and a hemocytometer (Hausser Scientific™ Bright-Line™ Counting Chamber, Fisher Scientific).

Isolated cells were cultured in 10 cm culture dishes (CellStar®, VWR, Radnor, PA) at 5×10^3 cells/cm^2 with stromal medium that was refreshed after 24 h and then every 2-3 days (5% CO_2, 37°C, 90% humidity). Cells were detached [25% trypsin (HyClone Laboratories)] and passaged at 80% confluence. Subsequently, P0 cells (10^6 cells/mL) in cryopreservation medium [80% FBS, 10% DMEM-Ham F12, 10% DMSO (Thermo Fisher Scientific)] within cryopreservation vials (Fisherbrand™ Externally and Internally Threaded Cryogenic Storage Vials, Thermo Fisher Scientific) were cooled to −80°C in a freezing container (Corning® CoolCell™ Freezer Container, Sigma Aldrich) and then transferred to liquid nitrogen (−150°C). Samples in cryopreservation vials were thawed in a water bath (37°C), and cryopreservation medium was removed by resuspension of cell pellets in PBS after centrifugation (2.6×10^2 g, 5 min) 3-4 times. Cells were subcultured to P1 in stromal medium as described above. Passage 2 cells on COLI templates were cultured in tenogenic (DMEM-high glucose (HyClone Laboratories), 1% FBS, 10 ng/mL transforming growth factor (TGF)-β1 (Shenandoah Biotechnology, Inc., Warminster, PA), 50 μM L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate (Sigma Aldrich), 0.5 μg/mL insulin (Sigma-Adrich), 1% antibiotic/antimycotic) (Theiss et al., 2015) or stromal medium [(Dulbecco's modified Eagle medium (DMEM)-Ham F12 (Hyclone Laboratories), 10% fetal bovine serum (FBS, HyClone Laboratories), 1% antibiotic/antimycotic solution (HyClone Laboratories)].

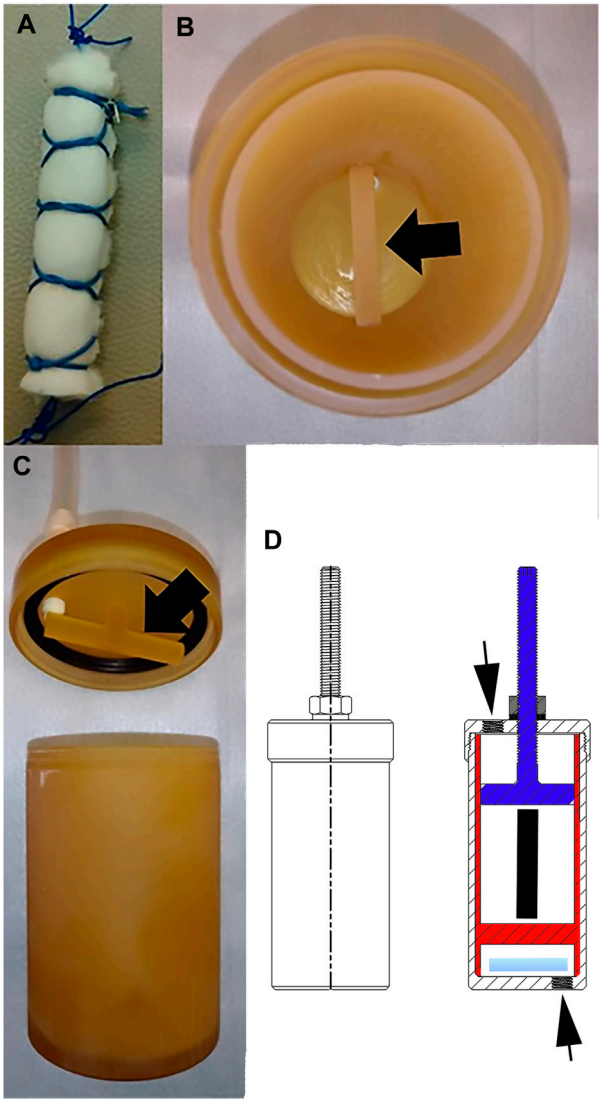


FIGURE 2
Bioreactor. Photographs of a COLI template **(A)**, the immobile horizontal bar (black arrow) at the lowest end of the frame insert within a bioreactor chamber **(B)**, the adjustable horizontal bar (black arrow) on the lid of the bioreactor chamber **(C)**, and drawings of the outside (left) and inside (right) of the bioreactor **(D)**. The adjustable top bar attached to the bioreactor lid (blue) and secured with a nut (gray) on a rubber washer (horizontal black line beneath the washer), the insert (red) with the lower bar, the stir bar (light blue), the position of the construct within the bioreactor (black vertical line) and the ports on the cap and the bottom of the bioreactor (black arrows) are illustrated.

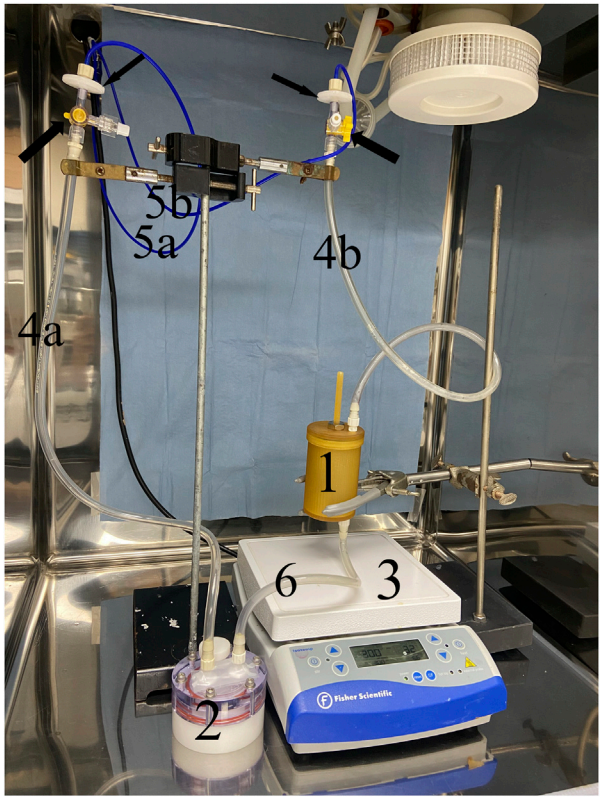


FIGURE 3
Perfusion bioreactor system. Medium perfusion was driven by a computer-controlled peristaltic pump connected to the bioreactor chamber (1) and a medium reservoir (2). The pump was attached with 1.0 mm tubing (5a) to a 0.22 µm syringe filter which was connected to a segment of 4.8 mm tubing (4a) attached to one port on the upper surface of a 10 mL medium reservoir (2). Another port on the upper surface of the medium reservoir was attached to the lower port of the bioreactor chamber with 4.8 mm tubing (6). The pump was also connected with 1.0 mm tubing (5b) to another 0.22 µm syringe filter (small black arrow) which was connected with a 3-way stopcock (large black arrow) 4.8 mm tubing (4b) attached to the upper port on the bioreactor chamber (1). A magnetic stir bar at the lowest end of the bioreactor chamber driven by a stir plate (3) provided centrifugal fluid motion.

Perfusion bioreactor system

Templates were composed of a commercially available COLI sponge consisting of a partial hydrochloric acid salt of purified bovine corium (Avitene™ Ultrafoam™ Collagen Sponge, Davol Inc.) that is approved for use as microfibrillar collagen hemostat by the US Food and Drug Administration (Zhou et al., 2016; Cziperle, 2021). The sponge is porous, pliable, water insoluble and bioabsorbable, and it is produced by lyophilization of a slurry of water and purified collagen. The manufacturing process permits noncovalent attachment of hydrochloric acid to amine groups on the collagen molecules and preserves their native morphology.

For each template, a COLI sponge section, 6.0 × 4.0 × 1.0 cm (length × width × height), was rolled into a column with a diameter of 1.0 cm and height of 6.0 cm that was surrounded by a finger trap composed of #0 polydioxanone suture (PDS® II, Ethicon, Inc., Somerville, NJ) (Figure 2A). There were 1 cm long loops on each end. The bioreactor was a custom design by the authors that was produced by stereolithography out of liquid resin (SOMOS® WaterShed® XC 11122, Stratasys, Waltham, MA) by a commercial printing company (Proto Labs, Inc., Maple Plain, MN). A frame with an immobile cross beam on the lowest end fit within a single bioreactor chamber measuring 80 × 45 mm (height × diameter) (Figures 2B–D). The bioreactor lid was secured to the top of the chamber with matching threads. A threaded, vertically adjustable cross beam attached to the upper

lid with matching threads extended downward into the bioreactor chamber within the frame insert. The distance of the bar from the lid (and the lower beam on the insert) was adjusted by turning the beam to advance toward or away from the lid, and the position was fixed with a nut on the top of the lid. The space between the nut and the lid was sealed with sterile rubber gasket material. Constructs were secured to the upper beam on the lid and to the lower beam on the frame with the suture loops on each end. The distance between the beams was initially set at the correct distance for a 10% construct strain based on the length of the construct with no tension applied as determined with an electronic caliper (Mitutoyo #500–196, Mitutoyo Corp., Japan). When medium was exchanged every 7 days, the height of the upper beam was adjusted if necessary to maintain 10% strain based on the construct length determined at that time. A magnetic stir bar (2.5 × 0.7 cm) was placed in the chamber beneath the lowest bar of the frame insert.

Medium perfusion was driven by a computer-controlled peristaltic pump (Ismatec model ISM404b, Huiyu Weiye (Beijing) Fluid Equipment Co., Ltd., Beijing, China) connected to the bioreactor chamber and a medium reservoir (Figure 3). Specifically, tubing (1.0 mm inner diameter; Tygon®; Compagnie de Saint-Gobain, Courbevoie, Centre, France) extended from the pump to a 0.22 µm sterile syringe filter (MilliporeSigma™, Thermo Fisher Scientific) which was connected with a 3-way stopcock to 55 cm of additional tubing (4.8 mm inner diameter; Tygon®, Compagnie de Saint-Gobain) connected to a port on the bioreactor. The pump was separately attached with tubing (1.0 mm inner diameter; Tygon®, Compagnie de Saint-Gobain) to a 0.22 µm sterile syringe filter (MilliporeSigma™, Thermo Fisher Scientific) which was connected to another 55 cm segment of tubing (4.8 mm inner diameter; Tygon®, Compagnie de Saint-Gobain) attached to one port on the upper surface of a 10 mL medium reservoir (High Aspect Ratio Vessel, Synthecon, Inc., Houston, TX). Another port on the upper surface of the medium reservoir was attached to the lower port of the bioreactor chamber with 10 cm of tubing (4.8 mm inner diameter; Tygon®, Compagnie de Saint-Gobain). The reservoir provided medium oxygenation via a flat, silicone rubber gas transfer membrane.

A fluid flow rate of 10 mL/min was maintained by a computer program (LabView™, National Instruments, Austin, TX), and the direction of fluid flow was reversed when it reached the upper and lower ends of the tubing between the bioreactor lid and the syringe filter. Centrifugal medium motion within the chamber was generated with the magnetic stir bar beneath the frame in the chamber (300 rpm) that was driven by a stir plate (Isotemp™, Thermo Fisher Scientific) positioned beneath the chamber system. The fluid flow was both ingress-egress and centrifugal. Fluid flow rate and stir speed were based on previous work with custom perfusion and spinner flask bioreactors as well as progressive iterations of the current system to optimize viable cell distribution in the scaffold (Xie et al., 2013; Duan et al., 2017; Taguchi et al., 2021). All bioreactor system parts were sterilized with ethylene oxide prior to assembly and use. The perfusion system was maintained in a CO_2 incubator (5% CO_2, 37°C) for the duration of the culture period.

A previously reported method was used to seed P2 ASCs on the COLI template (Taguchi et al., 2021). Specifically, a template was premoistened and added to the bioreactor after the system was filled with stromal or tenogenic culture medium. It was maintained in the incubator for 1 h with fluid motion. Subsequently, the fluid motion was paused. Cells (1×10^6 cells/cm^3 template) were added to the medium of the bioreactor chamber through the 3-way port on the tubing attached to the bioreactor lid, and fluid motion was restarted. Medium was exchanged every 7 days through the reservoir (Taguchi et al., 2021).

Construct sample collection

Constructs were harvested after 7, 14, or 21 days of culture. To ensure collection of representative samples from the entire construct length and width, constructs were gently unrolled following suture removal. The long axis of each construct rectangle was divided into three equal regions (approximately 2 cm each) that corresponded to the highest, middle, and lowest point of the bioreactor. A 4.0 mm diameter biopsy punch (Integra™ Miltex™ Standard Biopsy Punches, Thermo Fisher Scientific) was used to collect full thickness samples from each region. Differences in cell viability staining among harvest regions were subjectively assessed. Samples from each region were combined for all other outcome measures.

Viable cell distribution and relative cell number

To evaluate viable cell distribution, specimens were incubated in darkness with 4.0 µM calcein acetoxymethyl ester (Invitrogen™, calcein-AM, Thermo Fisher Scientific) and ethidium homodimer-1 (Invitrogen™ EthD-1, Thermo Fisher Scientific) in PBS at 20°C for 30 min to stain viable and nonviable cells, respectively. After incubation, specimens were washed with PBS, compressed between a glass slide and cover glass, and evaluated with a confocal laser microscope (TCS SP8, Leica, Wetzlar, Germany). Cell morphology and viability were assessed in multiple z planes to examine the full thickness of each specimen. Digital images were generated with a camera integrated into the microscope.

The number of viable cells in each specimen was indirectly quantified based on cell metabolic activity measured by resazurin reduction (alamarBlue™, Thermo Fisher Scientific). Individual biopsy samples were incubated in 100 µL of 50 µM resazurin at 37°C for 3 h. After incubation, 50 µL was mixed with 50 µL of PBS, and resorufin fluorescence was measured at an excitation wavelength of 540 nm and an emission wavelength of 590 nm using a microplate reader (SPARK® Multimode Microplate Reader, Tecan, Männdorf, Switzerland). Fluorescence values were normalized to background fluorescence measured from resazurin solution incubated with no specimen.

Gene expression

Specimens were digested at 37°C in 0.1% type I collagenase in PBS for 1 h, centrifuged (3×10^2 g, 10 min, 4°C), and the supernatant discarded. One milliliter of TRI reagent® (Sigma Aldrich) was immediately added to the precipitate, the solution homogenized by aspirating through an 18-gauge needle 30 times, and the homogenate centrifuged (2.1×10^4 g, 15 min, 4°C). Total RNA was initially extracted with phenol-chloroform (Sigma Aldrich) and then with a commercially available kit (RNeasy® Mini Kit, QIAGEN Sciences, Germantown, MD). One microgram of total

RNA was used for cDNA synthesis (QuantiTect® Reverse Transcription Kit, QIAGEN Sciences).

Equine-specific primers for tendon-related genes, *scleraxis* (*Scx*), *mohawk* (*Mkx*), *early growth response 1* (*Egr1*), *connective tissue growth factor* (*CTGF*), *lysyl oxidase* (*LOX*), *collagen 1a1* (*Col1a1*), *collagen 3a1* (*Col3a1*), *decorin* (*Dcn*), *elastin* (*Eln*), *tenascin-c* (*Tnc*), *biglycan* (*Bgn*), *fibromodulin* (*Fbmd*), *collagen 14a1* (*Col14a1*), and *truncated hemoglobin 4* (*THBS4),* were prepared using previously published sequences or designed with Primer-BLAST (Ye et al., 2012; Miyabara et al., 2014; Mohanty et al., 2014; Jacobson et al., 2015; Yang et al., 2019) (Table 1). The PCR cycles included an initial denaturation step (95°C, 15 min) followed by 40 denaturation cycles (94°C, 15 s), annealing (52°C, 30 s), and elongation (72°C, 30 s) using SYBR Green (QuantiTect® SYBR® Green PCR Kits, QIAGEN Sciences). Target gene expression in constructs cultured in tenogenic and stromal medium was normalized to the reference gene *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*). Fold change between constructs cultured in tenogenic versus stromal medium was calculated as $2^{-\Delta\Delta CT}$. The relative gene expression for constructs cultured in stromal medium (reference) was set to 1 since when ΔΔCT is equal to 0 (no change), 2^0 is equal to 1 (Livak and Schmittgen, 2001; Abate et al., 2009).

Histological analysis

Specimens were fixed in 4% paraformaldehyde, serially dehydrated in increasing concentrations of ethanol and xylene, paraffin embedded, sectioned (5 μm) and stained with hematoxylin and eosin (H&E). Cell morphology, distribution, and extra cellular matrix were evaluated on digital images generated with a slide scanner (NanoZoomer S20, Hamamatsu Photonics, Shimokanzo, Iwata City, Shizuoka Pref., 438-0193, Japan) or with a light microscope (DM4500B, Leica) fitted with a camera (DFC480, Leica).

Immunohistochemical fibromodulin staining

Paraffin sections on glass slides were incubated in PBST (0.1% Triton X-100 (Sigma Aldrich) in PBS) at 20°C for 10 min, in antigen retrieval buffer [100 mM Tris (Sigma Aldrich), 5% Urea (Sigma Aldrich) pH 9.5] at 121°C for 30 min, and then in blocking buffer (1% BSA and 22.5 mg/mL glycine (Sigma Aldrich) in PBST) at 20°C for 30 min. Subsequently, slides were incubated with rabbit anti-human fibromodulin (PA5-26250, Thermo Fisher Scientific) polyclonal antibody at concentration of 1:100 in incubation buffer (1% BSA in PBST) overnight at 4°C. Sections were washed with PBS at 20°C 3 times for 15 min each, then stained with goat anti-rabbit IgG conjugated with Alexa Fluor™ 488 (A11070, Thermo Fisher Scientific) at a concentration of 1:200 in incubation buffer for 1 h at 20°C. Following washing with PBS 3 times, nuclei were counter-stained with 4',6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific) at a concentration of 10 μM in PBS at 20°C for 10 min. Sections were washed with PBS once and mounted with mounting medium (Vectashield® Antifade Mounting Medium, Vector Laboratories, Newark, CA) beneath a cover glass. Digital images were obtained at an excitation wavelength of 490 nm and an emission wavelength of 525 nm using a camera integrated into a confocal microscope (TCS SP8, Leica). Equine deep digital flexor tendon sections were stained as positive controls and construct sections were stained with secondary antibody alone as negative controls. Staining was assessed subjectively.

Scanning electron microscopy (SEM)

Specimens were fixed in 2% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium cacodylate (CAC) buffer (pH 7.4) for 1 h at 25°C and transferred to buffer (3% glutaraldehyde in 0.1 M CAC buffer, pH 7.4) for 30 min. They were rinsed with washing buffer (5% sucrose in 0.1 M CAC buffer, pH 7.4), post-fixative buffer (1% osmium tetroxide in 0.1 M CAC buffer, pH 7.4), and water. Specimens were serially dehydrated, critical point dried, and sputter coated with gold. Digital images were created with a scanning electron microscope and camera at 15 kVp (Quanta 200, FEI Company, Hillsboro, OR).

Transmission electron microscopy (TEM)

Specimens collected from constructs cultured in stromal or tenogenic medium for 21 days were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M PBS (pH 7.4) at 4°C overnight. They were washed 3 times in 0.1 M PBS for 30 min each, and post-fixed in 2% osmium tetroxide (OsO_4) in 0.1 M PBS at 4°C for 3 h. They were dehydrated in graded ethanol, infiltrated with propylene oxide twice for 30 min each, and placed in a 70:30 mixture of propylene oxide and resin for 1 h followed by polymerization in 100% resin at 60°C for 48 h. Polymerized resins were sectioned (70 nm) with a diamond knife using an ultramicrotome (Ultratome Leica EM UC7, Leica), mounted on copper grids, and stained with 2% uranyl acetate at 20°C for 15 min. They were stained with lead stain solution at 20°C for 3 min. Images were obtained with a transmission electron microscope [JEM-1011, JEOL Ltd., Zhubei City, Hsinchu County 302,004, Taiwan (R.O.C.)] at an acceleration voltage of 80 kV.

Statistical analysis

Individual data points are shown on scatter plots with mean ± standard error of the mean (SEM) indicated. Differences in RFU were evaluated with two-way ANOVA and post-hoc pairwise Tukey's tests with treatment, culture period and their interaction as the fixed effects (JMP Pro 17.0.0, JMP Statistical Discovery LLC, Cary, NC). Differences in gene expression fold change from baseline (1) in constructs cultured in tenogenic medium for each culture period were determined with one sample t-tests for normally distributed results and Wilcoxon signed rank tests for non-normally distributed results (Prism, v7, GraphPad Software Inc., La Jolla, CA). Significance was set at $p \leq 0.05$.

TABLE 1 Equine-specific primer sequences.

Gene		Sequence (5′->3′)	Amplicon Length	Accession Number
Scx[c]	Forward	TCTGCCTCAGCAACCAGAGA	246	NM_001105150.1
	Reverse	AAAGTTCCAGTGGGTCTGGG		
Mkx[c]	Forward	AGTGGCTTTACAAGCACCGT	217	XM_023632371.1
	Reverse	ACACTAAGCCGCTCAGCATT		
Egr1[d]	Forward	CCTACGAGCACCTGACCTCAG	241	XM_001502553.5
	Reverse	GATGGTGCTGAAGATGAAGTGG		
CTGF	Forward	ACCCGCGTTACCAATGACAA	140	XM_023651101.1
	Reverse	GGCTTGGAGATTTTGGGGGT		
LOX	Forward	CAGGCGATTTGCGTGTACTG	301	XM_023617821.1
	Reverse	ACTTCAGAACACCAGGCACT		
Col1a1[c]	Forward	CAAGAGGAGGGCCAAGAAGA	261	XM_023652710.1
	Reverse	TCCTGTGGTTTGGTCGTCTG		
Col3a1	Forward	TCCTGGGGCTAGTGGTAGTC	255	XM_008508902.1
	Reverse	GGCGAACCATCTTTGCCATC		
Dcn[b]	Forward	TTATCAAAGTGCCTGGTG	204	XM_005606467.3
	Reverse	CATAGACACATCGGAAGG		
Eln[d]	Forward	CTATGGTGTCGGTGTCGGAG	247	XM_023655466.1
	Reverse	GGGGGCTAACCCAAACTGAG		
Tnc	Forward	TACTGATGGGGCCTTCGAGA	330	XM_023628745.1
	Reverse	AGCAGCTTCCCAGAATCCAC		
Bgn[a]	Forward	TGATTGAGAACGGGAGCCTGAG	143	XM_023633175.1
	Reverse	TTTGGTGATGTTGTTGGTGTGC		
Fbmd[b]	Forward	GCTTCTGCTGAGGGACAC	91	NM_001081777.1
	Reverse	GATTTCTGGGGTTGGGAC		
Col14a1[b]	Forward	CTGGACGATGGAAGTGAG	215	XM_005613197.3
	Reverse	GTGACCCTGAACTGCTGC		
THBS4	Forward	ACGTAAACACCCAGACGGAC	359	XM_023618094.1
	Reverse	CACCAACTCGGAGCCTTCAT		
GAPDH[b]	Forward	GTGTCCCCACCCCTAACG	131	NM_001163856.1
	Reverse	AGTGTAGCCCAGGATGCC		

[a](Jacobson et al., 2015).
[b](Miyabara et al., 2014).
[c](Mohanty et al., 2014).
[d](Yang et al., 2019).

Results

Construct gross appearance

After removal from the bioreactor and a thorough PBS rinse, the gross appearance of constructs cultured in stromal medium (Figures 4A–C) was different from those cultured in tenogenic medium (Figures 4D–F) beginning after 7 days of culture. The size of constructs cultured in stromal medium did not change much after 7 days of culture evidenced by construct bulging between finger trap suture loops (Figure 4A), like dry templates (Figure 2A). Notably, the template material alone does not expand when moistened (Zhou et al., 2016). After 14 days, there was no appreciable change in size, but the surface of the lower half of constructs cultured in stromal medium was lighter in color and roughened compared to the upper half (Figure 4B). After 21 days of

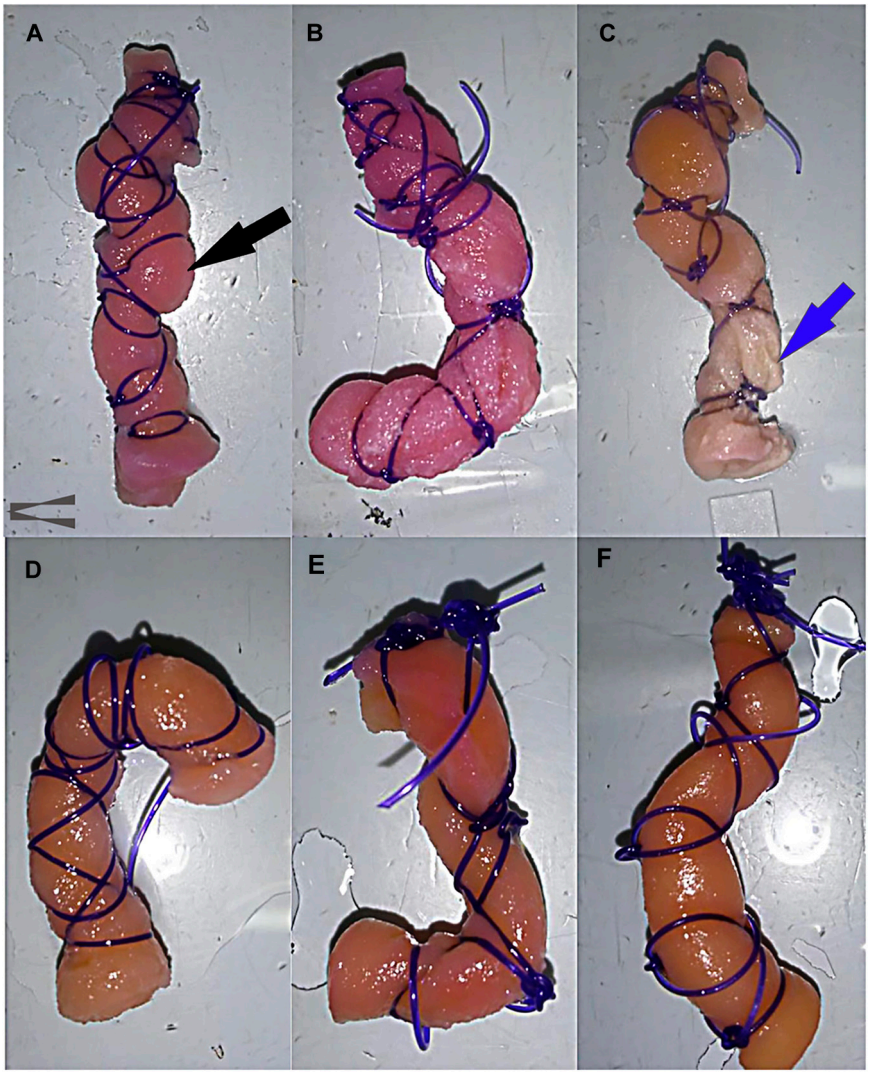


FIGURE 4
Gross appearance of COLI-ASC constructs. Photographs of untensioned constructs cultured in stromal **(A–C)** or tenogenic **(D–F)** medium for 7 **(A,D)**, 14 **(B,E)**, or 21 **(C,F)** days with construct bulging through the finger trap suture (black arrow) and construct erosion (blue arrow) indicated. The upper portion of each image corresponds to the upper end of the bioreactor.

culture, the upper half of the constructs were a tan color, the lower half an off-white color, and construct material was frequently missing at the lowest end (Figure 4C). Constructs cultured in tenogenic medium contracted after 7 days of culture based on loose finger trap suture loops. The constructs were a tan color with occasional red patches and surfaces were roughened (Figure 4D). After 14 days of culture in tenogenic medium, the constructs were a similar color to after 7 days, but had a smooth surface and had contracted more (Figure 4E). The constructs contracted such that the suture loops rarely contacted the template, more so in the upper half, were a solid tan color, and had an even smoother surface after 21 days of culture (Figure 4F). Additionally, the constructs were less compressible and the template layers more tightly adhered than those cultured in stromal medium for 21 days or uncultured template material.

Viable cell distribution and relative cell number

Viable cells were apparent in both stromal and tenogenic medium up to 21 days of culture, and they were present throughout the width and length of the constructs at all time points (Figure 5). They were spherical in stromal medium for all culture periods and in tenogenic medium on day 7. Many viable cells had a spindle-shape and parallel alignment in tenogenic medium after 14 and 21 days of culture. Viable cells were frequently in clusters in tenogenic medium while individual cells were prevalent in stromal medium. Differences in the relative fluorescence units among time points for each culture medium were not significant, so time points were combined. The relative fluorescence units of constructs cultured in tenogenic was

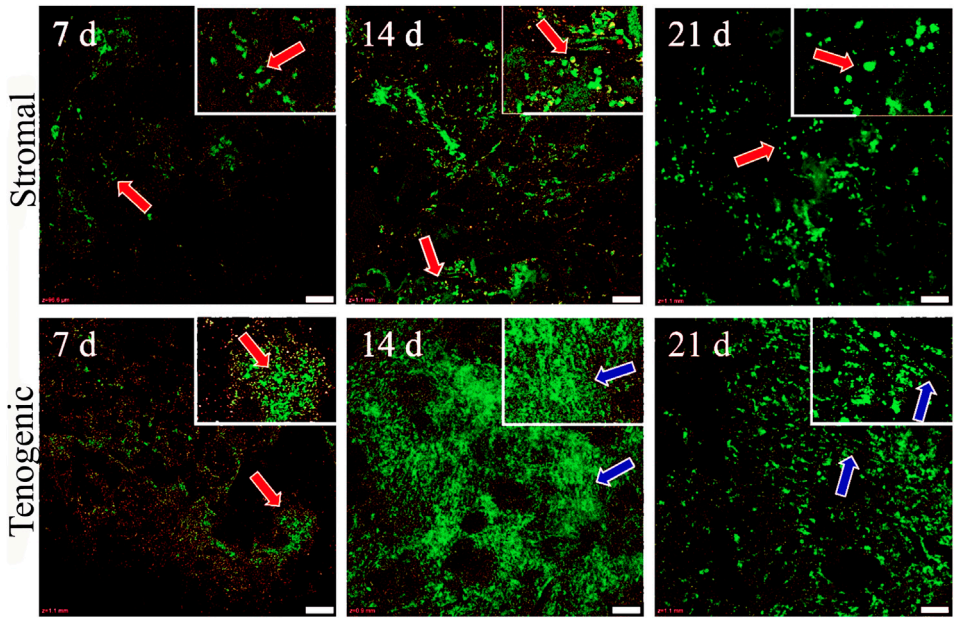


FIGURE 5
Viable cell distribution in COLI-ASC constructs. Photomicrographs of constructs cultured in stromal (upper) or tenogenic (lower) medium for 7, 14, or 21 days with green viable and red nonviable cells shown. Cells with spherical (orange arrows) and elongated (blue arrows) morphology are indicated. Insets at the top right corners are enlargements of areas around the arrows in the larger images. Scale bars = 100 μm.

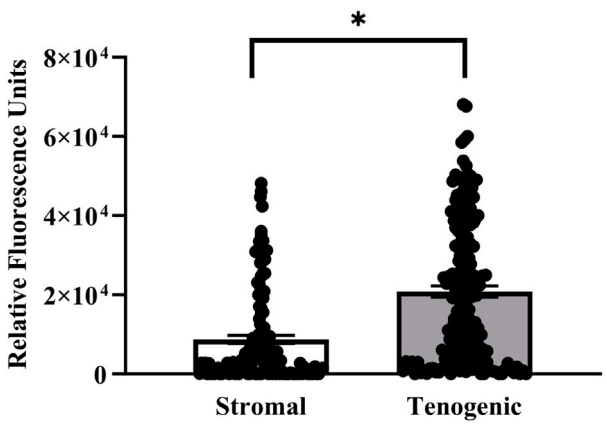


FIGURE 6
Relative viable cell number in COLI-ASC constructs. Fluorescent intensity of resorufin (mean ± SEM) in constructs cultured in stromal or tenogenic medium with all time points combined. A difference between culture medium columns is indicated by an asterisk ($p < 0.05$).

significantly higher than in stromal medium with all time points combined ($p < 0.0001$, Figure 6).

Gene expression

Among the transcription factors, *Mkx* levels were lower (0.75 ± 0.05-fold, $p < 0.0001$) after 14 days and higher (2.28 ± 0.46-fold, $p = 0.03$) after 21 days, respectively, in constructs cultured in tenogenic medium (Figure 7A). The *Egr1* (7.31 ± 1.71-fold, $p = 0.008$) and *LOX* (86.96 ± 78.50 -fold, 0.03) were higher after 7, and *CTGF* after 7 (5.48 ± 2.47-fold, $p = 0.03$), 14 (20.4 ± 8.76-fold, $p = 0.02$), and 21 (7.69 ± 2.50-fold, $p = 0.008$) days of culture. Among tenogenic genes, mRNA levels of *Col1a1* ($2.78 \times 10^3 \pm 2.63 \times 10^3$-fold, $p = 0.02$), *Col3a1* ($2.98 \times 10^1 \pm 2.08 \times 10^1$-fold, $p = 0.008$), *Eln* ($3.03 \times 10^2 \pm 1.44 \times 10^2$-fold, $p = 0.008$), and *Bgn* (8.52 ± 3.03-fold, $p = 0.02$) were higher after 7 days of culture; *Col3a1* ($2.62 \times 10^1 \pm 1.59 \times 10^1$-fold, $p = 0.03$), *Eln* ($4.63 \times 10^2 \pm 3.65 \times 10^2$-fold, 0.05), and *Tnc* (7.29 ± 2.40-fold, 0.03) were higher after 14 days of culture; and *Tnc* mRNA levels were higher after 21 (5.12 ± 1.35-fold, $p = 0.03$) days of culture (Figure 7B). The mRNA levels of mature tendon markers, *Fbmd* (9.75 ± 4.68-fold, $p = 0.02$) and *Col14a1* ($2.10 \times 10^1 \pm 1.1 \times 10^1$-fold, $p = 0.04$) were higher after 7 days, and *THBS4* ($5.85 \times 10^1 \pm 3.55 \times 10^1$-fold, $p = 0.03$) after 14 days of culture (Figure 7C).

Histological analysis

There was no evidence of cell necrosis or apoptosis in constructs cultured in either medium at any time point based on intact cell and nuclear membranes and little to no cytosolic vacuolation (Figure 8). Rare, individual spheroid cells containing large nuclei, attached to template COLI fibers, and with little to no surrounding ECM were evident in constructs cultured in stromal medium for 7 and 14 days. After 21 days of culture in stromal medium, individual, or clustered, spindle- or rhomboid-shaped cells with small, dense nuclei and scant ECM were evident.

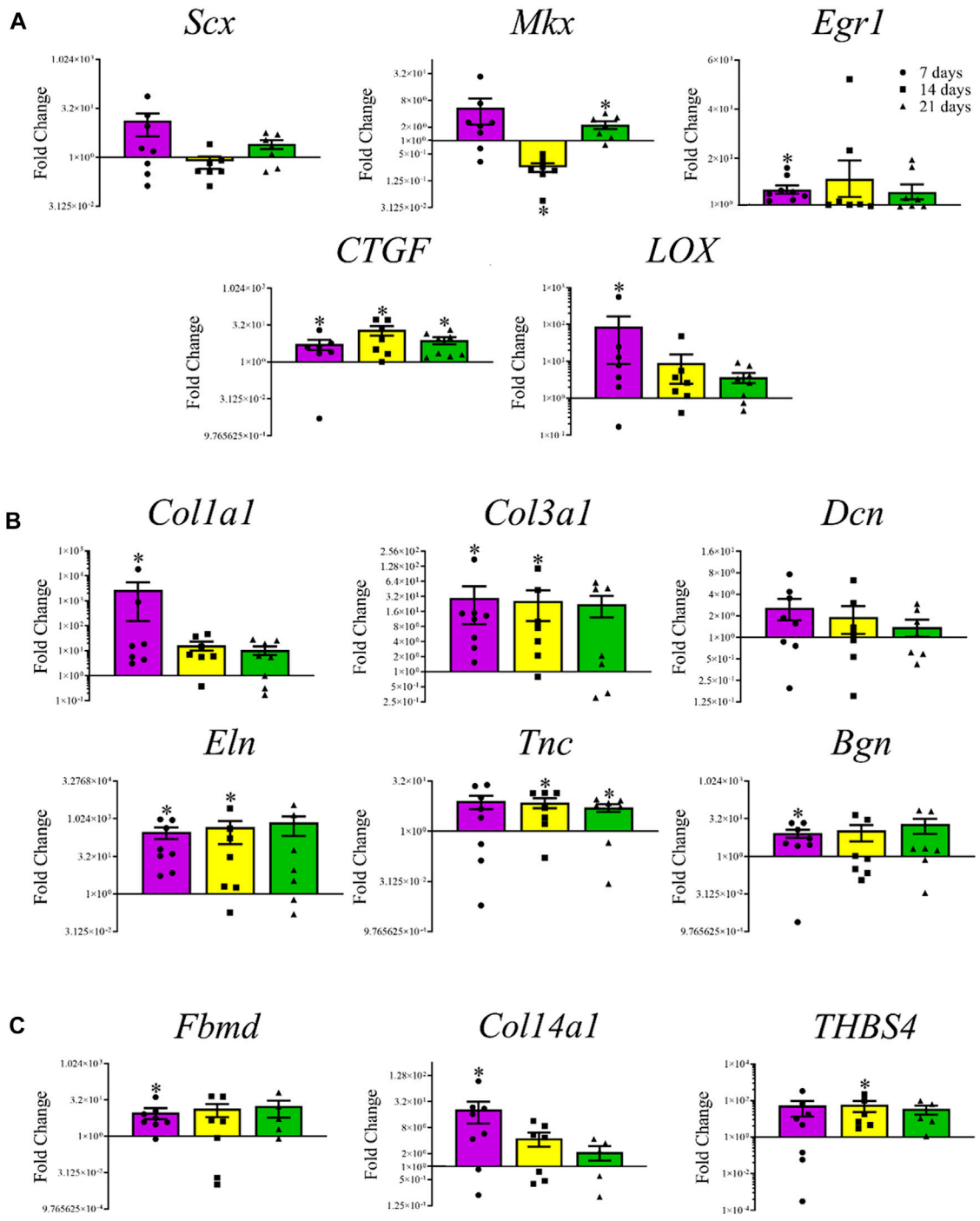


FIGURE 7
Tendon-related gene expression of construct cells. Fold change (mean ± SEM) in mRNA levels of tenogenic transcription factors *Scx*, *Mkx*, *Egr1*, *CTGF*, and *LOX* **(A)**, tenogenic ECM genes *Col1a1*, *Col3a1*, *Dcn*, *Eln*, *Tnc*, and *Bgn* **(B)**, and mature tendon markers, *Fbmd*, *Col14a1*, and *THBS4* **(C)** by cells in COLI-ASC constructs cultured in tenogenic medium relative to those cultured in stromal medium for 7 (purple), 14 (yellow), or 21 (green) days. Asterisks indicate a difference from 1-fold within each culture period.

Within constructs cultured in tenogenic medium, there were clusters of spindle-shaped cells with large, round to oblong nuclei that were adhered to template COLI fibers and surrounded by ECM after 7 days of culture. After 14 days of culture, cell density and ECM were higher, most cells had a spindle-shaped morphology with dense, elongated nuclei, and cells were positioned in a more parallel arrangement compared to day 7. By day 21 of culture, closely packed, elongated cells with a dense, rod-like nucleus and surrounded by well-organized ECM were aligned in parallel with each other and template COLI fibers.

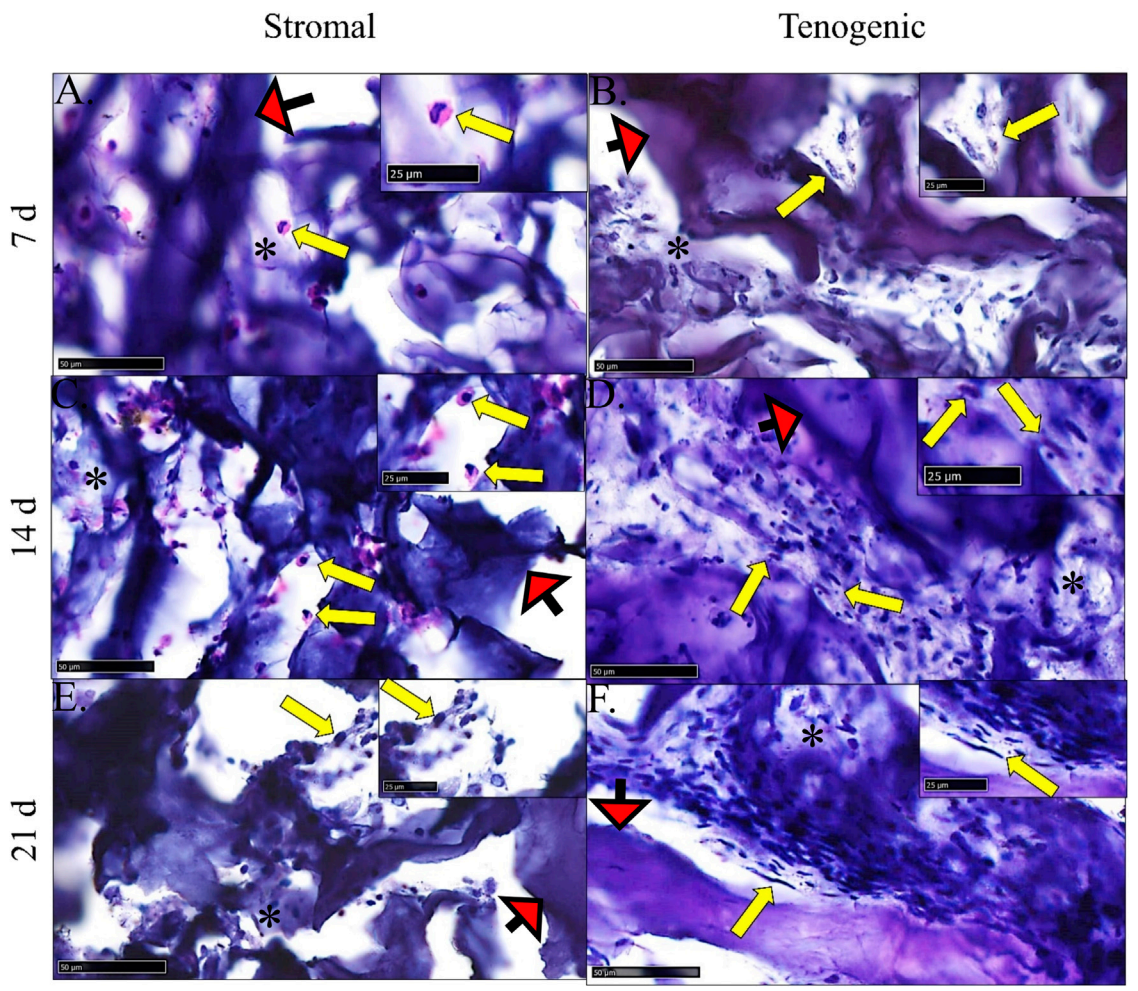


FIGURE 8
Construct histology. Photomicrographs of COLI-ASC constructs cultured in stromal **(A,C,E)** or tenogenic **(B,D,F)** medium for 7 **(A,B)**, 14 **(C,D)**, or 21 **(E,F)** days. Cells (yellow arrows) within variable amounts of ECM (*) as well as template material (red arrows) were apparent. The region surrounding each arrow is enlarged in the inset at the right upper corner. Stain: H&E; Scale bars = 50 µm **(A–C,E)**, 100 µm **(D,F)**.

Immunohistochemical fibromodulin staining

There was no fibromodulin staining in constructs cultured in stromal medium for 7 or 14 days, and only scant, non-specific fibromodulin staining was present after 21 days (Figure 9). In constructs cultured in tenogenic medium, no staining was evident after 7 days, scant, non-specific staining was apparent after 14 days, and, after 21 days of culture, well circumscribed rings of fibromodulin deposition were evident within constructs. Cells within constructs were identified by nuclear staining.

Scanning electron microscopy (SEM)

Individual or clusters of round cells loosely adhered to template fibers with minimal, poorly organized ECM were present in constructs cultured in stromal medium up to 21 days (Figures 10A–C). The amount of ECM was higher after 14 and 21 versus 7 days of culture. Constructs cultured in tenogenic medium appeared to have small, round cells attached in clusters to template and minimal ECM after 7 days of culture. In contrast, elongated cells that were tightly adhered to collagen template within some ECM were apparent after 14 days, and after 21 days, numerous, rhomboid cells, covered by ECM were tightly adhered to template fibers (Figures 10D–F).

Transmission electron microscopy (TEM)

Cells on ASC-COLI constructs cultured in stromal medium for 21 days were round, had a high nucleus to cytosol ratio, and were rich in mitochondria and rough endoplasmic reticulum (Figure 11A). Some were attached to template COLI fibers. Cells on constructs cultured in tenogenic medium were oval and had minimal cytoplasm surrounding a rod-shaped nucleus with abundant heterochromatin (Figure 11B). Fibrils were present in the surrounding ECM.

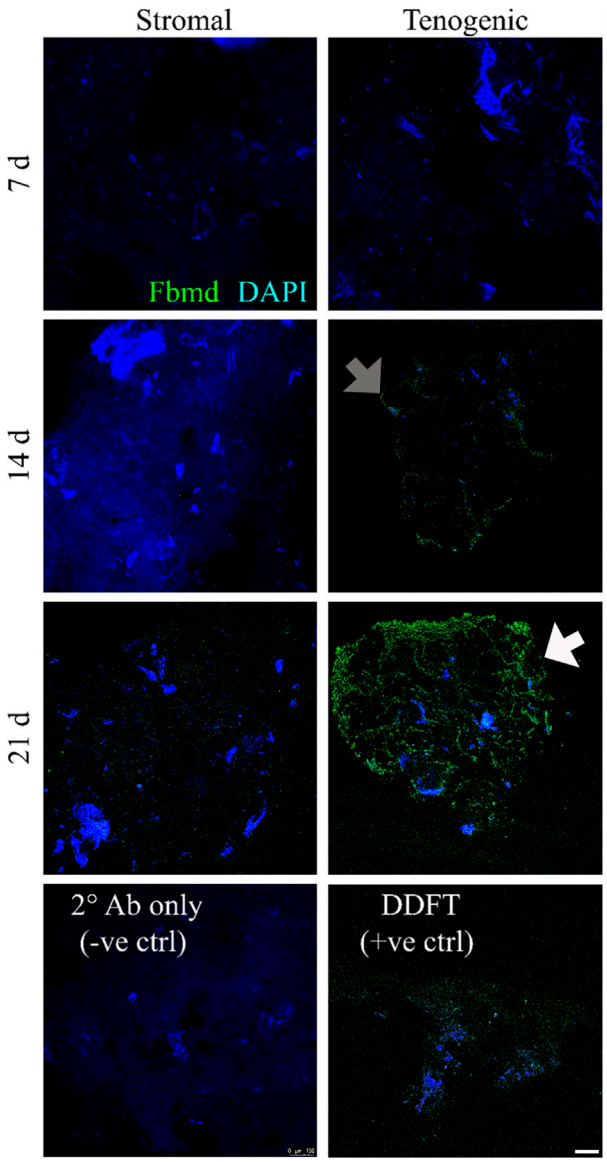


FIGURE 9
Immunohistochemical fibromodulin staining. Fibromodulin (Fbmd, green) localization in COLI-ASC constructs cultured in stromal (left) or tenogenic (right) medium for 7, 14, or 21 days, and in a section of deep digital flexor tendon (DDFT). A construct section stained with only secondary antibody (2° Ab only) is also shown. Scant, non-specific staining (gray arrow) was apparent after 14 days in constructs cultured in tenogenic medium, and, after 21 days of culture in tenogenic medium, rings of fibromodulin surrounded well-circumscribed regions within constructs (white arrow). Cell clusters (blue) were apparent in all constructs. DAPI counterstain; Scale bar = 100 μm.

Discussion

The results of this study confirm that culture in custom bioreactors with combined perfusion and centrifugal tenogenic medium circulation supports differentiation of equine adult ASCs into tendon progenitor-like cells capable of neotissue formation. Viable cells were present throughout constructs cultured in stromal and tenogenic medium for up to 21 days of culture. However, viable cell numbers, gene expression, fibromodulin localization, micro- and ultrastructure, were distinct between culture media. The hypothesis that ASCs assume a tendon progenitor cell-like morphology, express tendon-related genes, and produce more organized extracellular matrix in tenogenic versus stromal medium with perfusion and centrifugal fluid motion was accepted. *De novo* generation of viable tendon neotissue using the methods described has the potential to contribute to novel tendon therapies for multiple species.

Collagen-based templates to support MSC differentiation come in many forms. Hydrogels, decellularized tendon tissue, and various sponge configurations are among the most common (Noth et al., 2005; Theiss et al., 2015; Clements et al., 2016; Youngstrom et al., 2016). In general, cell distribution in COLI sponge is more challenging than in the stable architecture of decelluarized tissue or malleable, semi-solid hydrogels with low fiber density and stiffness. When COLI sponge is hydrated, the fibers adhere to each other, which reduces porosity and can interfere with cell migration and gas and nutrient exchange. Use of culture mechanisms with dynamic fluid flow are designed to amelioriate these limitations. Shear stresses and better gas and nutrient delivery from centrifugal fluid motion generated with a stir bar (50 rpm) reportedly improves MSC proliferation on collagen sponges reinforced with polyethylene terephthalate compared to no fluid flow (Takamoto et al., 2012). The benefits of dynamic perfusion are similar to those of centrifugal forces, but the shear forces are typically normal, or aligned with the long axis of the construct, versus the tangential shear forces of centrifugal fluid flow (Xie et al., 2013; Duan et al., 2017; Taguchi et al., 2021). Centrifugal and perfusion fluid flow were uniquely combined in the custom bioreactors within this study. Perfusion through the medium reservoir ensured adequate medium gas exchange during long term culture, and the normal and tangential shear forces provided unique mechanical stimulation. It is possible that the combined forces improved fluid motion within the construct, though this was not measured directly. Damage to the constructs cultured in stromal medium in this study was likely a result of shear forces that were highest near the stir bar. The effect was not apparent in constructs cultured in tenogenic medium. This could have been from better tissue stability from abundant, organized ECM. The magnitude of dynamic perfusion and centrifugal fluid motion will need to be customized for bioreactor and template configuration and size.

Tenogenic differentation is a result of cross talk between chemical and mechanical signals, and neotissue is defined as a combination of cells and the proteinaceous extracellular they produce (Mace et al., 2016; Mehrian et al., 2018). Construct strain and shear fluid forces combined with tenogenic medium were designed to drive ASC differentiation and generation of tendon neotissue in this study. Current knowledge supports that construct strain is imperative to effective tenogenic differentiation of progenitor cells, and variations in static or dynamic strain affect cell proliferation and the rate and efficiency of tendon neotissue generation (Mace et al., 2016; Engebretson et al., 2017; Mehrian et al., 2018; Zhang et al., 2018). During culture, cells align with the direction of construct strain (Mozdzen et al., 2016). The maintenance of a construct strain of 10% in this study likely promoted cell distribution and alignment along tensioned COLI fibers, and may have supported cell proliferation (Kuo and Tuan, 2008; Mozdzen et al., 2016; Engebretson et al., 2017). However, the culture conditions alone were not sufficient to drive cell differentiation and ECM deposition without medium originally designed for equine tenocyte culture (Theiss et al., 2015). Combined with the other medium

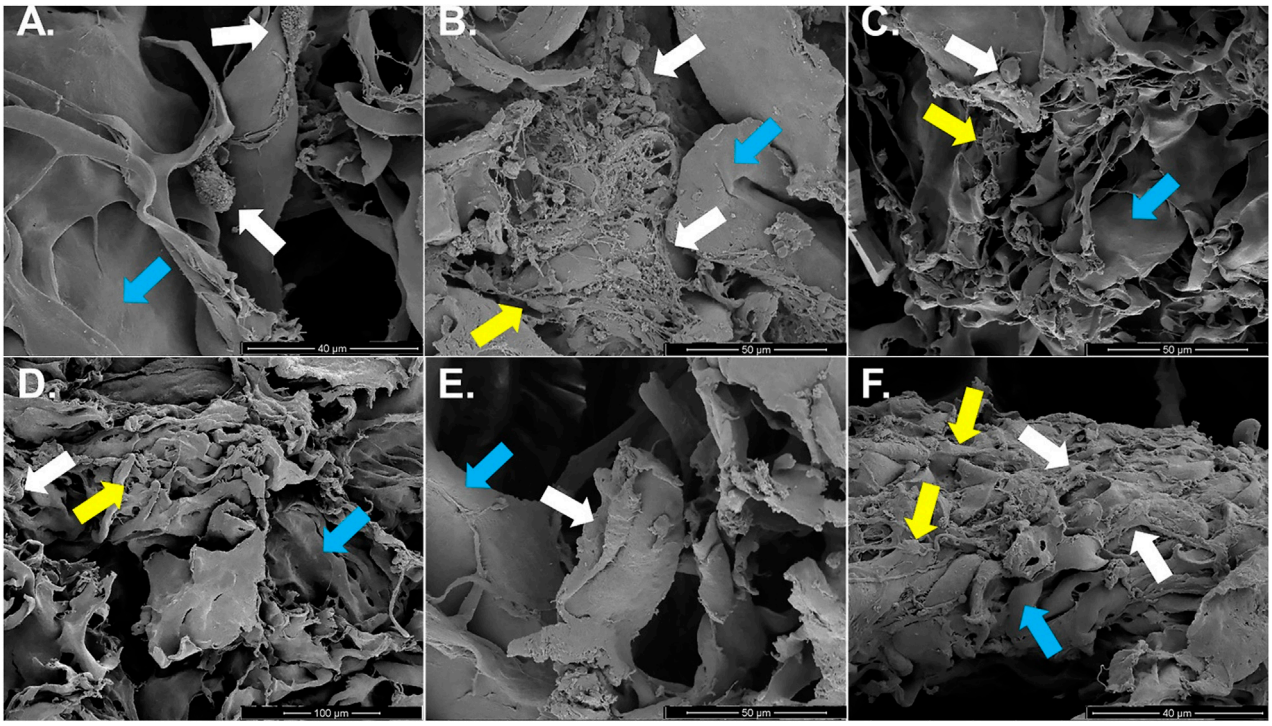


FIGURE 10
Construct surface ultrastructure. Scanning electron photomicrographs of COL1-ASC constructs cultured in stromal **(A–C)** or tenogenic **(D–F)** medium for 7 **(A,D)**, 14 **(B,E)**, or 21 **(C,F)** days with cells (white arrows), on template collagen fibers (blue arrows) and variable amounts of ECM (yellow arrows). Scale bars = 40 µm **(A,F)**, 50 µm **(B,C,E)**, 100 µm **(D)**.

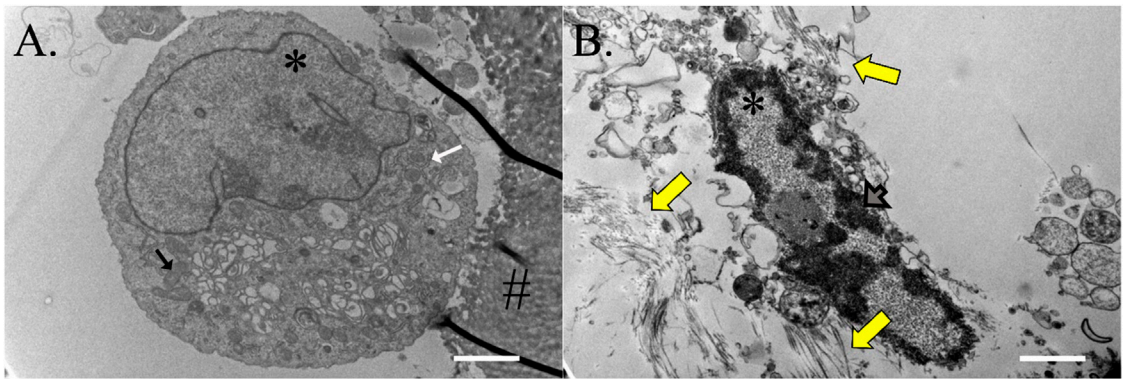


FIGURE 11
Construct cell ultrastructure. Electron microphotographs of cells from ASC-COLI constructs cultured in stromal **(A)** or tenogenic **(B)** medium for 21 days with fibrils in the ECM surrounding the latter (yellow arrows). Cells within constructs cultured in stromal medium had abundant rough endoplasmic reticulum (white arrow) and mitochondria (black arrow), and heterochromatin was apparent in the nuclei of construct cells cultured in tenogenic medium (gray arrow). Nuclei are indicated with an asterisk (*) and template material with a hash (#). Scale bars = 2 µm **(A)**, 1 µm **(B)**.

components, TGF-β is a vital growth factor for differentiation of progenitor cells into tenocytes. Differentiation is regulated by TGF-β/Smad2/3 signal transduction, and TGF-β also promotes collagen production by the cells (Y. Li et al., 2022). The authors, acknowledge, however, the presence of growth factors that were not quantified in the FBS that was used in both culture media. Further refinement of strain and culture conditions to produce tendon neotissue on equine ASC-COLI constructs could improve efficiency to scale up production (Engebretson et al., 2017; Grier et al., 2017; Mozdzen et al., 2016; Taguchi et al., 2021; Youngstrom et al., 2016).

Within the acknowledged limits of the RT-PCR methods used in this study, the change in gene expression profile of cells within constructs cultured in tenogenic medium over the 21 day culture period supports early cell differentiation to a tenogenic lineage. Early, high expression of *Scx*, *Mkx*, *Egr1*, *CTFG*, and *LOX*, transcription factors essential for initiation of progenitor cell

tenogenic differentiation, suggests that the differentiation process began early in the culture period at the genetic level (Derby et al., 2012; Sakabe et al., 2018; Korcari et al., 2022). Lower expression of Mkx after 14 days of culture could have been due to a smaller population of undifferentiated cells compared to after 7 days, while, due to cell proliferation, there was a larger population of undifferentiated cells after 21 versus 14 days. Since *CTFG* is a constitutively expressed tenogenic transcription factor that inhibits maturation of tenogenic progenitors, highest expression after 14 days followed by 21 days of culture indicates cell immaturity and suggests proliferation capabilities (Chen et al., 2008; Li et al., 2019; Rui et al., 2019). Given that LOX enzymes play a role in collagen crosslinking that occurs during embryonic tendon development, decreases in LOX mRNA levels with culture time suggest some progression in ECM maturity (Marturano et al., 2014; de Aro et al., 2018; Pan et al., 2018; Ellingson et al., 2022).

The ECM gene mRNA levels in constructs cultured in tenogenic medium support deposition and subsequent organization of fibrous ECM observed with light and electron microscopy. Higher levels of *Col1a1* RNA initially may be related to the differentiation process, after which stable levels of *Col1a1* and *Col3a1* at a ratio characteristic of healthy versus abnormal tissue deposition were maintained (de Girolamo et al., 2015; Stanco et al., 2019). Tropoelastin is important for adult tendon function, though less is known about its role in tendon development (Brown et al., 2015; Halper, 2021). Tenacins contribute to coordinated generation and repair of musculotendinous tissue, and decorin and biglycan are small leucine-rich proteoglycans that are critical to collagen fibrillogenesis for tendon development (Taylor et al., 2009; Dyment et al., 2013; Leiphart et al., 2020; Halper, 2021).

Genes associated with mature tendon tissue evaluated in this study, *Fbmd, Col14a,* and *THBS4*, are also associated with embryonic tendon formation. Specifically, *Fbmd* is upregulated in tendon tissue during embryogenesis and during the neonatal period, *Col14a1* gene and protein levels are elevated in neonatal tissue, and *THBS4* is highly expressed in ovine calcaneal tendon during early gestation and gradually decreases with age to low levels in adults (Ansorge et al., 2009; Dyment et al., 2013; Liu et al., 2012; Russo et al., 2015). Gene expression results are relative and limited to the comparisons between culture conditions in this study. Taken together, the transcription factor, extra-cellular matrix, and mature tendon gene expression profiles suggest tenogenic differentiation of ASCs on COLI constructs cultured in tenogenic medium relative to those cultured in stromal medium.

Changes in cell and ECM morphology paralleled gene expression and were consistent with tenogenic differentiation (Chen et al., 2014; Schulze-Tanzil et al., 2004). A different appearance, greater adhesion between construct layers, and firmer texture of constructs cultured in tenogenic medium are consistent with tenogenic differentation and aligned with the more highly organized, fibrillar ECM visible at the micro- and ultra-structural levels. The cell morphology and rudimentary ECM evident in constructs cultured in stromal medium could represent some level of differentiation since COLI-based matrix, with and without strain, is reported to induce progenitor cell differentiation (Cardwell et al., 2014; Grier et al., 2017; Grier et al., 2019; Kim et al., 2015). Transmission electrophotomicrographs highlighted distinct cell maturities. Specifically, those in tenogenic medium had the appearance of differentiated cells surrounded by collagen-like fibrils, while those in stromal medium had the appearance of immature progenitor cells (Schneider et al., 2011; Ugarte et al., 2015). Immunolocalization of fibromodulin, a bioactive factor of native tendon ECM suggested to be a differentiation marker of tendon, additionally supports the presence of tendon progenitor cells in the constructs cultured in tenogenic medium (Miyabara et al., 2014; Ning et al., 2021). The protein is a vital component of the tendon progenitor cell ECM niche that controls tendon progenitor cell self-renewal and differentiation (Bi et al., 2007). It is also thought to regulate collagen fibril size by controlling premature cross-linking via LOX modulation (Taye et al., 2020). The pattern of fibromodulin labeling in the constructs cultured in tenogenic medium resembled that of mature equine where the strongest staining is in the intrafasicular matrix surrounding bundles of collagen called fascicles (Thorpe et al., 2016; Taye et al., 2020). Lower, poorly organized labeling in constructs cultured in stromal medium highlights some low level of cell differentiation as mentioned above. The gross appearance, micro- and ultrastructure, and immunohistochemical labeling of constructs cultured in tenogenic medium are consistent with early tendon neotissue.

The authors acknowledge limitations to this *in vitro* study. Constructs were surrounded by suture in a pattern that allowed strain to be applied to them within the bioreactor chamber as previously reported. The suture may have impacted shear forces on the construct surface and cell migration from compression of construct material directly beneath suture strands. A mechanism to apply strain without focal compression could potentially improve cell movement and consistency in shear forces. Similarly, centrifugal forces were not identical along the length of the construct due to the position of the stir bar at the lowest end of the bioreactor. As previously mentioned, higher forces at the lower end of the construct may have damaged constructs cultured in stromal medium. The method for RNA isolation from the constructs included collagenase digestion like descriptions of RNA isolation from fibrous tissues (Burja et al., 2022; Grinstein et al., 2018; Takacs et al., 2023). The potential that RNA quantity was compromised cannot be entirely ruled out, however. Comparisons in this investigation are limited to the specific primary cell isolates and culture conditions described here.

The unique combination of static strain and centrifugal and perfusion fluid flow of tenogenic medium supported *de novo* tendon neotissue from adult equine ASCs. *De novo* equine tendon neotissue tissue production could be a useful resource for investigations surrounding tendon formation and pathology. This is especially important to reduce animal use for screening therapeutic compounds and technology. Injectable, collagen- and lyophilized tendon-based hydrogels with and without progenitor cells have been explored for treatment of tendon pathology (Crowe et al., 2016; Liu et al., 2020). Ultrasound is the established imaging modality to identify and monitor healing of equine tendon lesions, and injection of cell- and cell product-based therapies into equine tendon lesions with ultrasound guidance is a contemporary standard of care (Dyson, 2004; Geburek et al., 2017; Giunta et al., 2019; Romero et al., 2017; Witte et al., 2016). Tendon neotissue stiffness is compatible with injection, so it is feasible that it could be administered with ultrasound guidance following appropriate safety and efficacy testing. Taken together, the unique culture system and methods of this investigation have the potential expand the current repertoire of adult multipotent stromal cell-based products to the neotissue level.

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

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because tissue samples for primary cell isolation were obtained immediately post-mortem with signed owner consent.

Author contributions

TT: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing–original draft. ML: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing–original draft, Writing–review and editing. CT: Formal analysis, Validation, Methodology, Writing - review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Funding for this study was provided in part by the Tynewald Foundation, American College of Veterinary Surgeons Research Foundation, Grayson Jockey Club Research Foundation, United States Department of Agriculture 1433, Louisiana State University Equine Health Studies Program, and the Louisiana State University Veterinary Clinical Sciences Department.

Acknowledgments

Authors thank past and present members of the Laboratory for Equine and Comparative Orthopedic Research for technical support.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

Abate, M., Silbernagel, K. G., Siljeholm, C., Di Iorio, A., De Amicis, D., Salini, V., et al. (2009). Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Res. Ther.* 11, 235. doi:10.1186/ar2723

Aimaletdinov, A., Mindubaeva, G., Khalikova, S., Kabwe, E., Salmakova, A., Alexandrova, N., et al. (2020). Application of gene therapy in the treatment of superficial digital flexor tendon injury in horses. *Open Vet. J.* 10, 261–266. doi:10.4314/ovj.v10i3.3

Ansorge, H. L., Meng, X., Zhang, G., Veit, G., Sun, M., Klement, J. F., et al. (2009). Type xiv collagen regulates fibrillogenesis: premature collagen fibril growth and tissue dysfunction in null mice. *J. Biol. Chem.* 284, 8427–8438. doi:10.1074/jbc.M805582200

Aurich, H., Sgodda, M., Kaltwasser, P., Vetter, M., Weise, A., Liehr, T., et al. (2009). Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue *in vitro* promotes hepatic integration *in vivo*. *Gut* 58, 570–581. doi:10.1136/gut.2008.154880

Banos, C. C., Thomas, A. H., and Kuo, C. K. (2008). Collagen fibrillogenesis in tendon development: current models and regulation of fibril assembly. *Birth Defects Res. C Embryo Today* 84, 228–244. doi:10.1002/bdrc.20130

Beerts, C., Suls, M., Broeckx, S. Y., Seys, B., Vandenberghe, A., Declercq, J., et al. (2017). Tenogenically induced allogeneic peripheral blood mesenchymal stem cells in allogeneic platelet-rich plasma: 2-year follow-up after tendon or ligament treatment in horses. *Front. Vet. Sci.* 4, 158. doi:10.3389/fvets.2017.00158

Bertuglia, A., Bullone, M., Rossotto, F., and Gasparini, M. (2014). Epidemiology of musculoskeletal injuries in a population of harness standardbred racehorses in training. *BMC Vet. Res.* 10, 11. doi:10.1186/1746-6148-10-11

Bi, Y., Ehirchiou, D., Kilts, T. M., Inkson, C. A., Embree, M. C., Sonoyama, W., et al. (2007). Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat. Med.* 13, 1219–1227. doi:10.1038/nm1630

Bostrom, A., Bergh, A., Hyytiainen, H., and Asplund, K. (2022). *Systematic review of complementary and alternative veterinary medicine in sport and companion animals: extracorporeal shockwave therapy*, 12. doi:10.3390/ani12223124*Anim. (Basel)*

Brown, J. P., Galassi, T. V., Stoppato, M., Schiele, N. R., and Kuo, C. K. (2015). Comparative analysis of mesenchymal stem cell and embryonic tendon progenitor cell response to embryonic tendon biochemical and mechanical factors. *Stem Cell Res. Ther.* 6, 89. doi:10.1186/s13287-015-0043-z

Burja, B., Paul, D., Tastanova, A., Edalat, S. G., Gerber, R., Houtman, M., et al. (2022). An optimized tissue dissociation protocol for single-cell RNA sequencing analysis of fresh and cultured human skin biopsies. *Front Cell Dev Biol* 10, 872688. doi:10.3389/fcell.2022.872688

Cardwell, R. D., Dahlgren, L. A., and Goldstein, A. S. (2014). Electrospun fibre diameter, not alignment, affects mesenchymal stem cell differentiation into the tendon/ligament lineage. *J. Tissue Eng. Regen. Med.* 8, 937–945. doi:10.1002/term.1589

Carlier, S., Depuydt, E., Suls, M., Bocque, C., Thys, J., Vandenberghe, A., et al. (2023). Equine allogeneic tenogenic primed mesenchymal stem cells: a clinical field study in horses suffering from naturally occurring superficial digital flexor tendon and suspensory ligament injuries. *Equine veterinary J.* doi:10.1111/evj.14008

Chen, C. H., Cao, Y., Wu, Y. F., Bais, A. J., Gao, J. S., and Tang, J. B. (2008). Tendon healing *in vivo*: gene expression and production of multiple growth factors in early tendon healing period. *J. Hand Surg. Am.* 33, 1834–1842. doi:10.1016/j.jhsa.2008.07.003

Chen, J., Yu, Q., Wu, B., Lin, Z., Pavlos, N. J., Xu, J., et al. (2011). Autologous tenocyte therapy for experimental achilles tendinopathy in a rabbit model. *Tissue Eng. Part A* 17, 2037–2048. doi:10.1089/ten.TEA.2010.0492

Chen, X., Yin, Z., Chen, J. L., Liu, H. H., Shen, W. L., Fang, Z., et al. (2014). Scleraxis-overexpressed human embryonic stem cell-derived mesenchymal stem cells for tendon tissue engineering with knitted silk-collagen scaffold. *Tissue Eng. Part A* 20, 1583–1592. doi:10.1089/ten.TEA.2012.0656

Clements, L. E., Garvican, E. R., Dudhia, J., and Smith, R. K. (2016). Modulation of mesenchymal stem cell genotype and phenotype by extracellular matrix proteins. *Connect. Tissue Res.* 57, 443–453. doi:10.1080/03008207.2016.1215442

Cockerham, K., and Hsu, V. J. (2009). Collagen-based dermal fillers: past, present, future. *Facial plast. Surg. FPS* 25, 106–113. doi:10.1055/s-0029-1220650

Crowe, C. S., Chattopadhyay, A., McGoldrick, R., Chiou, G., Pham, H., and Chang, J. (2016). Characteristics of reconstituted lyophilized tendon hydrogel: an injectable scaffold for tendon regeneration. *Plast. Reconstr. Surg.* 137, 843–851. doi:10.1097/01.prs.0000480012.41411.7c

Cziperle, D. J. (2021). Avitene microfibrillar collagen hemostat for adjunctive hemostasis in surgical procedures: a systematic literature review. *Med. Devices (Auckl)* 14, 155–163. doi:10.2147/MDER.S298207

de Aro, A. A., Carneiro, G. D., Teodoro, L. F. R., da Veiga, F. C., Ferrucci, D. L., Simoes, G. F., et al. (2018). Injured achilles tendons treated with adipose-derived stem cells transplantation and gdf-5. *Cells* 7. doi:10.3390/cells7090127

de Girolamo, L., Vigano, M., Galliera, E., Stanco, D., Setti, S., Marazzi, M. G., et al. (2015). *In vitro* functional response of human tendon cells to different dosages of low-frequency pulsed electromagnetic field. *Knee Surg. sports traumatology, Arthrosc. official J. ESSKA* 23, 3443–3453. doi:10.1007/s00167-014-3143-x

Derby, B. M., Reichensperger, J., Chambers, C., Bueno, R. A., Suchy, H., and Neumeister, M. W. (2012). Early growth response factor-1: expression in a rabbit flexor tendon scar model. *Plast. Reconstr. Surg.* 129, 435e–442e. doi:10.1097/PRS.0b013e3182402d81

Duan, W., Haque, M., Kearney, M. T., and Lopez, M. J. (2017). Collagen and hydroxyapatite scaffolds activate distinct osteogenesis signaling pathways in adult adipose-derived multipotent stromal cells. *Tissue Eng. Part C. Methods*, 23, 592–603. doi:10.1089/ten.TEC.2017.0078

Dyment, N. A., Liu, C. F., Kazemi, N., Aschbacher-Smith, L. E., Kenter, K., Breidenbach, A. P., et al. (2013). The paratenon contributes to scleraxis-expressing cells during patellar tendon healing. *PLoS One* 8, e59944. doi:10.1371/journal.pone.0059944

Dyson, S., Pinilla, M. J., Bolas, N., and Murray, R. (2018). Proximal suspensory desmopathy in hindlimbs: magnetic resonance imaging, gross post-mortem and histological study. *Equine veterinary J.* 50, 159–165. doi:10.1111/evj.12756

Dyson, S. J. (2004). Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992-2000). *Equine veterinary J.* 36, 415–419. doi:10.2746/0425164044868422

Ellingson, A. J., Pancheri, N. M., and Schiele, N. R. (2022). Regulators of collagen crosslinking in developing and adult tendons. *Eur. Cell Mater* 43, 130–152. doi:10.22203/eCM.v043a11

Engebretson, B., Mussett, Z. R., and Sikavitsas, V. I. (2017). Tenocytic extract and mechanical stimulation in a tissue-engineered tendon construct increases cellular proliferation and ecm deposition. *Biotechnol. J.* 12. doi:10.1002/biot.201600595

Fahy, N., de Vries-van Melle, M. L., Lehmann, J., Wei, W., Grotenhuis, N., Farrell, E., et al. (2014). Human osteoarthritic synovium impacts chondrogenic differentiation of mesenchymal stem cells via macrophage polarisation state. *Osteoarthr. Cartil.* 22, 1167–1175. doi:10.1016/j.joca.2014.05.021

Geburek, F., Roggel, F., van Schie, H. T. M., Beineke, A., Estrada, R., Weber, K., et al. (2017). Effect of single intralesional treatment of surgically induced equine superficial digital flexor tendon core lesions with adipose-derived mesenchymal stromal cells: a controlled experimental trial. *Stem Cell Res. Ther.* 8, 129. doi:10.1186/s13287-017-0564-8

Grinstein, M., Dingwall, H. L., Shah, R. R., Capellini, T. D., Galloway, J. L., et al. (2018). A robust method for RNA extraction and purification from a single adult mouse tendon. *PeerJ* 6, e4664. doi:10.7717/peerj.4664

Giunta, K., Donnell, J. R., Donnell, A. D., and Frisbie, D. D. (2019). Prospective randomized comparison of platelet rich plasma to extracorporeal shockwave therapy for treatment of proximal suspensory pain in western performance horses. *Res. Vet. Sci.* 126, 38–44. doi:10.1016/j.rvsc.2019.07.020

Grier, W. G., Moy, A. S., and Harley, B. A. (2017). Cyclic tensile strain enhances human mesenchymal stem cell smad 2/3 activation and tenogenic differentiation in anisotropic collagen-glycosaminoglycan scaffolds. *Eur. Cell Mater* 33, 227–239. doi:10.22203/eCM.v033a14

Grier, W. K., Sun Han Chang, R. A., Ramsey, M. D., and Harley, B. A. C. (2019). The influence of cyclic tensile strain on multi-compartment collagen-gag scaffolds for tendon-bone junction repair. *Connect. Tissue Res.* 60, 530–543. doi:10.1080/03008207.2019.1601183

Halper, J. (2021). Basic components of connective tissues and extracellular matrix: fibronectin, fibrinogen, laminin, elastin, fibrillins, fibulins, matrilins, tenascins and thrombospondins. *Adv. Exp. Med. Biol.* 1348, 105–126. doi:10.1007/978-3-030-80614-9_4

Harris, M. T., Butler, D. L., Boivin, G. P., Florer, J. B., Schantz, E. J., and Wenstrup, R. J. (2004). Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. *J. Orthop. Res. official Publ. Orthop. Res. Soc.* 22, 998–1003. doi:10.1016/j.orthres.2004.02.012

Howell, K., Chien, C., Bell, R., Laudier, D., Tufa, S. F., Keene, D. R., et al. (2017). Novel model of tendon regeneration reveals distinct cell mechanisms underlying regenerative and fibrotic tendon healing. *Sci. Rep.* 7, 45238. doi:10.1038/srep45238

Jacobson, E., Dart, A. J., Mondori, T., Horadogoda, N., Jeffcott, L. B., Little, C. B., et al. (2015). Focal experimental injury leads to widespread gene expression and histologic changes in equine flexor tendons. *PLoS One* 10, e0122220. doi:10.1371/journal.pone.0122220

Kaji, D. A., Howell, K. L., Balic, Z., Hubmacher, D., and Huang, A. H. (2020). Tgfbeta signaling is required for tenocyte recruitment and functional neonatal tendon regeneration. *Elife* 9. doi:10.7554/eLife.51779

Kannus, P., and Jozsa, L. (1991). Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. *J. Bone Jt. Surg. Am.* 73, 1507–1525.

Kim, T., Sridharan, I., Zhu, B., Orgel, J., and Wang, R. (2015). Effect of cnt on collagen fiber structure, stiffness assembly kinetics and stem cell differentiation. *Mater Sci. Eng. C Mater Biol. Appl.* 49, 281–289. doi:10.1016/j.msec.2015.01.014

Korcari, A., Muscat, S., McGinn, E., Buckley, M. R., and Loiselle, A. E. (2022). Depletion of scleraxis-lineage cells during tendon healing transiently impairs multi-scale restoration of tendon structure during early healing. *PLoS One* 17, e0274227. doi:10.1371/journal.pone.0274227

Kovac, M., Litvin, Y. A., Aliev, R. O., Zakirova, E. Y., Rutland, C. S., Kiyasov, A. P., et al. (2018). Gene therapy using plasmid DNA encoding vegf164 and fgf2 genes: a novel treatment of naturally occurring tendinitis and desmitis in horses. *Front. Pharmacol.* 9, 978. doi:10.3389/fphar.2018.00978

Kuo, C. K., and Tuan, R. S. (2008). Mechanoactive tenogenic differentiation of human mesenchymal stem cells. *Tissue Eng. Part A*, 14, 1615–1627. doi:10.1089/ten.tea.2006.0415

Lam, K. H., Parkin, T. D., Riggs, C. M., and Morgan, K. L. (2007). Descriptive analysis of retirement of thoroughbred racehorses due to tendon injuries at the Hong Kong jockey club (1992-2004). *Equine veterinary J.* 39, 143–148. doi:10.2746/042516407x159132

Leiphart, R. J., Shetye, S. S., Weiss, S. N., Dyment, N. A., and Soslowsky, L. J. (2020). Induced knockdown of decorin, alone and in tandem with biglycan knockdown, directly increases aged murine patellar tendon viscoelastic properties. *J. Biomech. Eng.* 142. doi:10.1115/1.4048030

Li, X., Pongkitwitoon, S., Lu, H., Lee, C., Gelberman, R., and Thomopoulos, S. (2019). Ctgf induces tenogenic differentiation and proliferation of adipose-derived stromal cells. *J. Orthop. Res. official Publ. Orthop. Res. Soc.* 37, 574–582. doi:10.1002/jor.24248

Li, Y., Liu, X., Liu, X., Peng, Y., Zhu, B., Guo, S., et al. (2022). Transforming growth factor-beta signalling pathway in tendon healing. *Growth factors.* 40, 98–107. doi:10.1080/08977194.2022.2082294

Liu, C. F., Aschbacher-Smith, L., Barthelery, N. J., Dyment, N., Butler, D., and Wylie, C. (2012). Spatial and temporal expression of molecular markers and cell signals during normal development of the mouse patellar tendon. *Tissue Eng. Part A* 18, 598–608. doi:10.1089/ten.TEA.2011.0338

Liu, R., Zhang, S., and Chen, X. (2020). Injectable hydrogels for tendon and ligament tissue engineering. *J. Tissue Eng. Regen. Med.* 14, 1333–1348. doi:10.1002/term.3078

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. *Methods* 25, 402–408. doi:10.1006/meth.2001.1262

Longo, U. G., Franceschi, F., Ruzzini, L., Rabitti, C., Morini, S., Maffulli, N., et al. (2007). Light microscopic histology of supraspinatus tendon ruptures. *Knee Surg. sports traumatology, Arthrosc. official J. ESSKA* 15, 1390–1394. doi:10.1007/s00167-007-0395-8

Longo, U. G., Lamberti, A., Maffulli, N., and Denaro, V. (2010). Tendon augmentation grafts: a systematic review. *Br. Med. Bull.* 94, 165–188. doi:10.1093/bmb/ldp051

Lui, P. P., Rui, Y. F., Ni, M., and Chan, K. M. (2011). Tenogenic differentiation of stem cells for tendon repair-what is the current evidence? *J. Tissue Eng. Regen. Med.* 5, e144–e163. doi:10.1002/term.424

Ma, R., Schar, M., Chen, T., Wang, H., Wada, S., Ju, X., et al. (2019). Use of human placenta-derived cells in a preclinical model of tendon injury. *J. Bone Jt. Surg. Am.* 101, e61. doi:10.2106/JBJS.15.01381

Mace, J., Wheelton, A., Khan, W. S., and Anand, S. (2016). The role of bioreactors in ligament and tendon tissue engineering. *Curr. Stem Cell Res. T* 11, 35–40. doi:10.2174/1574888x10666150904113827

Marr, C. M., Love, S., Boyd, J. S., and McKellar, Q. (1993). Factors affecting the clinical outcome of injuries to the superficial digital flexor tendon in national hunt and point-to-point racehorses. *Veterinary Rec.* 132, 476–479. doi:10.1136/vr.132.19.476

Marturano, J. E., Xylas, J. F., Sridharan, G. V., Georgakoudi, I., and Kuo, C. K. (2014). Lysyl oxidase-mediated collagen crosslinks may be assessed as markers of functional properties of tendon tissue formation. *Acta biomater.* 10, 1370–1379. doi:10.1016/j.actbio.2013.11.024

Mehrian, M., Guyot, Y., Papantoniou, I., Olofsson, S., Sonnaert, M., Misener, R., et al. (2018). Maximizing neotissue growth kinetics in a perfusion bioreactor: an *in silico*

strategy using model reduction and bayesian optimization. *Biotechnol. Bioeng.* 115, 617–629. doi:10.1002/bit.26500

Meyer, M. (2019). Processing of collagen based biomaterials and the resulting materials properties. *Biomed. Eng. Online* 18, 24. doi:10.1186/s12938-019-0647-0

Miyabara, S., Yuda, Y., Kasashima, Y., Kuwano, A., and Arai, K. (2014). Regulation of tenomodulin expression via wnt/beta-catenin signaling in equine bone marrow-derived mesenchymal stem cells. *J. Equine Sci.* 25, 7–13. doi:10.1294/jes.25.7

Mohanty, N., Gulati, B. R., Kumar, R., Gera, S., Kumar, P., Somasundaram, R. K., et al. (2014). Immunophenotypic characterization and tenogenic differentiation of mesenchymal stromal cells isolated from equine umbilical cord blood. *Vitro Cell Dev. Biol. Anim.* 50, 538–548. doi:10.1007/s11626-013-9729-7

Mozdzen, L. C., Thorpe, S. D., Screen, H. R., and Harley, B. A. (2016). The effect of gradations in mineral content, matrix alignment, and applied strain on human mesenchymal stem cell morphology within collagen biomaterials. *Adv. Healthc. Mater* 5, 1731–1739. doi:10.1002/adhm.201600181

Muller, S. A., Durselen, L., Heisterbach, P., Evans, C., and Majewski, M. (2016). Effect of a simple collagen type i sponge for achilles tendon repair in a rat model. *Am. J. sports Med.* 44, 1998–2004. doi:10.1177/0363546516641942

Ning, L. J., Zhang, Y. J., Zhang, Y. J., Zhu, M., Ding, W., Jiang, Y. L., et al. (2021). Enhancement of migration and tenogenic differentiation of macaca mulatta tendon-derived stem cells by decellularized tendon hydrogel. *Front. Cell Dev. Biol.* 9, 651583. doi:10.3389/fcell.2021.651583

Noth, U., Schupp, K., Heymer, A., Kall, S., Jakob, F., Schutze, N., et al. (2005). Anterior cruciate ligament constructs fabricated from human mesenchymal stem cells in a collagen type i hydrogel. *Cytotherapy* 7, 447–455. doi:10.1080/14653240500319093

Olivier, A., Nurton, J. P., and Guthrie, A. J. (1997). An epizoological study of wastage in thoroughbred racehorses in gauteng, South Africa. *J. South Afr. Veterinary Assoc.* 68, 125–129. doi:10.4102/jsava.v68i4.893

O'Sullivan, C. B. (2007). Injuries of the flexor tendons: focus on the superficial digital flexor tendon. *Clin. Tech. Equine Pract.* 6, 189–197.

Pan, X. S., Li, J., Brown, E. B., and Kuo, C. K. (2018). Embryo movements regulate tendon mechanical property development. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 373. doi:10.1098/rstb.2017.0325

Pillai, D. S., Dhinsa, B. S., and Khan, W. S. (2017). Tissue engineering in achilles tendon reconstruction; the role of stem cells, growth factors and scaffolds. *Curr. Stem Cell Res. Ther.* 12, 506–512. doi:10.2174/1574888X12666170523162214

Rashedi, I., Talele, N., Wang, X. H., Hinz, B., Radisic, M., and Keating, A. (2017). Collagen scaffold enhances the regenerative properties of mesenchymal stromal cells. *PLoS One* 12, e0187348. doi:10.1371/journal.pone.0187348

Reed, S. A., and Leahy, E. R. (2013). Growth and development symposium: stem cell therapy in equine tendon injury. *J. animal Sci.* 91, 59–65. doi:10.2527/jas.2012-5736

Romero, A., Barrachina, L., Ranera, B., Remacha, A. R., Moreno, B., de Blas, I., et al. (2017). Comparison of autologous bone marrow and adipose tissue derived mesenchymal stem cells, and platelet rich plasma, for treating surgically induced lesions of the equine superficial digital flexor tendon. *Vet. J.* 224, 76–84. doi:10.1016/j.tvjl.2017.04.005

Rossdale, P. D., Hopes, R., Digby, N. J., and offord, K. (1985). Epidemiological study of wastage among racehorses 1982 and 1983. *Veterinary Rec.* 116, 66–69. doi:10.1136/vr.116.3.66

Rui, Y. F., Chen, M. H., Li, Y. J., Xiao, L. F., Geng, P., Wang, P., et al. (2019). Ctgf attenuates tendon-derived stem/progenitor cell aging. *Stem Cells Int.*, 2019, 6257537. doi:10.1155/2019/6257537

Russo, V., Mauro, A., Martelli, A., Di Giacinto, O., Di Marcantonio, L., Nardinocchi, D., et al. (2015). Cellular and molecular maturation in fetal and adult ovine calcaneal tendons. *J. Anat.* 226, 126–142. doi:10.1111/joa.12269

Sakabe, T., Sakai, K., Maeda, T., Sunaga, A., Furuta, N., Schweitzer, R., et al. (2018). Transcription factor scleraxis vitally contributes to progenitor lineage direction in wound healing of adult tendon in mice. *J. Biol. Chem.* 293, 5766–5780. doi:10.1074/jbc.RA118.001987

Sakabe, T., and Sakai, T. (2011). Musculoskeletal diseases--tendon. *Br. Med. Bull.* 99, 211–225. doi:10.1093/bmb/ldr025

Schneider, P. R., Buhrmann, C., Mobasheri, A., Matis, U., and Shakibaei, M. (2011). Three-dimensional high-density co-culture with primary tenocytes induces tenogenic differentiation in mesenchymal stem cells. *J. Orthop. Res. official Publ. Orthop. Res. Soc.* 29, 1351–1360. doi:10.1002/jor.21400

Schulze-Tanzil, G., Mobasheri, A., Clegg, P. D., Sendzik, J., John, T., and Shakibaei, M. (2004). Cultivation of human tenocytes in high-density culture. *Histochem Cell Biol.* 122, 219–228. doi:10.1007/s00418-004-0694-9

Shojaee, A., and Parham, A. (2019). Strategies of tenogenic differentiation of equine stem cells for tendon repair: current status and challenges. *Stem Cell Res. Ther.* 10, 181. doi:10.1186/s13287-019-1291-0

Smith, R. K., Werling, N. J., Dakin, S. G., Alam, R., Goodship, A. E., and Dudhia, J. (2013). Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. *PLoS One* 8, e75697. doi:10.1371/journal.pone.0075697

Snedeker, J. G., and Foolen, J. (2017). Tendon injury and repair - a perspective on the basic mechanisms of tendon disease and future clinical therapy. *Acta biomater.* 63, 18–36. doi:10.1016/j.actbio.2017.08.032

Stanco, D., Caprara, C., Ciardelli, G., Mariotta, L., Gola, M., Minonzio, G., et al. (2019). Tenogenic differentiation protocol in xenogenic-free media enhances tendon-related marker expression in ascs. *PLoS One* 14, e0212192. doi:10.1371/journal.pone.0212192

Taguchi, T., Zhang, N., Angibeau, D., Spivey, K. P., and Lopez, M. J. (2021). Evaluation of canine adipose-derived multipotent stromal cell differentiation to ligamentoblasts on tensioned collagen type i templates in a custom bioreactor culture system. *Am. J. Vet. Res.* 82, 924–934. doi:10.2460/ajvr.82.11.924

Takamoto, T., Ichinohe, N., and Tabata, Y. (2012). Proliferation of rat mesenchymal stem cells in collagen sponges reinforced with poly(ethylene terephthalate) fibers by stirring culture method. *J. Biomater. Sci. Polym. Ed.* 23, 1741–1753. doi:10.1163/156856211X598184

Takacs, R., Poliska, S., Juhasz, T., Barna, K. B., and Matta, C. (2023). Isolation of high-quality total RNA from small animal articular cartilage for next-generation sequencing. *Curr Protoc* 3, e692. doi:10.1002/cpz1.692

Taye, N., Karoulias, S. Z., and Hubmacher, D. (2020). The "other" 15-40%: the role of non-collagenous extracellular matrix proteins and minor collagens in tendon. *J. Orthop. Res. official Publ. Orthop. Res. Soc.* 38, 23–35. doi:10.1002/jor.24440

Taylor, S. E., Vaughan-Thomas, A., Clements, D. N., Pinchbeck, G., Macrory, L. C., Smith, R. K., et al. (2009). Gene expression markers of tendon fibroblasts in normal and diseased tissue compared to monolayer and three dimensional culture systems. *BMC Musculoskelet. Disord.* 10, 27. doi:10.1186/1471-2474-10-27

Theiss, F., Mirsaidi, A., Mhanna, R., Kummerle, J., Glanz, S., Bahrenberg, G., et al. (2015). Use of biomimetic microtissue spheroids and specific growth factor supplementation to improve tenocyte differentiation and adaptation to a collagen-based scaffold *in vitro*. *Biomaterials* 69, 99–109. doi:10.1016/j.biomaterials.2015.08.013

Thorpe, C. T., Karunaseelan, K. J., Ng Chieng Hin, J., Riley, G. P., Birch, H. L., Clegg, P. D., et al. (2016). Distribution of proteins within different compartments of tendon varies according to tendon type. *J. Anat.* 229, 450–458. doi:10.1111/joa.12485

Ugarte, F., Sousae, R., Cinquin, B., Martin, E. W., Krietsch, J., Sanchez, G., et al. (2015). Progressive chromatin condensation and h3k9 methylation regulate the differentiation of embryonic and hematopoietic stem cells. *Stem Cell Rep.* 5, 728–740. doi:10.1016/j.stemcr.2015.09.009

Vadaye Kheiry, E., Fazly Bazzaz, B. S., and Kerachian, M. A. (2021). Implantation of stem cells on synthetic or biological scaffolds: an overview of bone regeneration. *Biotechnol. Genet. Eng. Rev.* 37, 238–268. doi:10.1080/02648725.2021.2003590

Van Loon, V. J., Scheffer, C. J., Genn, H. J., Hoogendoorn, A. C., and Greve, J. W. (2014). Clinical follow-up of horses treated with allogeneic equine mesenchymal stem cells derived from umbilical cord blood for different tendon and ligament disorders. *Vet. Q.* 34, 92–97. doi:10.1080/01652176.2014.949390

Vidal, M. A., Kilroy, G. E., Lopez, M. J., Johnson, J. R., Moore, R. M., and Gimble, J. M. (2007). Characterization of equine adipose tissue-derived stromal cells: adipogenic and osteogenic capacity and comparison with bone marrow-derived mesenchymal stromal cells. *Vet. Surg.* 36, 613–622. doi:10.1111/j.1532-950X.2007.00313.x

Wang, A., Breidahl, W., Mackie, K. E., Lin, Z., Qin, A., Chen, J., et al. (2013). Autologous tenocyte injection for the treatment of severe, chronic resistant lateral epicondylitis: a pilot study. *Am. J. sports Med.* 41, 2925–2932. doi:10.1177/0363546513504285

Wang, A., Chen, J., and Zheng, M. (2005). Autologous tenocyte implantation on collagen bio-scaffolds improve healing of rotator cuff tendon defects in a rabbit model. *Orthop. Proc.* 87-B, 333. doi:10.1302/0301-620X.87BSUPP_III.0870333c

Williams, R. B., Harkins, L. S., Hammond, C. J., and Wood, J. L. (2001). Racehorse injuries, clinical problems and fatalities recorded on british racecourses from flat racing and national hunt racing during 1996, 1997 and 1998. *Equine veterinary J.* 33, 478–486. doi:10.2746/042516401776254808

Witte, S., Dedman, C., Harriss, F., Kelly, G., Chang, Y. M., and Witte, T. H. (2016). Comparison of treatment outcomes for superficial digital flexor tendonitis in national hunt racehorses. *Vet. J.* 216, 157–163. doi:10.1016/j.tvjl.2016.08.003

Witte, T. H., Yeager, A. E., and Nixon, A. J. (2011). Intralesional injection of insulin-like growth factor-i for treatment of superficial digital flexor tendonitis in thoroughbred racehorses: 40 cases (2000-2004). *J. Am. Vet. Med. Assoc.* 239, 992–997. doi:10.2460/javma.239.7.992

Xie, L., Zhang, N., Marsano, A., Vunjak-Novakovic, G., Zhang, Y., and Lopez, M. J. (2013). *In vitro* mesenchymal trilineage differentiation and extracellular matrix production by adipose and bone marrow derived adult equine multipotent stromal cells on a collagen scaffold. *Stem Cell Rev.* 9, 858–872. doi:10.1007/s12015-013-9456-1

Yang, F., Zhang, A., and Richardson, D. W. (2019). Regulation of the tenogenic gene expression in equine tenocyte-derived induced pluripotent stem cells by mechanical loading and mohawk. *Stem Cell Res.* 39, 101489. doi:10.1016/j.scr.2019.101489

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T. L. (2012). Primer-blast: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinforma.* 13, 134. doi:10.1186/1471-2105-13-134

Youngstrom, D. W., LaDow, J. E., and Barrett, J. G. (2016). Tenogenesis of bone marrow-, adipose-, and tendon-derived stem cells in a dynamic bioreactor. *Connect. Tissue Res.* 57, 454–465. doi:10.3109/03008207.2015.1117458

Zhang, B., Luo, Q., Deng, B., Morita, Y., Ju, Y., and Song, G. (2018). Construction of tendon replacement tissue based on collagen sponge and mesenchymal stem cells by coupled mechano-chemical induction and evaluation of its tendon repair abilities. *Acta biomater.* 74, 247–259. doi:10.1016/j.actbio.2018.04.047

Zhao, F., Bai, Y., Xiang, X., and Pang, X. (2023). The role of fibromodulin in inflammatory responses and diseases associated with inflammation. *Front. Immunol.* 14, 1191787. doi:10.3389/fimmu.2023.1191787

Zhao, Y., Zhang, S., Zhou, J., Wang, J., Zhen, M., Liu, Y., et al. (2010). The development of a tissue-engineered artery using decellularized scaffold and autologous ovine mesenchymal stem cells. *Biomaterials* 31, 296–307. doi:10.1016/j.biomaterials.2009.09.049

Zhou, L. B., Ding, R. Y., Xu, B. X., Fan, X., Li, B. W., Wang, G., et al. (2016). Application of microfibrillar collagen hemostat sponge for cartilage engineering. *Int. J. Clin. Exp. Med.* 9, 6127–6132.

frontiers | Frontiers in Medical Technology

TYPE Brief Research Report
PUBLISHED 15 February 2024
DOI 10.3389/fmedt.2024.1269861

OPEN ACCESS

EDITED BY
Leo R. Quinlan,
University of Galway, Ireland

REVIEWED BY
Sofija Pejkova,
University Clinic for Plastic and Reconstructive Surgery, North Macedonia
Georg Singer,
Medical University of Graz, Austria

*CORRESPONDENCE
Sarah Cartmell
✉ sarah.cartmell@manchester.ac.uk

RECEIVED 31 July 2023
ACCEPTED 31 January 2024
PUBLISHED 15 February 2024

CITATION
Xue R, Wong J, Imere A, King H, Clegg P and Cartmell S (2024) Current clinical opinion on surgical approaches and rehabilitation of hand flexor tendon injury—a questionnaire study. Front. Med. Technol. 6:1269861. doi: 10.3389/fmedt.2024.1269861

Current clinical opinion on surgical approaches and rehabilitation of hand flexor tendon injury—a questionnaire study

Ruikang Xue[1], Jason Wong[2,3], Angela Imere[1,4], Heather King[5], Peter Clegg[6,7] and Sarah Cartmell[1,4]*

[1]Department of Materials, Faculty of Science and Engineering, School of Natural Sciences, University of Manchester, Manchester, United Kingdom, [2]Division of Cell Matrix Biology & Regenerative Medicine, University of Manchester, Manchester, United Kingdom, [3]Department of Plastic & Reconstructive Surgery, Manchester Academic Health Science Centre, Manchester University NHS Foundation Trust, Manchester, United Kingdom, [4]The Henry Royce Institute, Royce Hub Building, The University of Manchester, Manchester, United Kingdom, [5]Addos Consulting Ltd, Winchester, United Kingdom, [6]Department and of Musculoskeletal and Ageing Science, Institute of Life Course and Medical Sciences, William Henry Duncan Building, University of Liverpool, Liverpool, United Kingdom, [7]MRC-Versus Arthritis Centre for Integrated Research in Musculoskeletal Ageing, William Henry Duncan Building, University of Liverpool, Liverpool, United Kingdom

The management of flexor tendon injury has seen many iterations over the years, but more substantial innovations in practice have been sadly lacking. The aim of this study was to investigate the current practice of flexor tendon injury management, and variation in practice from the previous reports, most troublesome complications, and whether there was a clinical interest in potential innovative tendon repair technologies. An online survey was distributed via the British Society for Surgery of the Hand (BSSH) and a total of 132 responses were collected anonymously. Results showed that although most surgeons followed the current medical recommendation based on the literature, a significant number of surgeons still employed more conventional treatments in clinic, such as general anesthesia, ineffective tendon retrieval techniques, and passive rehabilitation. Complications including adhesion formation and re-rupture remained persistent. The interest in new approaches such as use of minimally invasive instruments, biodegradable materials and additive manufactured devices was not strong, however the surgeons were potentially open to more effective and economic solutions.

KEYWORDS

flexor tendon, repair, retrieval, survey, clinical opinion

1 Introduction

Flexor tendon injuries are one of the most common ailments in hand surgery that can lead to long-term disability and significant negative social and economic impact (1, 2). Despite a wealth of research in the field, management of hand flexor tendon injury remains inconsistent in approaches and outcomes (3). Current evidence revealed several beneficial development and change in practice in the field, including the use of wide-awake local anesthesia no tourniquet (WALANT) technique (4), updated methods of

retrieving the retracted tendon stump (5–8), change in tendon repair approaches (9), the use of early active mobilization in post-surgery rehabilitation (10).

Use of endoscope and other minimally invasive surgical instrument has been described in the literature for the retrieval of the retracted tendon stump (11) and flexor tendon repair (12). With advances in the surgical instrumentation, minimally invasive surgeries have been widely used in other tendon repair such as Achilles tendon to deliver beneficial outcome (13). Development in tissue engineering, biomaterials and additive manufacturing has further potential in improving tendon repair outcome (14, 15).

In this study, we distributed an online questionnaire to hand surgeons via the British Society for Surgery of the Hand (BSSH) to survey the current clinical practice on hand flexor tendon injury management, including anesthesia, tendon retrieval, tendon repair, post-surgery rehabilitation, operative time and complications. Firstly, we aimed to assess the impact of the current medical research evidence on clinical practice, and identify if there is any trend from previous survey studies. Finally, we aimed to gather surgeons' opinions on the adapting potential novel solutions to treat hand flexor tendon injury enabled by minimally invasive instrumentation, biomaterials and additive manufacturing.

2 Methods

A 26-item online survey was developed, containing 22 single-answer multiple-choice questions and 4 open-ended questions (see Supplementary Table S1). "Other, please specify" option was included in all the multiple-choice questions to improve study flexibility. The first 3 open-ended questions were follow-on questions that were designed to enable the respondents to add any additional comments on tendon retrieval technique used, challenges in tendon retrieval, and estimated complication rate of the most common complication the respondents mentioned. The last open question was designed to enable the respondents to add any additional comments on any aspect of flexor tendon injury management. The survey covered a number of aspects of hand tendon injury management, including anesthesia, tendon retrieval, primary tendon repair, peripheral (i.e., epitendinous) repair, post-surgery rehabilitation, average operation time, complications, as well as opinions on some potential solutions. Demographics of respondents including gender, age, ethnic background, experience in hand specialty, type of their surgery were also collected. The survey was peer reviewed by the authors and in consultation with external hand surgeons, and subsequently approved by the University of Manchester Research Ethics Committee (2019-7707-11796).

The survey was electronically delivered to surgeons through the British Society for Surgery of the Hand (BSSH) communication channels (email and Twitter) to BSSH members and associates.

The results remained anonymous and were analyzed using GraphPad Prism 8.4.3 (GraphPad Software, USA) where, for each question, the total number of answers (N) was obtained, and the percentage of each option was calculated. The comments from the open-ended questions were categorized and the percentage of each category was calculated.

3 Results

A total of 132 individual survey responses were completed. The full demographic information collected can be found in Supplementary Table S2. Most respondents had been in hand specialty for over 11 years (48%, 61/128), with 23% (30/128) being in hand specialty for 8–11 years, 22% (28/128) for 4–7 years and 7% (9/128) for less than 4 years. The majority of respondents (71%, 91/129) were working in National Health Service (NHS) in the UK whereas 4% (5/129) respondents were working only in private surgery; the rest of the respondents (26%, 33/129) were involved in both NHS and private surgery.

In terms of anesthesia used for flexor tendon repair, most respondents preferred regional anesthesia (60%, 78/130), whilst others favored the use of general anesthesia (22%, 28/130) or WALANT (18%, 24/130, Figure 1A).

The majority of the respondents used atraumatic tendon retrieval techniques (85%, 110/130) if there was retraction of the proximal end of the tendon stump (Figure 1B). Most respondents described using sutures along with either a small gauge flexible feeding tube or equivalent material for tendon retrieval (46%, 51/112, Figure 1C). Other methods mentioned include milking (15%, 17/112), creating extra incisions (10%, 11/112), use of loop sutures (10%, 11/112), wrist or digit flexion (4%, 4/112), use of tendon retrievers (4%, 4/112), push and pull method (3%, 3/112), use of needle (3%, 3/112), use of tendon or skin hook (3%, 3/112), use of dental wire (3%, 3/112) and use of endoscope or microsurgical techniques (2%, 2/112).

Forty-one percent (52/128) respondents considered minimizing tissue damage as the key challenge in flexor tendon retrieval (Figure 1D); preservation of tendon sheath and pulley structure, and maintaining the tendon stump integrity were specifically mentioned. Furthermore, passing tendon stump through the pulley system was frequently mentioned (27%, 35/128), followed by locating proximal tendon end (9%, 12/128), maintain anatomic alignment to avoid decussation (6%, 8/128), tendon retraction (5%, 6/128) and keeping retrieved tendon ends apposed during surgical repair (4%, 5/128). Five other challenges were also mentioned once or twice by the respondents – tendon shortening, retrieval of *flexor pollicis longus* (FPL) tendon, availability of narrow feeding tube, lack of time, attaching the tendon to the feeding tube and lack of a surgical assistant.

For primary repair, a wide range of techniques were used, among which the Cruciate suture pattern (28%, 37/131) was the most popular (Figure 2A). Four-strand repairs (56%, 76/131) were preferred by the respondents to either the 2-strand (22%, 29/131) or 6-strand repairs (22%, 29/131). Prolene (52%, 69/132) suture material was most frequently used, followed by Ethibond (17%, 23/132), Fiberwire (15%, 20/132), Ticron (8%, 10/132), Nylon (5%, 6/132) and PDS (3%, 4/132). For suture gauge, 3-0 (57%, 78/136) and 4-0 (41%, 56/136) sutures were

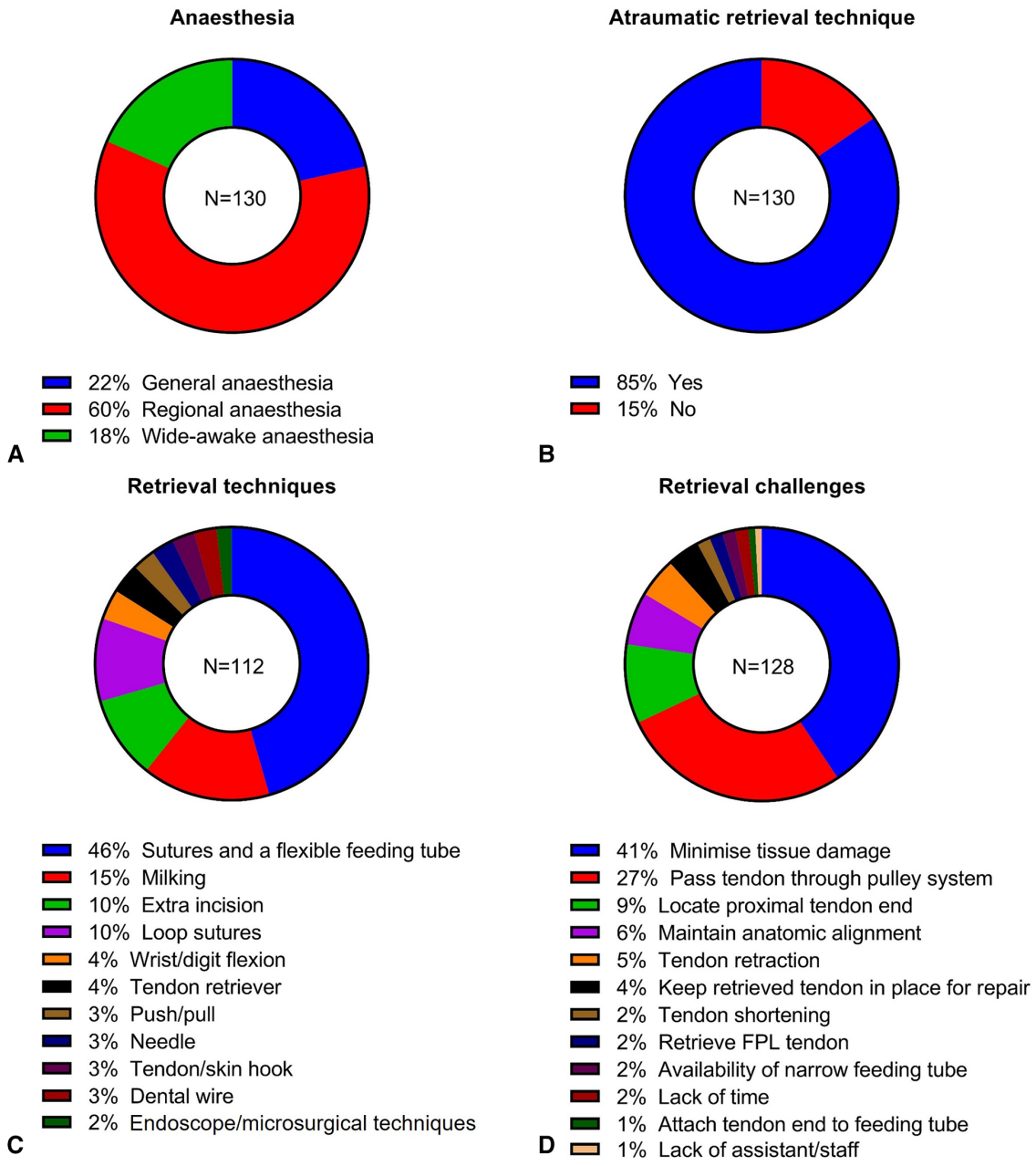


FIGURE 1
Results of anesthesia used for flexor tendon repair (A), use of atraumatic tendon retrieval (B), tendon retrieval techniques (C) and challenges in tendon retrieval (D).

used by most respondents whereas 2-0 gauge was only mentioned twice (2%, 2/136).

For the peripheral repair, simple running suture technique (51%, 66/128) was most preferred by the respondents, followed by cross-stitch (34%, 43/128) and simple locking (15%, 19/128, Figure 2B). Noticeably, under cross-stitch technique, Silfverskiöld repair was mentioned 27 times (21%, 27/128). Like primary repair, Prolene (74%, 95/129) was the most frequently used suture material; other materials reported include Nylon (16%, 21/129), PDS (8%, 11/129), Ethibond (1%, 1/129) and Vicryl (1%, 1/129). Sutures with 5-0 (61%, 81/132) and 6-0 gauge (38%, 50/132) were used by most respondents with only 1 individual using 8-0 gauge (1%, 1/132).

In terms of rehabilitation protocol, most respondents would support early active mobilization (84%, 108/128, Figure 3A). On the other hand, early passive mobilization protocol (15%, 19/128) was not uncommon. Immobilization was selected by one respondent (1%, 1/128).

On average, the majority the respondents (89%, 118/132) spent less than 1 h on hand tendon repair operations with 5%

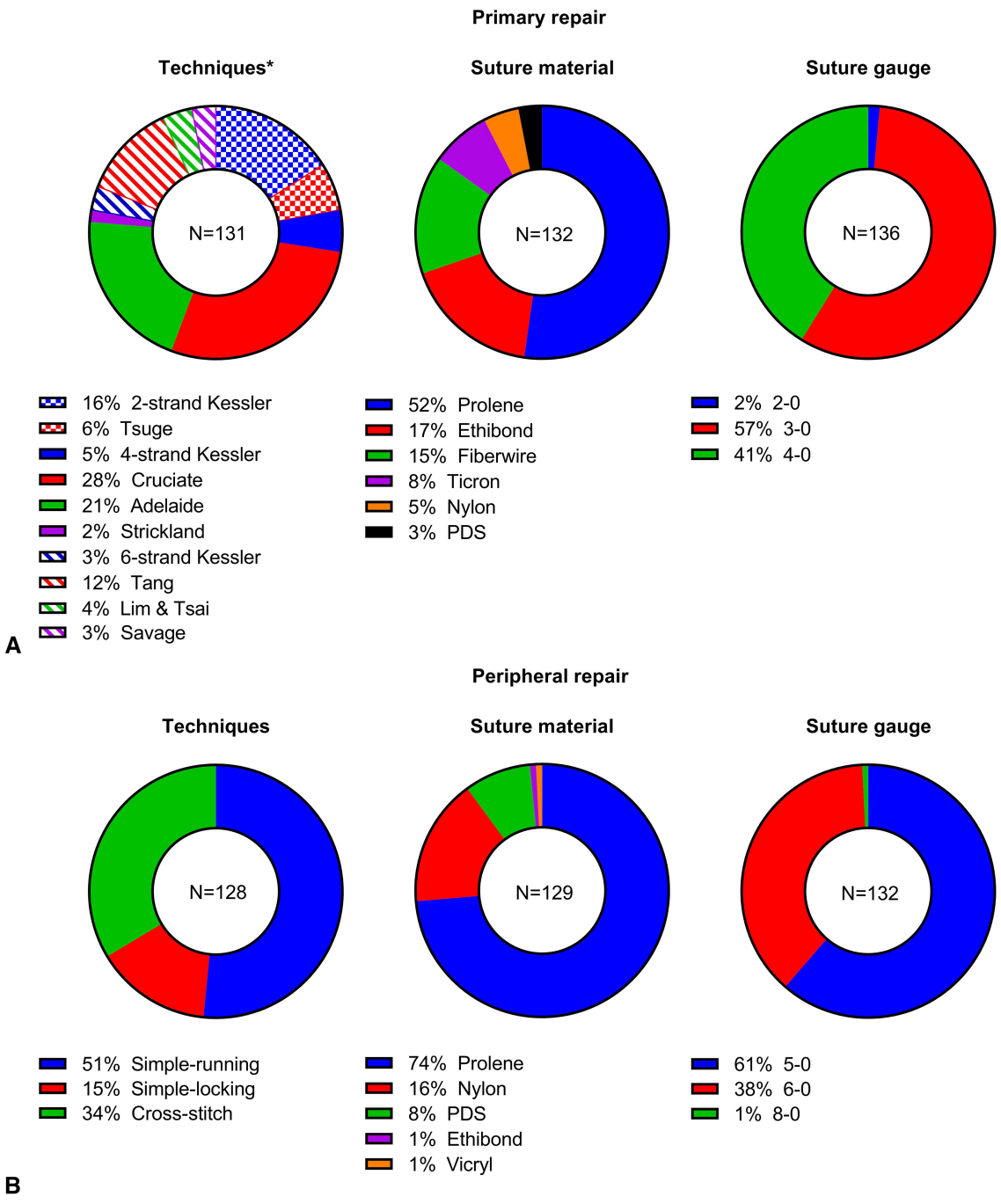


FIGURE 2
Results of repair techniques, suture materials and suture gauge on primary (A) and peripheral tendon repair (B). *Checked – 2 strand repairs, solid – 4 strand repairs and hatched – 6 strand repairs.

respondents under 20 min (7/132), 34% from 20 to 39 min (45/132), and 50% from 40 to 59 min (66/132). However, 12 respondents (9%) selected 60–79 min and 2 respondents (2%) spent over 79 min (Figure 3B).

Adhesion formation (83%, 104/126) was identified as the most common complication of flexor tendon repair by the majority of the respondents (Figure 3C). Other respondents considered re-rupture (11%, 14/126), joint stiffness (5%, 6/126) or infection (2%, 2/126) as the most common complication. Comments from the respondents on the estimated complication rate indicated that most patients would get some adhesion formation and complicated adhesion accounted for 5%–20% of the patients. For the estimated percentage of re-rupture, 45% (5/11) respondents indicated under 5% and another 45% (5/11) responses indicated 5%–10%, whereas one respondent answered 15%. Only 1 respondent commented on the complication rate

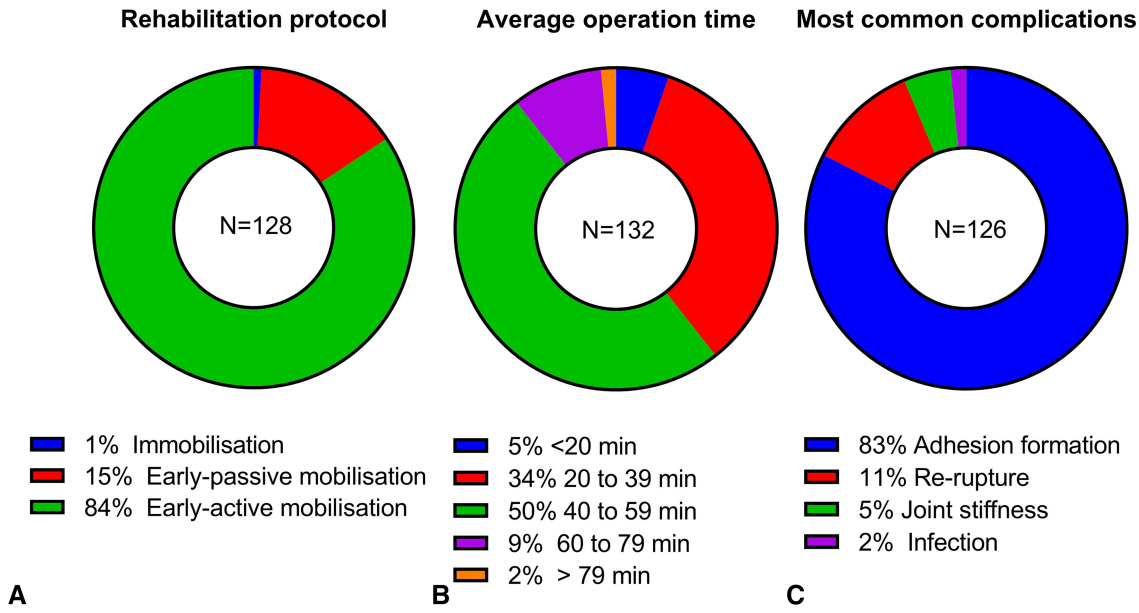


FIGURE 3
Results of rehabilitation protocol (A), average hand tendon repair operation time (B), and most common complications (C).

of joint stiffness – "majority cases". The estimated infection rate was below 5% from 2 respondents.

In Figure 4, questions regarding clinical opinions on novel approaches to tendon repair revealed that only 29% (38/130) of the respondents supported the use of endoscopes or fiber optic technology, whereas a significant portion of the respondents (43%, 55/130) reacted negatively. In terms of the use of degradable biomaterials, the majority of respondents had a neutral response (58%, 74/128); from the remaining participants, more negative responds (26%, 34/128) were found compared to positive ones (16%, 20/128). Similarly, for the use of additive manufacturing, neutral responses were received by most participants (68%, 88/129), followed by negative (24%, 32/129) and positive (7%, 9/129) responses. Interestingly, in contrary to results from the multiple-choice questions, six out of eight comments on the topic from the open-ended question indicated that there is still a room for more expensive novel approaches provided they are simple to use and can provide better outcome.

4 Discussion

Three previous questionnaire studies on hand flexor tendon injury management were identified from the literature. Healy et al. surveyed 22 consultant surgeons in Ireland in 2007 (16); Rudge et al. surveyed 39 hand units in the UK and Ireland with responses from their lead consultant surgeon (17); and Gibson et al. surveyed 410 individual surgeons with varied experience in the USA (18). In comparison, this study surveyed 132 individual surgeons with varied experience in the UK, which was the highest in the UK. The comparison between current medical evidence, previous survey findings and this this work is summarized in Table 1.

Traditional anesthesia approaches including general and regional anesthesia along with tourniquet application are routinely practiced in hand flexor tendon repair. In the past two decades, WALANT that injects lidocaine with epinephrine directly to the operative site, which enables patients to remain conscious during the operation, gained increasing popularity (30, 31). It showed comparative flexor tendon repair outcome to general and regional anesthesia (32), and a number of additional advantages, including intraoperative assessment of tendon repair, reduction in operation time and surgery cost, faster patient discharge, better patient education (4, 19). Gibson survey reported that 20% respondents performed WALANT in the past, and 45% of which preferred it when situation allows. In comparison, this study showed an increase in preference of using WALANT (18%) (18). Following the COVID-19 pandemic, numerous centers reported a shift towards WALANT due to its reduced risk to healthcare professionals from contracting COVID-19 by removing the use of aerosol-generating traditional anesthesia (20, 33), which is expected to cause further increase in the use of WALANT.

In zone II flexor tendon injury, retrieval of retracted proximal tendon stump can be problematic due to the presence of the relatively inelastic tendon sheath; trauma created during retrieval can lead to poor functional outcome and needs to be minimized. A plethora of tendon retrieval methods were published in literature (1, 11). Agreed with the current evidence, in this study, most respondents (85%) supported atraumatic tendon retrieval, and reported a variety of retrieval techniques. Recent development of flexor tendon retrieval techniques advocated the

TABLE 1 Comparison between current evidence, previous questionnaire studies and the current work on aspects of flexor tendon injury management investigated.

	Current evidence	Previous survey study			Current work
		Healy et al. (16)	Rudge et al. (17)	Gibson et al. (18)	
Respondent demographics		22 Individuals, Consultant surgeons, Ireland	39 Hand units, Lead consultant surgeon, UK and Ireland	410 Individuals, Varied experience, USA	132 Individuals, Varied experience, UK
Anaesthesia	WALANT offers better patient education, intra-operative assessment, reduced risk of Covid transmission, and economic benefits to patient and hospital (4, 19, 20).			20% performed WALANT, 45% of which preferred it	18% WALANT, majority (60%) preferred general anaesthesia
Retrieval of retracted tendon stump	A mix of retrieval methods are used. Atraumatic approach should be used where possible as minimizing tissue damage is the highest priority during tendon retrieval (1, 11). Recent techniques advocated the use of sutures, a flexible tube and a proximal palmar incision (5–8).				85% used atraumatic approach, 11 different techniques reported, Use of sutures and a flexible feeding tube (46%) most common approach, but "milking" still common (15%). Minimising tissue damage (41%) most common challenge, but numerous other challenges also reported.
Primary repair	A mix of repair methods are used, Multi-strand repair showed improved strength and rupture rate in biomechanical studies but its clinical evidence is less clear (21–23). Braided polyester sutures showed better strength than monofilament Prolene sutures (3, 18).	Two most popular repair methods			
		Kessler (68%), Adelaide (23%)	Kessler (64%), Strickland (18%)	Kessler (42%), Cruciate (26%)	Cruciate (28%), Adelaide (21%)
		2-strand vs. multi-strand methods			
		2-strand (64%), multi-strand (32%)	2-strand (36%), multi-strand (64%)	2-strand (6%), multi-strand (94%)	2-strand (16%), multi-strand (84%)
		Suture materials: Prolene vs. braided polyester (Fibrewire, Ethibond)			
		Prolene (45%), braided polyester (36%)	Prolene (64%), braided polyester (34%)	Prolene (8%), braided polyester (54%)	Prolene (52%), braided polyester (32%)
		Suture gauge			
		3-0 (50%), 4-0 (50%)	3-0 (82%), 4-0 (18%)	3-0 (52%), 4-0 (47%)	3-0 (57%), 4-0 (41%)
Peripheral repair	Cross-stitch offers higher mechanical strength than simple running and locking (24, 25).	Two most popular repair methods			
		Simple running (73%)		97% used peripheral repair	Simple running (51%), Cross-stitch (34%)
		Two most popular suture material			
		Prolene (64%), Nylon (27%)	Prolene (82%), Nylon (18%)		Prolene (74%), Nylon (16%)
		Suture gauge			
			5-0 (28%), 6-0 (72%)		5-0 (61%), 6-0 (38%)
Rehabilitation	EAM offers improved functional outcome and economic benefits with a trade-off for slight increase of re-rupture rates (26, 27).			EAM (51%), PEM (49%)	EAM (84%), EPM (15%)
Operation time	NHS UK indicates 45–60 min for a simple flexor tendon repair (28).				Most common 40–59 min (50%), and 20–39 min (34%)
Complications	4% re-rupture, 4% adhesion formation (29).				Most common (83%) with estimated rate of 5%–20% for complicated adhesion. Re-rupture (11%) with estimated rate of 5%–10% from 10/11 respondents.

WALANT, wide-awake local anesthesia no tourniquet; EAM, early active mobilization; EPM, early passive mobilization; NHS, national health service.

use of sutures, a flexible tube and a proximal palmar incision, in which the tendon stump was connected to the flexible tube and successfully retrieved through the tendon sheath (5–8). Most respondents (45%) mentioned the use of sutures and a flexible feeding tube, and some respondents (10%) also mentioned the creation of an extra incision. However, a number of the respondents (15%) still chose "Milking" in which the proximal tendon stump was milked down through the tendon sheath despite its low success rate (61%) (11). Supported by 10% of the respondents, looped sutures were able to retrieve the proximal stump through tendon sheath without the need of a feeding tube (34). It is worth noting that almost 1 in 5 respondents used other retrieval techniques, showing the diversity in tendon retrieval methods in practice. In alignment with the current evidence, minimizing tissue damage (commented by 41% respondents) is most challenging and is the highest priority during tendon retrieval (1). However, comments received from this work considered a variety of other factors as most common

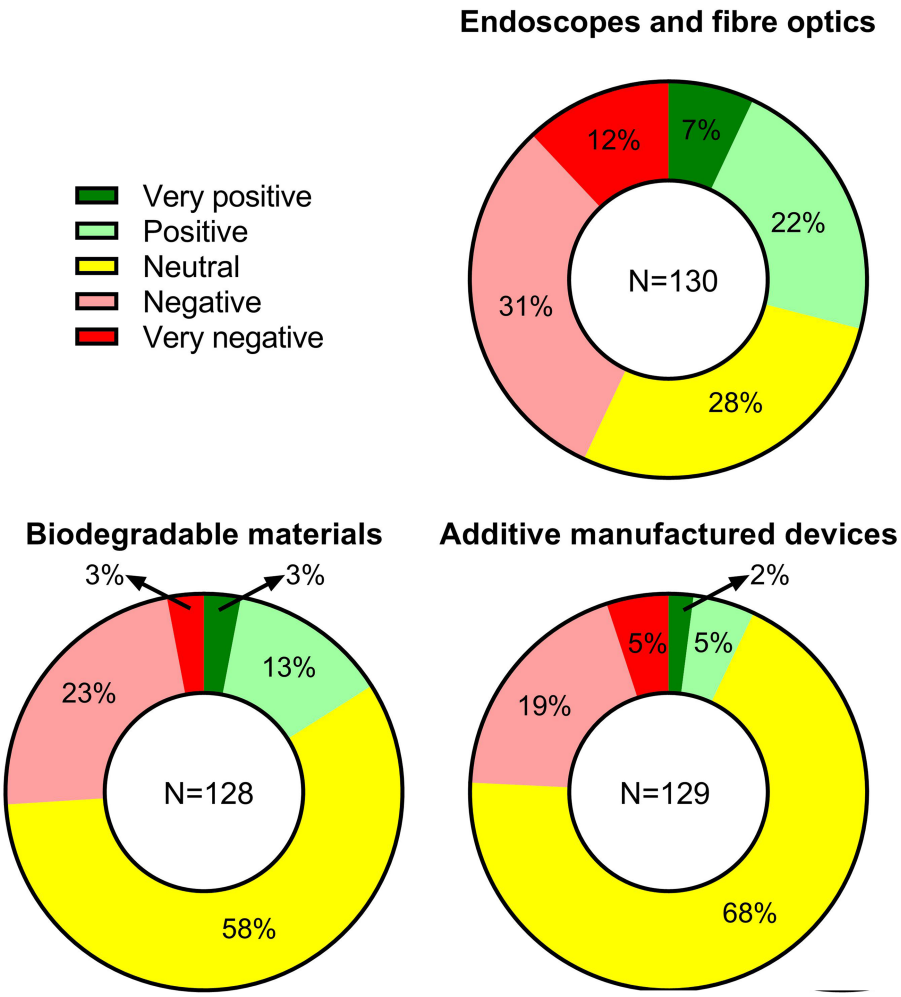


FIGURE 4
Clinical opinions on new approaches to treat hand flexor tendon injuries.

challenges, including passing tendon through pulley system (27%) that has an important role in translating force from muscles to phalanges (35), difficulty in locating the retracted tendon (9%) that sometimes requires multiple incisions (1), maintaining the anatomic alignment of retrieved tendons (6%) that can affect the mechanical efficiency of figure flexion (36), and eight additional items, each of which commented by less than 5% of the respondents. No information on flexor tendon retrieval was reported in the previous survey studies.

An ideal primary flexor tendon repair aims to provide strong repair strength to minimize risk of re-rupture with minimal bulkiness that can interfere with tendon gliding and ultimately result in adhesion formation (21). In the past decade, multi-strand core sutures (4-strand or more) with 3-0 or 4-0 sutures gained increasing popularity across the globe (9, 21). Laboratory biomechanical studies showed superior repair strength and lower re-rupture rate of multi-strand repairs; however, clinical studies showed no significance in overall re-rupture rate despite that re-rupture occurred later (after 4 weeks) in multi-strand repairs compared to 2-strand repairs (22, 23). Thinner sutures reduced tissue bulk and minimized tissue trauma whereas thicker sutures improved repair strength and ease of handling. 78% of respondents followed the current trend of using multi-strand core sutures (78%) and the majority of them (98%) used 3-0 or 4-0 sutures. 4-strand Cruciate and Adelaide repair techniques were used by almost half (49%) of the respondents, which was likely due to their good repair strength and ease of performance previously reported (37, 38). In terms of the suture materials, newer braided polyester sutures such as Fiberwire and Ethibond have been shown to have superior mechanical properties than monofilament sutures such as Prolene and Nylon (3, 18). However, Prolene (52%) was still the choice from the most respondents from the current survey, followed by braided polyesters (32%), and 16% other materials (Ticron, Nylon and PDS). Compared to previous surveys in the UK and Ireland (16, 17), an increased use of multi-strand core sutures and Cruciate suturing was observed, whereas there was a limited change in suture material; interestingly, more 3-0 sutures (82%) were reported despite of an uptake of multi-strand (64%) repair in Rudge's study. In contrast, Gibson's survey indicated that more

surgeons in the US supported the use of multi-strand core (94%), Kessler type repair (42%) and braided polyester materials (52%) (18).

Peripheral repairs are often used in combination with primary core suture repairs to improve repair strength, prevent fraying and decrease friction (24). In vitro biomechanical study revealed that Cross-stitch had higher mechanical strength than simple-running and simple-locking techniques (24, 25). Despite inferior mechanical strength, simple-running technique was identified by more than half of respondents (51%), which was likely due to its ease of use (24). Compared to previous surveys (16, 17), an increased use of Cross-stitch was identified, and there was not much change in suture materials as Prolene and Nylon remained the most popular choices; more 5-0 sutures were used in the current study compared to Rudge's.

Since 1940s, flexor tendon rehabilitation has progressed from immobilization to early passive mobilization to early active mobilization (39). Recent studies showed that early active mobilization improved functional outcome including increased range of motion, reduced joint stiffness and adhesions, with a trade-off for slight increase of re-rupture rates (26, 27). Furthermore, early active mobilization is the least therapist dependent method, leading to additional economic benefits (23). The majority of the correspondents (84%) followed the current scientific evidence to use early active mobilization rehabilitation, higher than the percentage (51%) in Gibson's report.

The NHS in the UK estimated the average operative time for a simple flexor tendon repair to be between 45 and 60 min (28), which was supported by half of respondents. Interestingly, 39% respondents were faster than the NHS suggestion, whilst 11% respondents were slower. Tang et al. reported the surgery time for performing a primary repair with various suturing techniques, ranging from 6.2 to 13.5 min (40). Apart from primary tendon repair, incision for tendon access, tendon retrieval, peripheral repair and pulley reconstruction can also affect the total operative time in flexor tendon surgery (1).

Meta-analysis on complications of flexor tendon repair from Dy et al. revealed a 6% average rate of re-operation, 4% of adhesion formation and 4% of re-rupture (29). Adhesion formation is frequently observed after surgery and complicated adhesions need surgical tenolysis; the rate of re-rupture has continuously decreased in the past decade but re-rupture still remains a persistent complication (41). Those two complications were mentioned by 94% of the respondents as the most common complications, with the their estimated rate ranging from 5% to 20% for complicated adhesions, and 5%–10% for re-rupture. Joint stiffness (5%) and infection (2%) were also mentioned by some respondents. Joint stiffness is common, but it normally improves with time through daily hand use; infection after flexor tendon repair is rare, which is most likely caused by contamination during initial trauma (41, 42). The functional outcome was reported to be over 80% with overall excellent and good recovery of functionality in recent years (41).

Minimally invasive instrumentation (e.g., endoscopes, fiber optics, microsurgical tools) has evolved rapidly and it can benefit hand tendon repair (43, 44). Recently, Kucukguven et al. employed an endoscope and 1 mm flexible forceps to atraumatically retrieve retracted tendons, showing significantly shorter operative duration, better pain score and higher total range of active motion in 11 patients compared to traditional retrieval methods (44). Biodegradable materials and tissue engineering are other ways to reduce flexor tendon repair complications since it was shown to enhance tendon healing and regeneration, leading to improved functional outcomes (45). Also, our previous studies indicated that suture repairs produced high stress regions and acellular zones on the tendon, which potentially contributed to early tendon failure (46, 47). Additive manufacturing and barbed connecting devices may provide unique solutions in reducing acellular zones and improving tendon repair (48, 49). This work revealed that the surgeons' interests in new approaches such as endoscopes, biodegradable materials and additive manufactured devices were not strong with most responses being neutral or negative. However, it was mentioned by several clinicians that there was still room for improvement in the field and approaches to reduce complications, improve functional outcome and shorten surgery time were still welcome.

As with most surveys, this study may have potential bias caused by incomplete sampling and underrepresentation of the non-responders (50, 51). Specifically, the response rate was not available due to the unknown number of total recipients of the survey. Also, this study did not take pediatric patients into consideration, which could provide more comprehensive knowledge on the current practice. Furthermore, geographical data was not collected although the majority of the respondents were believed to be in the UK because the questionnaire was distributed via the BSSH communications to its members and associates. Last but not least, some specific technical aspects of tendon repairs such as flexor digitorum superficialis tendon repair, A2/A4 pulley release, tendon sheath repair, comparison between delayed and primary repairs were not included in the survey, which could lead to enhancement of the impact of the publication.

In conclusion, this study revealed an increased number of surgeons followed the current medical evidence on flexor tendon injury management in the UK. However, a portion of surgeons still practiced suboptimal solutions, including costly general anesthesia, traumatic and ineffective tendon retrieval techniques, traditional two-strand repairs with monofilament suture materials, and passive rehabilitation protocols. Flexor tendon repair complications such as adhesion formation and re-rupture remained persistent. Variation in practice between surgeons from the UK and Ireland, and those from the US suggested that surgeons in the US were able to adopt new technology more quickly into clinic. Last but not least, in general, clinical interest in use of minimally invasive instruments, new biomaterials and additive manufactured devices was not strong; however, novel approaches that can improve repair outcome or provide economic benefits or both were welcomed by a portion of respondents.

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

This study was reviewed and approved by the University of Manchester Research Ethics Committee (2019-7707-11796). The participants provided their written informed consent to participate in this study.

Author contributions

RX: Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. JW: Conceptualization, Investigation, Resources, Supervision, Validation, Writing – review & editing. AI: Data curation, Investigation, Methodology, Writing – review & editing. HK: Methodology, Project administration, Writing – review & editing. PC: Conceptualization, Investigation, Methodology, Writing – review & editing. SC: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Validation, Writing – review & editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article.

The authors would like to thank the financial support from vs. Arthritis (POC075) and Engineering and Physical Sciences Research Council, Medical Research Council Centre for Doctoral Training in Regenerative Medicine (EP/L014904/1) and Henry Royce Institute for Advanced Materials, funded through EPSRC grants EP/R00661X/1, EP/S019367/1, EP/P025021/1 and EP/P025498/1.

Acknowledgments

The authors would like to thank British Society for Surgery of the Hand for distributing the online survey.

Conflict of interest

HK is Director of Addos Consulting Ltd.

The remaining 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.

The authors declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmedt.2024.1269861/full#supplementary-material

References

1. Khor WS, Langer MF, Wong R, Zhou R, Peck F, Wong JK. Improving outcomes in tendon repair: a critical look at the evidence for flexor tendon repair and rehabilitation. *Plast Reconstr Surg.* (2016) 138(6):1045e–58e. doi: 10.1097/PRS.0000000000002769

2. de Jong JP, Nguyen JT, Sonnema AJ, Nguyen EC, Amadio PC, Moran SL. The incidence of acute traumatic tendon injuries in the hand and wrist: a 10-year population-based study. *Clin Orthop Surg.* (2014) 6(2):196–202. doi: 10.4055/cios.2014.6.2.196

3. Wong JK, Peck F. Improving results of flexor tendon repair and rehabilitation. *Plast Reconstr Surg.* (2014) 134(6):913e–25e. doi: 10.1097/PRS.0000000000000749

4. Lalonde DH. Latest advances in wide awake hand surgery. *Hand Clin.* (2019) 35 (1):1–6. doi: 10.1016/j.hcl.2018.08.002

5. Karbalaeikhani A, Yavari M. Flexor tendon retrieval in zone I and II: a new modified technique. *Tech Hand Up Extrem Surg.* (2012) 16(1):45–7. doi: 10.1097/BTH.0b013e3182388561

6. Ozturk MB, Basat SO, Kayadibi T, Karahangil M, Akan IM. Atraumatic flexor tendon retrieval - a simple method. *Ann Surg Innov Res.* (2013) 7(1):11. doi: 10.1186/1750-1164-7-11

7. Ahed K, Moujtahid M, Nechad M. Retrieval of the retracted flexor tendons for long fingers: new tip. *Chir Main.* (2014) 33(4):247–50. doi: 10.1016/j.main.2014.05.003

8. Measuria HD, McBride TJ, Talwalkar SC. Flexor tendon retrieval—a modified technique. *J Hand Surg Eur.* (2014) 39(6):671–2. doi: 10.1177/1753193412460169

9. Tang JB, Lalonde D, Harhaus L, Sadek AF, Moriya K, Pan ZJ. Flexor tendon repair: recent changes and current methods. *J Hand Surg Eur.* (2022) 47(1):31–9. doi: 10.1177/17531934211053757

10. Fujihara Y, Ota H, Watanabe K. Utility of early active motion for flexor tendon repair with concomitant injuries: a multivariate analysis. *Injury.* (2018) 49 (12):2248–51. doi: 10.1016/j.injury.2018.10.022

11. Kadar A, Gur S, Schermann H, Iordache SD. Techniques for retrieval of lacerated flexor tendons: a scoping review. *Plastic Surg.* (2022) :22925503221088841. doi: 10.1177/22925503221088841

12. Cheng J, Feng Y, Liu B, Gong G, Yu H. The therapeutic effects of the minimally invasive repair of hand flexor tendon injuries. *Int J Clin Exp Med.* (2019) 12:13706–11. ISSN: 1940-5901/IJCEM0101060

13. Gatz M, Driessen A, Eschweiler J, Tingart M, Migliorini F. Open versus minimally-invasive surgery for achilles tendon rupture: a meta-analysis study. *Arch Orthop Trauma Surg.* (2021) 141(3):383–401. doi: 10.1007/s00402-020-03437-z

14. Guzzi EA, Tibbitt MW. Additive manufacturing of precision biomaterials. *Adv Mater.* (2020) 32(13):1901994. doi: 10.1002/adma.201901994

15. Hou J, Yang R, Vuong I, Li F, Kong J, Mao H-Q. Biomaterials strategies to balance inflammation and tenogenesis for tendon repair. *Acta Biomater.* (2021) 130:1–16. doi: 10.1016/j.actbio.2021.05.043

16. Healy C, Mulhall KJ, Bouchier-Hayes DJ, Kneafsey B. Practice patterns in flexor tendon repair. *Ir J Med Sci.* (2007) 176(1):41–4. doi: 10.1007/s11845-007-0009-y

17. Rudge W, James M. Flexor tendon injuries in the hand: a UK survey of repair techniques and suture materials—are we following the evidence? *ISRN Plast Surg.* (2014) 2014:687128. doi: 10.1155/2014/687128

18. Gibson PD, Sobol GL, Ahmed IH. Zone II flexor tendon repairs in the United States: trends in current management. *J Hand Surg Am.* (2017) 42(2):e99–108. doi: 10.1016/j.jhsa.2016.11.022

19. Tang JB, Gong KT, Zhu L, Pan ZJ, Xing SG. Performing hand surgery under local anesthesia without a tourniquet in China. *Hand Clin*. (2017) 33(3):415–24. doi: 10.1016/j.hcl.2017.04.013

20. Bamal R, Alnobani O, Bastouros E, Nolan G, Morris E, Griffiths S, et al. Wide-awake local anesthesia no tourniquet (WALANT) for flexor tendon repairs as change in practice during the COVID-19 pandemic: a retrospective cohort study with outcomes. *Cureus*. (2023) 15(3):e36728. doi: 10.7759/cureus.36728

21. Tang JB, Amadio PC, Boyer MI, Savage R, Zhao C, Sandow M, et al. Current practice of primary flexor tendon repair: a global view. *Hand Clin*. (2013) 29 (2):179–89. doi: 10.1016/j.hcl.2013.02.003

22. Hardwicke JT, Tan JJ, Foster MA, Titley OG. A systematic review of 2-strand versus multistrand core suture techniques and functional outcome after digital flexor tendon repair. *J Hand Surg Am*. (2014) 39(4):686–95.e2. doi: 10.1016/j.jhsa.2013.12.037

23. Vögelin E, Elliot D, Amadio PC. IFSSH scientific committee on flexor tendon repair (2015).

24. Wieskotter B, Herbort M, Langer M, Raschke MJ, Wahnert D. The impact of different peripheral suture techniques on the biomechanical stability in flexor tendon repair. *Arch Orthop Trauma Surg*. (2018) 138(1):139–45. doi: 10.1007/s00402-017-2836-2

25. Mishra V, Kuiper JH, Kelly CP. Influence of core suture material and peripheral repair technique on the strength of kessler flexor tendon repair. *J Hand Surg Br*. (2003) 28(4):357–62. doi: 10.1016/S0266-7681(03)00080-9

26. Dy CJ, Daluiski A. Update on zone II flexor tendon injuries. *J Am Acad Orthop Surg*. (2014) 22(12):791–9. doi: 10.5435/JAAOS-22-12-791

27. Starr HM, Snoddy M, Hammond KE, Seiler JG 3rd. Flexor tendon repair rehabilitation protocols: a systematic review. *J Hand Surg Am*. (2013) 38(9):1712–7 e1-14. doi: 10.1016/j.jhsa.2013.06.025

28. Hand tendon repair - How it's performed. Available online at: https://www.nhs.uk/conditions/hand-tendon-repair/what-happens/ (accessed September 15, 2022).

29. Dy CJ, Hernandez-Soria A, Ma Y, Roberts TR, Daluiski A. Complications after flexor tendon repair: a systematic review and meta-analysis. *J Hand Surg Am*. (2012) 37(3):543–51 e1. doi: 10.1016/j.jhsa.2011.11.006

30. Tang JB. Wide-awake primary flexor tendon repair, tenolysis, and tendon transfer. *Clin Orthop Surg*. (2015) 7(3):275–81. doi: 10.4055/cios.2015.7.3.275

31. Lalonde D, Higgins A. Wide awake flexor tendon repair in the finger. *Plast Reconstr Surg Glob Open*. (2016) 4(7):e797. doi: 10.1097/GOX.0000000000000756

32. Kadhum M, Georgiou A, Kanapathy M, Reissis D, Akhavani M, Burr N, et al. Operative outcomes for wide awake local anesthesia versus regional and general anesthesia for flexor tendon repair. *Hand Surg Rehabil*. (2022) 41(1):125–30. doi: 10.1016/j.hansur.2021.10.312

33. Khor W, Lazenby D, Campbell T, Bedford J, Winterton R, Wong J, et al. Reorganisation to a local anaesthetic trauma service improves time to treatment during the COVID-19 pandemic–experience from a UK tertiary plastic surgery centre. *J Plast Reconstr Aesthet Surg*. (2020) 74:890–930. doi: 10.1016/j.bjps.2020.10.011

34. Foo TL, Mak DS. Wire loop technique to retrieve flexor tendon. *J Hand Surg Am*. (2011) 36(6):1115. doi: 10.1016/j.jhsa.2011.04.008

35. Zafonte B, Rendulic D, Szabo RM. Flexor pulley system: anatomy, injury, and management. *J Hand Surg Am*. (2014) 39(12):2525–32; quiz 33. doi: 10.1016/j.jhsa.2014.06.005

36. Benjamin M, Kaiser E, Milz S. Structure-function relationships in tendons: a review. *J Anat*. (2008) 212(3):211–28. doi: 10.1111/j.1469-7580.2008.00864.x

37. Jordan MC, Schmitt V, Jansen H, Meffert RH, Hoelscher-Doht S. Biomechanical analysis of the modified kessler, lahey, adelaide, and becker sutures for flexor tendon repair. *J Hand Surg Am*. (2015) 40(9):1812–7. doi: 10.1016/j.jhsa.2015.05.032

38. Angeles JG, Heminger H, Mass DP. Comparative biomechanical performances of 4-strand core suture repairs for zone II flexor tendon lacerations. *J Hand Surg Am*. (2002) 27(3):508–17. doi: 10.1053/jhsu.2002.32619

39. Neiduski RL, Powell RK. Flexor tendon rehabilitation in the 21st century: a systematic review. *J Hand Ther*. (2019) 32(2):165–74. doi: 10.1016/j.jht.2018.06.001

40. Tang JB, Gu YT, Rice K, Chen F, Pan CZ. Evaluation of four methods of flexor tendon repair for postoperative active mobilization. *Plast Reconstr Surg*. (2001) 107 (3):742–9. doi: 10.1097/00006534-200103000-00014

41. Tang JB. New developments are improving flexor tendon repair. *Plast Reconstr Surg*. (2018) 141(6):1427–37. doi: 10.1097/PRS.0000000000004416

42. Momeni A, Grauel E, Chang J. Complications after flexor tendon injuries. *Hand Clin*. (2010) 26(2):179–89. doi: 10.1016/j.hcl.2009.11.004

43. Kumar A, Yadav N, Singh S, Chauhan N. Minimally invasive (endoscopic-computer assisted) surgery: technique and review. *Ann Maxillofac Surg*. (2016) 6 (2):159–64. doi: 10.4103/2231-0746.200348

44. Kucukguven A, Uzun H, Menku FD, Sert G, Aksu AE. Endoscopic retrieval of retracted flexor tendons: an atraumatic technique. *J Plast Reconstr Aesthet Surg*. (2019) 72(4):622–7. doi: 10.1016/j.bjps.2019.01.007

45. Gonzalez-Quevedo D, Martinez-Medina I, Campos A, Campos F, Carriel V. Tissue engineering strategies for the treatment of tendon injuries: a systematic review and meta-analysis of animal models. *Bone Joint Res*. (2018) 7(4):318–24. doi: 10.1302/2046-3758.74.BJR-2017-0326

46. Wong JK, Alyouha S, Kadler KE, Ferguson MW, McGrouther DA. The cell biology of suturing tendons. *Matrix Biol*. (2010) 29(6):525–36. doi: 10.1016/j.matbio.2010.06.002

47. Rawson SD, Margetts L, Wong JK, Cartmell SH. Sutured tendon repair; a multi-scale finite element model. *Biomech Model Mechanobiol*. (2015) 14(1):123–33. doi: 10.1007/s10237-014-0593-5

48. Gussous YM, Zhao C, Amadio PC, An KN. The resurgence of barbed suture and connecting devices for use in flexor tendon tenorrhaphy. *Hand*. (2011) 6(3):268–75. doi: 10.1007/s11552-011-9344-6

49. Jiang X, Wu S, Kuss M, Kong Y, Shi W, Streubel PN, et al. 3D Printing of multilayered scaffolds for rotator cuff tendon regeneration. *Bioact Mater*. (2020) 5 (3):636–43. doi: 10.1016/j.bioactmat.2020.04.017

50. Rathbone S, Maffulli N, Cartmell SH. Most British surgeons would consider using a tissue-engineered anterior cruciate ligament: a questionnaire study. *Stem Cells Int*. (2012) 2012:303724. doi: 10.1155/2012/303724

51. Sedgwick P. Questionnaire surveys: sources of bias. *Br Med J*. (2013) 347:f5265. doi: 10.1136/bmj.f5265

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Review
PUBLISHED 16 February 2024
DOI 10.3389/fbioe.2024.1357871

OPEN ACCESS

EDITED BY
Dai Fei Elmer Ker,
The Chinese University of Hong Kong, China

REVIEWED BY
Jayesh Dudhia,
Royal Veterinary College (RVC),
United Kingdom
Mohammad El Khatib,
University of Teramo, Italy

*CORRESPONDENCE
David A. Hart,
hartd@ucalgary.ca

RECEIVED 18 December 2023
ACCEPTED 02 February 2024
PUBLISHED 16 February 2024

CITATION
Hart DA, Ahmed AS, Chen J and Ackermann PW (2024), Optimizing tendon repair and regeneration: how does the *in vivo* environment shape outcomes following rupture of a tendon such as the Achilles tendon?
Front. Bioeng. Biotechnol. 12:1357871.
doi: 10.3389/fbioe.2024.1357871

Optimizing tendon repair and regeneration: how does the *in vivo* environment shape outcomes following rupture of a tendon such as the Achilles tendon?

David A. Hart[1]*, Aisha S. Ahmed[2], Junyu Chen[3] and Paul W. Ackermann[2]

[1]Department of Surgery, Faculty of Kinesiology, McCaig Institute for Bone and Joint Health, University of Calgary, Calgary, AB, Canada, [2]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, [3]Department of Orthopedics, Orthopedic Research Institute, West China Hospital of Sichuan University, Chengdu, China

Risk for rupture of the Achilles tendon, and other tendons increases with age. Such injuries of tissues that function in high load environments generally are believed to heal with variable outcome. However, in many cases, the healing does not lead to a good outcome and the patient cannot return to the previous level of participation in active living activities, including sports. In the past few years, using proteomic approaches and other biological techniques, reports have appeared that identify biomarkers that are prognostic of good outcomes from healing, and others that are destined for poor outcomes using validated criteria at 1-year post injury. This review will discuss some of these recent findings and their potential implications for improving outcomes following connective tissue injuries, as well as implications for how clinical research and clinical trials may be conducted in the future where the goal is to assess the impact of specific interventions on the healing process, as well as focusing the emphasis on regeneration and not just repair.

KEYWORDS

tendon repair, tendon regeneration, *in vivo* environment, immobilization, induction of atrophy, inflammation

Introduction

Tendons are complex tissues, consisting of a myotendinous junction, a mid-substance, and an insertion into bone. They are also heterogenous, existing in a variety of environments with differing mechanical requirements, differing fine structures, functioning in collaboration with a sheath or not, and changing with age. Unlike many ligaments which function in more in the toe region of the stress-strain curve, many tendons function in high load environments.

Functioning in high load environments increases risks for developing chronic conditions such as tendinopathies with accompanying pain and loss of function. The high load environment lead to a great metabolic demand, which results in that tendons are vulnerable to slight metabolic disorders (Ackermann and Hart, 2016). As humans age,

many tendons become stiffer [reviewed in Kwan et al. (2023)], and can lead to increased risk for tendon ruptures, such as for the Achilles tendon (AT) which functions in a high load environment and is an energy-returning tendon. Tendons, particularly the flexor tendons of the hand are frequently damaged or severed due to trauma. In both situations, the tissue often requires surgery to reconnect the torn ends in order to facilitate repair [discussed in Svedman et al. (2018)].

The outcomes of such repair surgery can be varied, in part, depending on the location and environment, but also on other factors such as genetics, epigenetics, co-morbidities (i.e., diabetes), age, and expectations of future use, such as a return to sport participation. Thus, repair of a tendon such as a flexor tendon of the hand that functions in the context of a sheath, adhesions can develop post-surgery leading to loss of function [discussed in Kuroiwa and Amadio (2023)], a condition that can be influenced by a variety of interventions (Wiig et al., 2014; Edsfeldt et al., 2017; Jiang K. et al., 2023). In the case of the AT, some individuals heal naturally after surgery with a long-term good outcome, while others provided the same surgical procedure ± later *versus* early loading experience a much less satisfactory outcome [(Addevico et al., 2019; Chen et al., 2021; Saarensilta et al., 2023a); discussed in (Hart et al., 2023)]. However, even with surgery the repaired tendon may still be compromised at 2 years post-surgery (Geremia et al., 2015), and thus functional repair likely does not yield regeneration. Furthermore, the tendon-muscle unit may not return to normal even after 10 years (Lantto et al., 2015).

While some aspects of outcomes may be related to "good genes", in addition the local environment after the initial injury could be contributing to long-term outcomes. The local injury likely induces an inflammatory response, and certainly a follow-up surgery to repair the tissue would also be pro-inflammatory, and inflammation would need to be regulated carefully to allow for successful healing. This of course would be acute inflammation, and if it was prolonged and became chronic inflammation, there could be adverse consequences to outcomes. Relevant to this point are previous studies where glucocorticoid (GC) treatment immediately post surgery in a preclinical model of anterior cruciate injury inhibited or abolished subsequent development of an osteoarthritis-like/joint damage phenotype in the animals (Barton et al., 2018; Heard et al., 2019; Heard et al., 2022). Similarly, in rat Achilles tendon healing dexamethasone treatment at 7–11 days post-rupture lead to improved material properties of the healing tendon (Dietrich-Zagonel et al., 2018; Dietrich-Zagonel et al., 2022). Interestingly, dexamethasone applied to human tendon cells alter the expression of neuro-inflammatory mediators, i.e., substance P, through a glucocorticoid receptor-dependent pathway (Mousavizadeh et al., 2023). Earlier it has been demonstrated that the peripheral nervous system including pro- and anti-inflammatory neuronal mediators exert essential regulatory functions on tendon healing (Ackermann et al., 2016). Thus, induction of an inflammatory response that is not regulated in a tightly controlled manner, can lead to adverse consequences, likely in the context of injury healing or injury to a soft tissue. While GC treatment may influence the local environment following a connective tissue injury, whether it would be useful in all injury environments remains to be confirmed. Their use may depend on timing, dose and the type of GC employed (Dietrich-Zagonel et al., 2018; Dietrich-Zagonel et al., 2022).

Based on the discussion above, the response of humans to rupture of a tendon such as the Achilles tendon leads to heterogeneity in outcomes, ranging from poor to good. Some of this heterogeneity may reside in the genetic make-up of the patient, but also the environment of the wound site, and potentially whether the rupture is initially repaired surgically or not. Thus, clinical trials focused on assessing the value and impact of an intervention and generated using unselected patient populations would contain both those destined for a good outcome as well as those destined for a poor outcome. This scenario would likely complicate the interpretation of results and any statistical evaluations as an intervention could improve those destined for a poor outcome while not improving those destined for a good outcome. Therefore, what is needed is tools to improve personalized treatment options, and biomarkers that identify subsets of patients could lead to more focused interventions and more directed understanding of the variables contributing to good *versus* poor outcomes. This review is thus focused on that premise.

Furthermore, as a rupture is an acute event, leading to tissue damage and induction of inflammation, the healing process and related events will likely be different from those associated with chronic conditions such as tendinosis and tendinitis. In addition, different tendons exist and function in different biomechanical environments and thus, their biology may also be location-specific and thus some molecular aspects of healing may be unique. Therefore, this review will focus on the ruptured Achilles tendon, but the approaches to identify biomarkers associated with outcomes should be applicable to injuries to other tendons in the future. While the healing of the AT is the major theme of this review, this tendon is used as an example, and the applicability of the approaches used to other tendons is discussed as to whether the findings regarding the healing of mid-substance AT ruptures can be extrapolated to injuries to the AT in other locations and whether the findings can be extended to other tendon injuries is important for the field of repair and regeneration of tendon injuries.

The wound healing environment after an acute tendon injury

If one suffers a transection or complete rupture of a tendon such as the AT or the flexor tendons of the hand, this injury often requires surgical repair followed by a period of immobilization [discussed in Ackermann et al. (2023); Hart et al. (2023)]. Furthermore, the faster the patients can receive the surgery, the better the outcomes (Svedman et al., 2018). However, in other locals, the leg is merely immobilized for a period of time followed by physiotherapy, and thus in this scenario, the injured tissue is left to its own devices in an immobilized state. In either circumstance, there is initiation of a healing response with a multitude of phases including the inflammatory phase, proliferative phase, matrix deposition phase, and then a prolonged matrix remodeling phase. Alternatively, these phases of healing are labelled as the induction, production, orchestration, and conduction phases of healing (Figure 1).

Even with a surgical intervention to join the torn ends of the AT together, patients can experience a good to excellent outcome at 1-year post-injury, or a poor outcome based on validated criteria [discussed in Chen et al. (2022); Chen et al. (2023); Hart et al. (2023)]. Recently,

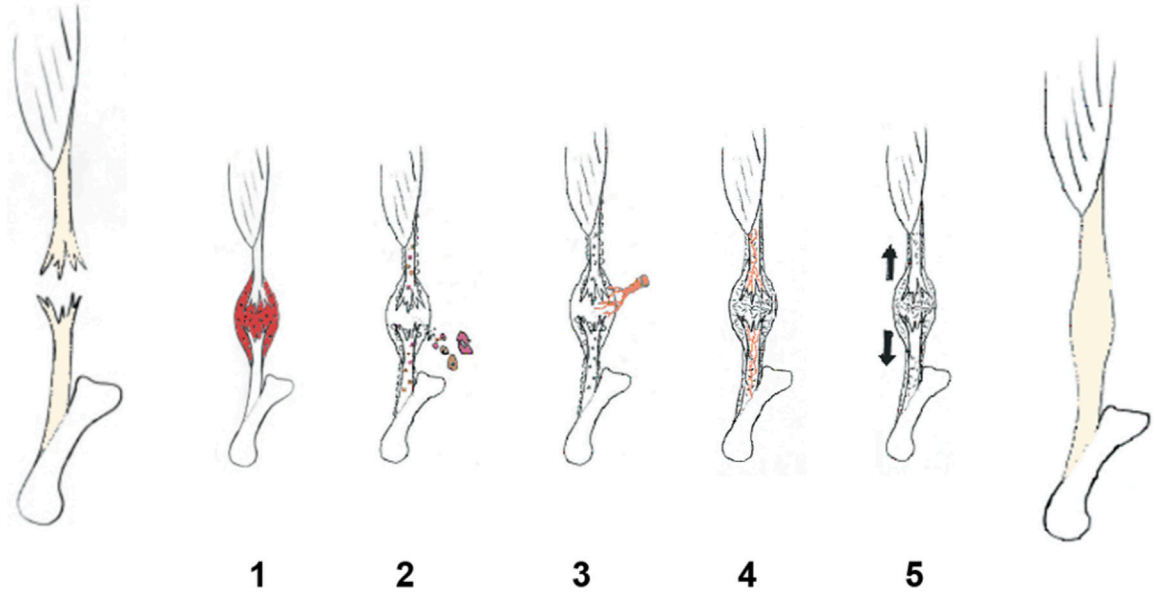


FIGURE 1
Tendon repair overview (Ackermann and Hart, 2016). (1) Induction (Kwan et al., 2023), (2) production (Svedman et al., 2018), (3) orchestration (Kuroiwa and Amadio, 2023), (4) conduction, and (Wiig et al., 2014) (5) modification of the healing process (Reproduced with permission from Ackermann [Ackermann PW. Healing and repair mechanisms. London: DJO Publications; 2014].

Chen et al. (2022) and Wu et al. (2023) reported that using proteomic approaches and shards of tissue from the torn ends of ruptured AT taken at the time of surgery led to the identification of biomarkers of good *versus* poor outcomes at 1-year post-injury. Thus, within days of injury, the local environment can predict whether a patient will have a good *versus* poor outcome at 1-year! How such biomarkers translate to good outcomes is currently not well described, but recent reports by Chen et al. (2023a); Chen et al. (2023) indicate that a biomarker of good outcomes, eukaryotic elongation factor-2 (eEF2) can directly affect a number of cell processes and protein expression levels. Whether all of the biomarkers identified directly influence healing outcome or are surrogate markers of outcome remains to be determined. However, the above-described findings indicate that the local environment early after injury to the AT can predict outcomes at 1-year and could help identify patients that may be in need of targeted interventions to improve outcomes. Such targeted interventions may need to be multifaceted, with one facet to enhance the local environment and another to exert a positive influence on the healing process. The reason for indicating a potential need for such an approach is that in the poor outcome patients, one does not really know of the outcome is poor due to a lack of some influence or due to the presence of an inhibitor of a good outcome.

It should be noted that connective tissues such as tendons, and nearly all other tissues of the musculoskeletal system, require mechanical loading to maintain their integrity and subscribe to the "use it or lose it" paradigm [discussed in Hart and Zernicke (2020); Hart (2021); Hart et al. (2022)]. Therefore, if one immobilizes a limb, the muscles and other connective tissues are removed from loading and undergo atrophy. Interestingly, it has been shown with menisci that removal from the knee of the animal leads to the rapid (4 h) induction of a "cassette" of genes that could contribute to catabolism of the tissue, including MMP-1, MMP-3, iNOS, COX-2 and IL-1beta and IL-6, but not MMP-13, collagens, biglycan or TIMP-4 (Natsu-ume et al., 2005). The induction of the expression of this subset of genes could be prevented by *in vitro* administration of intermittent cyclic hydrostatic compression (1 min every 15 min at 1 MPa). Thus, there is a set of genes that are repressed by loading. Whether a similar or different set of genes are also affected by a loss of loading in tendons remains to be determined, however, it is a likely scenario based on responses of individuals with immobilized limbs, prolonged bedrest, or astronauts [discussed in Hart et al. (2022)].

The above discussion is relevant to tendon repair as after surgery, the affected limb is usually immobilized for various periods of time and thus, subjected to conditions that foster atrophy of muscle bone and the surgically repaired AT. This may also affect the vascular system as such patients may incur a deep vein thrombosis at a high rate [~50%; discussed in Saarensilta et al. (2023b)]. However, this immobilization is occurring after surgery and based on the proteomic studies (Chen et al., 2022; Chen et al., 2023; Wu et al., 2023), biomarkers of good outcomes at 1-year were already evident prior to surgery. It should be noted that the torn AT was already unloaded after the rupture for 2–7 days before surgery, so the torn ends were in fact not only subjected to inflammation-associated with the injury, but also loss of biomechanical loading for several days prior to surgery. And then even after surgery, the limb was immobilized for a period of time. While it is recognized that immobilization is not good for connective tissue health, and the length of the immobilization period should be kept to a minimum so as not to downregulate tendon repair genes (Bring et al., 2009; Bring et al., 2010), with gradual return to minimal loading initially and then increasing as the healing tissue regains strength. Thus, loading is recognized as a positive influence on the healing progression.

While there is still much to understand about what contributes to how the good outcomes are manifested in the early stages after injury, there are at least two processes that are evident, inflammation and loss of loading and its consequences. Early acute inflammation may be very critical to the initial phases of healing and thus a positive (although often viewed as a negative), while the possible catabolic influence of unloading the tissue could be a negative influence on outcomes. Therefore, a good outcome may require some innate ability in the local environment to balance those contributions and regulate their influence to contribute to a good outcome at 1 year. This environment may also influence how effective cellular, biochemical, and drug interventions are to enhance outcomes. One might also expect that the incidence of a good outcome would be associated with early surgery after AT rupture [Svedman et al., 2018), and in those jurisdictions that do not use surgery and only immobilization, there would be fewer good outcomes and more adequate or poor outcomes that may need specific interventions to enhance the quality of the healing process. (Svedman et al., 2018) reported that surgery for an AT rupture within 48 h post-injury led to more good outcomes at 1 year for patients than did those patients receiving surgery >72 h post-injury, although some patients receiving surgery >72 h post-injury still had a good outcome. Therefore, there is some heterogeneity in the response pattern.

Based on the above discussion, there are several options for how the local environment early after a tendon injury may influence long term outcomes at 1-year post-injury. These include: 1) extended time for induction of catabolic atrophy genes before surgery (negative); 2) extended time for development of an inflammatory response with negative elements before surgery; and 3) extended time for an inflammatory response to impact a non-loaded atrophy-induced torn tissue before surgery. It should be noted that the surgical procedure itself is actually a second inflammatory stimulus and thus, can complicate the local environment. These are not mutually exclusive options, and because of human heterogeneity, genetic, epigenetic, and potentially sex-related differences could also influence how the above options evolve in the injured tissue environment and are implemented. However, future studies may have to develop interventions to optimize the local environment to enhance the success of other modalities to improve healing outcomes (Hart and Nakamura, 2022), such as those discussed in later sections of this review.

Surgical versus non-surgical treatment of at ruptures: Outcomes

The options for treatment after an AT rupture are varied, ranging from immediate surgery to merely casting the affected lower limb in an immobilized state for a period of time, with some variations in between. Immediate surgery will put some tension on the sutured tissue while casting alone will provide a prolonged period of immobilization where the healing process will progress in an initial environment that has no load. Thus, in the latter scenario one may expect that the outcomes at 1-year and beyond would be inferior for such patients compared to those that received immediate surgical repair. However, that is apparently not the case based on the report of Keating and Will (Keating and Will, 2011), but a majority of surgeons prefer surgical treatment for young, active patients (Parisien et al., 2021). In addition, some reports indicate there is a lower rate of re-rupture with surgical repair (Lynch, 2004), potentially indicating that surgical repair leads to better quality repair tissue and/or there is less scar-like repair tissue when the torn ends are sutured. Recently, a high impact multicenter, randomized, controlled trial by Myhrvold et al. confirmed a lower rate of re-rupture with surgical AT repair, although the patient-reported outcome between surgically and non-surgically treated patients showed no differences (Myhrvold et al., 2022). Therefore, patient selection and expectations of participating in an active lifestyle may influence the choice of treatment. In conservative treatment protocols, early mobilization is likely recommended compared to prolonged immobilization via casting (Kangas et al., 2003; Van der Eng et al., 2013), but issues around re-rupture rate and other complications still remain to be resolved in detail. Given the heterogeneity in patient outcomes even within the surgical treatment cohorts [(Svedman et al., 2018); discussed in Hart et al. (2023)], it is also likely, but not proven, that heterogeneity may also exist within the conservative treatment population as well. Thus, comparing two heterogeneous populations within both the surgical and non-surgical groups may lead to an obscuring of differences in outcomes. It may also depend on when the long-term assessments are performed (i.e., 1, 2, 10 years) as a good *versus* poor outcome at 1-year may be overcome by 2 or 10 years dependent on activity level and other parameters. The potential that good outcomes *versus* poor at 1 year is actually based on the rate of healing to yield a good outcome and this may be obscured at 2 or 10 years as those with an initial poor outcome progress to what is now a good outcome. Some of these issues may also depend on the age and sex of the cohorts assessed as a younger population may use the repaired/healed AT differently than an older patient population.

Attempts to improve outcomes after a tendon injury: Focusing on the at

The average healing of a ruptured tendon, including the AT, is quite variable, leading to the conclusion that they do not heal well. In part, this is due to the fact that the tendon is healing in an environment that is very different from that in which it developed during fetal life [discussed in (He et al., 2022)], and thus expecting complete regeneration may be an unreasonable expectation. However, tendons do contain cells with stem cell-like properties [(Lu et al., 2023)], but their role in healing is not well characterized. In the adult stage of life, how the tendon heals may in part be due to whether it is surgically repaired or just immobilized, but as discussed above, some patients heal with a good outcome while others heal with a poorer outcome at 1-year post-injury. Prior to the reports of good *versus* poor outcomes following natural healing of the AT, and continuing to today, many studies have attempted to improve healing using a variety of interventions without attempting to segregate naturally occurring good and poor healers. While not all of the interventions have assessed efficacy for AT healing, the interventions utilized include growth factors [reviewed in (El-Sherif et al., 2023; Lin et al., 2023; Miescher et al., 2023; Rieber et al., 2023; Wang and Li, 2023)],

acupuncture (Stewman, 2023), platelet-rich plasma (PRP) and variations (Markazi et al., 2022; Everts et al., 2023), other cell therapies including mesenchymal stem cells (MSC) (Chamberlain et al., 2017; Alt et al., 2021; Zhang et al., 2021; Jiang L. et al., 2023; Yuan et al., 2023; Zulkifli et al., 2023), and extracellular vesicles (EV) derived from MSC and related stem cells (Lu et al., 2021; Lyu et al., 2022; Wang and Li, 2023; Xue et al., 2023; Zou et al., 2023) and other cellular preparations (Aydin et al., 2023). Use of glucocorticoid injections to ostensibly control inflammation to enhance tendon healing or improve the local injury environment was variable and dependent on a variety of factors (Dietrich-Zagonel et al., 2018; Dietrich-Zagonel et al., 2022), and was often detrimental to healing (Dean et al., 2014). Some of these approaches have been used for treatment of tendinopathies other than ruptures, and in such cases, the outcomes are more related to pain rather than tissue regeneration.

While some of the approaches to improve tendon healing are still experimental in preclinical models, attempts to enhance tendon healing with some interventions such as PRP have been reported to not enhance tendon healing in a significant manner (Keene et al., 2022). However, PRP is used in an autologous manner so the failure to enhance healing could be due to limitations related to the source of the PRP or the local injury environment they were injected into, the age of the donor, the timing of the injection or the volume. As the preparation of PRP can also vary [discussed in (Kydd and Hart, 2020; Godoi et al., 2022; Bagheri et al., 2023; Everts et al., 2023; Giannotti et al., 2023)], this may also influence outcomes.

While the interventions identified above are quite diverse in their chemical, biochemical and cellular basis, their impact on improving clinical outcomes is variable, in part due to the heterogeneity of the patients and the quality of the local post-injury environment (discussed in the last paragraph of the Introduction section), as well as the fact that nearly all patients receiving cellular interventions (i.e., PRP, stem cells, and other cellular preparations) prefer to receive autologous materials which may not be optimal to impact outcomes [discussed in Kydd and Hart (2020); Hart and Nakamura (2022)]. Going forward, using tools such as biomarkers of good *versus* poor clinical outcomes could enhance the use of some of those interventions identified above to improve the outcomes of selected patient subsets (Hart et al., 2023).

Of the cellular interventions discussed above, likely the approach that may offer the best opportunity to improve healing is the use of extracellular vesicles (EVs) that exhibit low immunogenicity (Sarcinella et al., 2023) and thus can be optimized for allogeneic use. EV contain a variety of molecules including miRNAs (Ragni et al., 2020; Ragni et al., 2021; F-Palama et al., 2023) which are reported to influence tendon healing (Liu et al., 2021). The effectiveness of EV can also be influenced by the culture conditions (Hanai et al., 2023; Phelps et al., 2023) and thus, potentially targeted for specific applications.

The way forward and the next steps

The finding of biomarkers prognostic for good *versus* poor long-term tendon healing outcomes can change the approaches to clinical trials, as well as clinical research. Identifying such biomarkers within days of injury also has implications for how one approaches the evaluation of interventions with proteins, drugs or cellular therapies. The ability to identify biomarkers which relate to outcomes at the different phases of healing should enhance the evaluation of interventions to improve outcomes (summarized in Table 1). As outlined in Table 1, the process of healing is complex so having biomarkers at different stages of the process to assist in such evaluations may be critical. Some approaches to address current gaps in our knowledge and understanding of the process are outlined below.

Clinical research

A. Identification of biomarkers via proteomics is really a first step. One next has to determine how the biomarkers are affecting outcomes, or whether they are just surrogates for outcomes. One option of what is needed has been reported by Chen et al. (2023a); Chen et al. (2023), where how one of the biomarkers identified as being related to good healing outcomes (eEF-2) affects cellular processes was investigated. In addition, morphological and immunolocalization studies (I.e., proteins and cells) are needed to assess where the biomarker may be exerting an effect on healing. In addition, one may want to include the use of approaches such as Shear Wave Propagation (Blank et al., 2022) to assess the progression of the healing process from early to later (i.e., 1, 2, 5 years post-surgery) to assess the remodeling stage of healing and whether it is accelerated in some patients compared to those with poor outcomes.
B. The biomarkers identified as prognostic for good vs. poor outcome at 1-year after AT rupture was focused on patients with mid-substance injuries. Are the same biomarkers associated with outcomes after an injury to the myotendinous junction or the insertion into bone? The environments and tissues involved in such injuries are very different from those involved in mid-substance injuries.
C. Do injuries to other tendons (i.e., flexor tendons, supraspinatus, patellar) that require surgical interventions yield similar biomarkers to those identified for AT ruptures, or are they different. Likely both the environments and the tissues/cells are different so one may need to perform studies similar to those reported for the ruptured AT with patients suffering from injuries to other tendons.
D. Are the biomarkers identified as being prognostic of good vs. poor outcomes after AT rupture characteristic of general healing processes (i.e., ligament, skin, etc.) or unique to the ruptured AT? That is, are some people "good healers" and other "poor healers" irrespective of the healing site? This might imply that there is a strong genetic component to the process.

Clinical trials

A. Clinical trials designed to assess the impact of specific interventions on outcomes could target those destined to

TABLE 1 Healing phases, novel biomarkers of tendon repair and established approaches to enhance tendon repair.

Healing phase	Specification of healing phase	Novel biomarkers. Association with healing outcome	Various approaches to enhance tendon repair
1. Induction	Inflammation. Blood-derived cells, which subsequently releases growth factors	*ITIH4* Higher ITIH4 levels are positively associated with better clinical outcomes after ATR.	*Platelet rich plasma* Derived from centrifugation of whole blood. Such platelet preparations contain many growth factors and anabolic molecules
2. Production	Proliferation.Tissue-derived cells are attracted and transformed into myofibroblasts at the healing site. The myofibroblasts subsequently activate production of tendon callus	*eEF2* Higher eEF2 levels are positively associated during both inflammatory and proliferative healing with improved clinical outcomes after ATR.	*Stem cells,* E.g., Mesenchymal stem cells (MSC) (Hart et al., 2022), bone marrow stem cells (BMSC) (Natsu-ume et al., 2005), and genetically modified cells that synthesize and deliver the desired growth factor in a temporally and spatially orchestrated manner. *Growth factors* IGF TGF-B BMP VEGF
3. Orchestration	Proliferation. New pathways for delivery of healing substances are built with neuro-vascular ingrowth into a tendon-matrix normally devoid of nerves and vessels	*CFD* Lower CFD levels are associated with improved patient outcomes after ATR.	*NGF* and *neuropeptides* are released, which guide neurovascular ingrowth. Subsequently to healing factors that regulate nerve and blood vessel retraction are released. *Early mobilization* accelerates the nerve plasticity, i.e., nerve regeneration, expression of neuromediators and their receptors, and nerve retraction (Lu et al., 2023; Miescher et al., 2023)
4. Conduction	Proliferation. A prerequisite for healing to commence and to initiate the development of a functioning tissue matrix into which cells, vessels, and nerves can grow in and where production of new granulation tissue can occur	*FGF-2* Higher FGF-2 gene expression is positively associated with better patient outcomes after ATR.	Tendon tissue, flap techniques, or *tendon grafts* are used. *Scaffolding techniques*–either biogenic or synthetic (e.g., bioresorbable polymers) scaffolds
5. Modification	Remodeling. Transition from Col III to Col I is essential for scar maturation. Increasing mechanical loading activates myofibroblasts and fibroblasts to increase the production of relevant matrix molecules leading to structural reorganization to enhance the capacity of the tissue to withstand high mechanical load	*Pyruvate.* Higher pyruvate levels are associated with better patient outcomes after ATR.	*Pyruvate* is involved in tendon repair associated with the transition from Col III to Col I in the scar tissue during remodeling. *Increasing mechanical loading* activates myofibroblasts and fibroblasts to increase the production of collagen type I to increase the callus size and enhance the capacity to withstand high mechanical load

achieve good outcomes vs. a poor outcome even after the fact so as to determine whether the two populations would be influenced differently. Thus, converting those destined for a poor outcome to a good outcome could be one set of outcomes for a specific intervention and whether an intervention could further improve those destined for a good outcome naturally would be a second goal of the trial. The results from the proteomics studies could be done independently from the intervention so as not to impact of the timing of the intervention and could also be done in a blinded manner.

B. As discussed previously, healing outcomes likely reflect the effectiveness of repair processes and do not lead to regeneration. Therefore, if regeneration is the goal of the research, then it may be more appropriate to investigate the potential for regeneration with those already destined for a good outcome *versus* those destined for a poor outcome as in the latter, one may have to also overcome deficiencies that the intervention is not capable of addressing.

C. Clinical trials should also be undertaken to determine whether the same of different biomarkers are identified with good vs. poor outcomes depending on the location of the injury in a tendon such as the Achilles tendon. Injuries to this tendon can occur at the bone-tendon interface or at the myotendinous junction, as well as the mid-substance which the current biomarkers have been associated with thus far. As reports indicate that healing outcomes are better the more proximal the injury to the Achilles tendon (Qureshi et al., 2023), this will be important to establish whether separate biomarkers are associated with outcomes following injury at these transition points in the tendon *versus* within the tendon mid-substance.

Thus, the advent of biomarkers of outcomes after a tendon rupture may impact both clinical research and clinical trials in new ways and with enhanced potential to yield benefits to both understanding healing processes and patients in their post-injury life.

Conclusion

Wound healing after an extensive injury is complex, involving many steps in the process. Healing in tissues designed to function in high load environments, are particularly complex. Therefore, the finding of a number of biomarkers prognostic of good *versus* poor outcomes has the potential to change the way some clinical research and clinical trials are conducted. However, even when biomarkers prognostic for healing outcomes are identified, it is critical to determine how the biomarkers impact healing, how the local environment affects outcomes, and whether one can utilize the information to further enhance the healing potential of specific patient subpopulations. Thus, do biomarkers reflect a critical stage

of healing and therefore, an important initial step (s) in this complex process, or are they surrogates for some other process that can be deduced by network analysis of the proteomic data? The answers to such questions are critical to moving the field forward and will require significant new research efforts before they can translate to patient populations. However, these are achievable goals with the right investment and commitment in the not so distant future.

Author contributions

DH: Writing–original draft, Writing–review and editing. AA: Funding acquisition, Writing–review and editing. JC: Funding acquisition, Writing–review and editing. PA: Funding acquisition, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Preparation of this review was supported by grants to PA [The Swedish Research Council (2017-00202) and the regional agreement on medical training and clinical research (ALF; SLL20180348)], AA [Swedish National Centre for Sports Research (2022-0066; 2023-0060)] and JC (Chinese Scholarship Council (CSC).

Acknowledgments

The authors thank Drs. Cyril B. Frank (deceased), Gail Thornton and Ronald Zernicke for many discussions relevant to this review, as well as the many Trainees who have contributed to the findings from the author's laboratories.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

Ackermann, P. W., Ahmed, A. S., and Hart, D. A. (2023). "Medical considerations in tendinopathy," in *Tendon regeneration: understanding tissue physiology and development to engineer functional substitutes*. Editors M. E. Gomes, R. Reis, M. T. Rodrigues, A. Goncalves, D. Zeugolis, and D. Docheva 2nd Edition (Elsevier). In Press.

Ackermann, P. W., and Hart, D. A. (2016). General overview and summary of concepts regarding tendon disease topics addressed related to metabolic disorders. *Adv. Exp. Med. Biol.* 930, 293–298. doi:10.1007/978-3-319-33943-6_28

Ackermann, P. W., Salo, P., and Hart, D. A. (2016). Tendon innervation. *Adv. Exp. Med. Biol.* 920, 35–51. doi:10.1007/978-3-319-33943-6_4

Addevico, F., Svedman, S., Edman, G., and Ackerman, P. W. (2019). Pyruvate and lactate as local prognostic biomarkers of patient outcome after Achilles tendon rupture. *Scand. J. Med. Sci. Sports.* 29, 1529–1536. doi:10.1111/sms.13469

Alt, E., Rothoerl, R., Hoppert, M., Frank, H.-G., Wuerfel, T., Alt, C., et al. (2021). First immunohistochemical evidence of human tendon repair following stem cell injection: a case report and review of literature. *World J. Stem cells.* 13, 944–970. doi:10.4252/wjsc.v13.i7.944

Aydin, E. Y., Asik, M., Aydin, H. M., Cay, N., Gumuskaya, B., Caglayan, A., et al. (2023). The co-use of stromal vascular fraction and bone marrow concentrate for tendon healing. *Curr. Stem. Cell Res.* 18, 1150–1159. doi:10.2174/1574888X18666230221141743

Bagheri, K., Krez, A., Anastasio, A. T., and Adams, S. B. (2023). The use of platelet-rich plasma in pathologies of the foot and ankle: a comprehensive review of the recent literature. *Foot Ankle Surg.* 26 (23), 551–559. doi:10.1016/j.fas.2023.07.010

Barton, K. I., Heard, B. J., Sevick, J. L., Martin, C. R., Shekarforoush, S. M. M., Chung, M., et al. (2018). Posttraumatic osteoarthritis development and progression in an ovine model of partial anterior cruciate ligament transection and effect of repeated intra-articular methylprednisolone acetate injections on early disease. *Am. J. Sports Med.* 46, 1596–1605. doi:10.1177/0363546518765098

Blank, J., Blomquist, M., Arant, L., Cone, S., and Roth, J. (2022). Characterizing musculoskeletal tissue mechanics based on shear Wave propagation: a systematic review of current methods and reported measurements. *Ann. Biomed. Eng.* 50, 751–768. doi:10.1007/s10439-022-02935-y

Bring, D., Reno, C., Renstrom, P., Salo, P., Hart, D., and Ackermann, P. (2010). Prolonged immobilization compromises up-regulation of repair genes after tendon rupture in a rat model. *Scand. J. Med. Sci. Sports.* 20, 411–417. doi:10.1111/j.1600-0838.2009.00954.x

Bring, D. K.-I., Reno, C., Renstrom, P., Salo, P., Hart, D. A., and Ackermann, P. W. (2009). Joint immobilization reduces the expression of sensory neuropeptide receptors and impairs healing after tendon rupture in a rat model. *J. Orthop. Res.* 27, 274–280. doi:10.1002/jor.20657

Chamberlain, C. S., Saether, E. E., Aktas, E., and Vanderby, R. (2017). Mesenchymal stem cell therapy on tendon/ligament healing. *J. Cytokine Bio.* 2, 112. doi:10.4172/2576-3881.1000112

Chen, J., Svensson, J., Sundberg, C.-J., Ahmed, A. S., and Ackermann, P. W. (2021). FGF gene expression in injured tendons as a prognostic biomarker of 1-year patient outcome after Achilles tendon repair. *J. Exp. Orthop.* 8, 20. doi:10.1186/s40634-021-00335-0

Chen, J., Wang, J., Hart, D. A., Ahmed, A. S., and Ackermann, P. W. (2022). Complement factor D as a predictor of Achilles tendon healing and long-term patient outcomes. *FASEB J.* 36, e22365. doi:10.1096/Fj.202200200RR

Chen, J., Wang, J., Hart, D. A., Zhou, Z., Ackermann, P. W., and Ahmed, A. S. (2023a). Complement factor D regulates collagen type I expression and fibroblast migration to enhance human tendon repair and healing outcomes. *Front. Immunol.* 14, 1225957. doi:10.3389/fimmu.2023.1225957

Chen, J., Wang, J., Wu, X., Simon, N., Svensson, C. I., Yuan, J., et al. (2023). eEF2 improves dense connective tissue repair and healing outcome by regulating cellular death, autophagy, apoptosis, proliferation and migration. *Cell Mol. Life Sci.* 80, 128. doi:10.1007/s00018-023-04776-x

Dean, B. J. F., Franklin, S. L., Murphy, R. J., Javaid, M. K., and Carr, A. J. (2014). Glucocorticoids induce specific ion-channel-mediated toxicity in human rotator cuff tendon: a mechanism underpinning the ultimately deleterious effect of steroid injection in tendinopathy? *Br. J. Sports Med.* 48, 1620–1626. doi:10.1136/bjsports-2013-093178

Dietrich-Zagonel, F., Aspenberg, P., and Eliasson, P. (2022). Dexamethasone enhances Achilles tendon healing in an animal injury model, and the effects are dependent on dose, administration time, and mechanical loading stimulation. *Am. J. Sports Med.* 50, 1306–1316. doi:10.1177/03635465221077101

Dietrich-Zagonel, F., Mannerman, M., Tatting, L., Dietrich, F., Ljunggren, M. K., Blomgran, P., et al. (2018). Stimulation of tendon healing with delayed dexamethasone treatment is modified by the microbiome. *Am. J. Sports Med.* 46, 3281–3287. doi:10.1177/0363546518799442

Edsfeldt, S., Holm, B., Mahlapuu, M., Reno, C., Hart, D. A., and Wiig, M. (2017). PXL01 in sodium hyaluronate results in increased PRG4 expression: a potential mechanism for anti-adhesion. *Ups. J. Med. Sci.* 122, 28–34. doi:10.1080/03009734.2016.1230157

El-Sherif, S. M., Abdel-Hamid, M. M., Noureldin, J. M. A. M., Fahmy, H. M., and Abdel-Naby, H. M. A. (2023). Effectiveness of lyophilized growth factors injection for subacromial impingement syndrome: a prospective randomized double-blind placebo-controlled study. *J. Orthop. Surg. Res.* 18, 78. doi:10.1186/s13018-023-03548-4

Everts, P. A., Lana, J. F., Onishi, K., Buford, D., Peng, J., Mahmood, A., et al. (2023). Angiogenesis and tissue repair depend on platelet dosing and bioformulation strategies following orthobiological platelet-rich plasma procedures: a narrative review. *Biomedicines* 11, 1922. doi:10.3390/biomedicines11071922

F-Palama, M. E., Coco, S., Shaw, G. M., Reverberi, D., Ghelardoni, M., Ostano, P., et al. (2023). Xeno-free cultured mesenchymal stromal cells release extracellular vesicles with a "therapeutic" miRNA cargo ameliorating cartilage inflammation *in vitro*. *Theranostics* 13, 1470–1489. doi:10.7150/thno.77597

Geremia, J. M., Bobberrt, M. F., Nova, M. C., Ott, R. D., de Aguiar Lemos, F., de Oliverira Lupion, R., et al. (2015). The structural and mechanical properties of the Achilles tendon 2 years after surgical repair. *Clin. Biomech. (Bristol, Avon)* 30, 485–492. doi:10.1016/jclinbiomech.2015.03.005

Giannotti, L., Stanca, B. D. C., Spedicato, F., Nitti, P., Damiano, F., Demitri, C., et al. (2023). Progress in regenerative medicine: exploring autologous platelet concentrates and their clinical applications. *Genes (Basel).* 14, 1669. doi:10.3390/genes14091669

Godoi, T. T. F., Rodrigues, B. L., Huber, S. C., Santana, M. H. A., da Fonseca, L. F., Santos, G. S., et al. (2022). Platelet-rich plasma gel matrix (PRP-GM): description of a new technique. *Bioeng. (Basel).* 9, 817. doi:10.3390/bioengineering9120817

Hanai, H., Hart, D. A., Jacob, G., Shimomura, K., Ando, W., Yoshioka, Y., et al. (2023). Small extracellular vesicles derived from human adipose-derived mesenchymal stromal cells cultured in a new chemically-defined contaminate-free media exhibit enhanced biological and therapeutic effects on human chondrocytes *in vitro* and in a mouse osteoarthritis model. *J. Extracell. Vesicles.* 12, e12337. doi:10.1002/jev2.12337

Hart, D. A. (2021). Learning from human responses to deconditioning environments: improved understanding of the "use it or lose it" principle. *Front. Sports Act. Living.* 3, 685845. doi:10.3389/fspor.2021.685845

Hart, D. A., Ahmed, A. S., and Ackermann, P. (2023). Optimizing repair of tendon ruptures and chronic tendinopathies: integrating the use of biomarkers with biological interventions to improve patient outcomes and clinical trial design. *Front. Sports Act. Living.* 4, 1081129. doi:10.3389/fspor.2022.1081129

Hart, D. A., and Nakamura, N. (2022). Creating an optimal *in vivo* environment to enhance outcomes using cell therapy to repair/regenerate injured tissues of the musculoskeletal system. *Biomedicines* 10, 1570. doi:10.3390/biomedicines10071570

Hart, D. A., and Zernicke, R. F. (2020). Optimal human functioning requires exercise across the lifespan: mobility in a 1g environment is intrinsic to the integrity of multiple biological systems. *Front. Physiol.* 11, 156. doi:10.3389/fphys.2020.00156

Hart, D. A., Zernicke, R. F., and Shrive, N. G. (2022). *Homo sapiens* may incorporate daily acute cycles of "conditioning-deconditioning" to maintain musculoskeletal integrity: need to integrate with biological clocks and circadian rhythm mediators. *Int. J. Mol. Sci.* 23, 9949. doi:10.3390/ijms23179949

He, P., Ruan, D., Huang, Z., Wang, C., Xu, Y., Cai, H., et al. (2022). Comparison of tendon development versus tendon healing and regeneration. *Front. Cell Dev. Biol.* 10, 821667. doi:10.3389/fcell.2022.821667

Heard, B. J., Barton, K. I., Abubacker, S., Chung, M., Martin, C. R., Schmidt, T. A., et al. (2022). Synovial and cartilage responsiveness to peri-operative hyaluronic acid +/_ dexamethasone administration following limited injury to the rabbit stifle joint. *J. Orthop. Res.* 40, 8380845. doi:10.1002/jor.25108

Heard, B. J., Barton, K. I., Agbojo, O. M., Chung, M., Sevick, J. L., Bader, T. J., et al. (2019). Molecular response of rabbit menisci to surgically induced hemarthrosis and a single intra-articular dexamethasone treatment. *J. Orthop. Res.* 37, 2043–2052. doi:10.1002/jor.24346

Jiang, K., Li, Y., Xiang, C., Xiong, Y., and Jia, J. (2023a). Rebalancing SMAD7/SMAD3 signaling reduces adhesion formation during flexor tendon healing. *J. Microbiol. Biotechnol.* 33, 339–347. doi:10.4014/jmb.2209.09033

Jiang, L., Lu, J., Chen, Y., Lyu, K., Long, L., Wang, X., et al. (2023b). Mesenchymal stem cells: an efficient cell therapy for tendon repair (review). *Int. J. Mol. Med.* 52, 70. doi:10.3892/ijmm.2023.5273

Kangas, J., Pajala, A., Siira, P., Hamalainen, M., and Leppilahti, J. (2003). Early functional treatment versus early immobilization in tension of the musculotendinous unit after Achilles rupture repair: a prospective, randomized, clinical study. *J. Trauma.* 54, 1171–1180. doi:10.1097/01.ta.0000047945.20863.a2

Keating, J. F., and Will, E. M. (2011). Operative versus non-operative treatment of acute rupture of tendo Achillis: a prospective randomized evaluation of functional outcome. *J. Bone Jt. Surg. Br.* 93, 107101078. doi:10.1302/0301-620X.93B8.25998

Keene, D. J., Alsousou, J., Harrison, P., O'Conner, H. M., Wagland, S., Dutton, S. J., et al. (2022). Platelet-rich plasma injection for acute Achilles tendon rupture. *Bone Jt. J.* 104-B, 1256–1265. doi:10.1302/0301-620x.104b11.bjj-2022-0653.r1

Kuroiwa, T., and Amadio, P. C. (2023). Flexor tendon adhesion formation: current concepts. *Hand Clin.* 39, 171–180. doi:10.1016/j.hcl.2022.08.018

Kwan, K. Y. C., Ng, K. W. K., Rao, Y., Zhu, C., Qi, S., Tuan, R. S., et al. (2023). Effect of aging on tendon biology, biomechanics and implications for treatment approaches. *Int. J. Mol. Sci.* 24, 15183. doi:10.3390/ijms242015183

Kydd, A. S. R., and Hart, D. A. (2020). Efficacy and safety of platelet-rich plasma injections for osteoarthritis. *Curr. Treat. Options Rheum.* 6, 87–98. doi:10.1007/s40674-020-00142-1

Lantto, I., Heikkinen, J., Flinkkila, T., Ohtonen, P., Kangas, J., Siira, P., et al. (2015). Early functional treatment versus cast immobilization in tension after achilles rupture repair: results of a prospective randomized trial with 10 or more years of follow-up. *Am. J. Sports Med.* 43, 2302–2309. doi:10.1177/0363546515591267

Lin, M., Li, W., Ni, X., Sui, Y., Li, H., Chen, X., et al. (2023). Growth factors in the treatment of Achilles tendon injury. *Front. Bioeng. Biotechnol.* 11, 1250533. doi:10.3389/fbioe.2023.1250533

Liu, Q., Zhu, Y., Zhu, W., Zhang, G., Yang, Y. P., and Zhao, C. (2021). The role of microRNAs in tendon injury, repair and related tissue engineering. *Biomaterials* 277, 121083. doi:10.1016/j.biomaterials.2021.121083

Lu, J., Chen, H., Lyu, K., Jiang, L., Chen, Y., Long, L., et al. (2023). The functions and mechanisms of tendon stem/progenitor cells in tendon healing. *Stem Cells Int.* 2023, 1–18. doi:10.1155/2023/1258024

Lu, V., Tennyson, M., Zhang, J., and Khan, W. (2021). Mesenchymal stem cell-derived extracellular vesicles in tendon and ligament repair-a systematic review of *in vivo* studies. *Cells* 10, 2553. doi:10.3390/cells10102553

Lynch, R. M. (2004). Achilles tendon rupture: surgical versus non-surgical treatment. *Accid. Emerg. Nurs.* 12, 149–158. doi:10.1016/j.aaen.2003.11.004

Lyu, K., Liu, T., Chen, X., Lu, J., Jiang, L., Liu, X., et al. (2022). A "cell-free treatment" for tendon injuries: adipose stem cell-derived exosomes. *Eur. J. Med. Res.* 27, 75. doi:10.1186/s40001-022-00707-x

Markazi, R., Soltani-Zangbar, M. S., Zamani, M., Eghbal-Fard, S., Motavalli, R., Kamrani, A., et al. (2022). Platelet lysate and tendon healing: comparative analysis of autologous frozen-thawed PRP and ketorolac tromethamine in the treatment of patients with rotator cuff tendinopathy. *Growth factors.* 40, 163–174. doi:10.1080/08977194.2022.2093198

Miescher, I., Rieber, J., Calcagni, M., and Buschmann, J. (2023). *In vitro* and *in vivo* effects of IGF-1 delivery strategies on tendon healing: a review. *Int. J. Mol. Sci.* 24, 2370. doi:10.3390/ijms24032370

Mousavizadeh, R., Backman, L., McCormack, R. G., and Scott, A. (2023). Dexamethasone decreases substance P expression in human tendon cells: an *in vitro* study. *Rheumatol. Oxf.* 54, 318–323. doi:10.1093/rheumatology/keu315

Myhrvold, S. B., Brower, E. F., Andresen, T. K. M., Rydevik, K., Amundsen, M., Grun, W., et al. (2022). Nonoperative or surgical treatment of acute Achilles' tendon rupture. *N. Engl. J. Med.* 386, 1409–1420. doi:10.1056/NEJMoa2108447

Natsu-ume, T., Majima, T., Reno, C., Shrive, N. G., Frank, C. B., and Hart, D. A. (2005). Menisci of the rabbit knee require mechanical loading to maintain homeostasis: cyclic hydrostatic compression *in vitro* prevents derepression of catabolic genes. *J. Orthop. Sci.* 10, 396–405. doi:10.1007/s00776-005-0912-x

Parisien, R. L., Trofa, D. P., Gualtien, A. P., Dodson, C. C., Li, X., Levine, W. N., et al. (2021). How do sports medicine and foot and ankle specialists treat acute Achilles tendon ruptures? *Foot Ankle Spec.* 14, 114–119. doi:10.1177/1938640019901055

Phelps, J., Hart, D. A., Mitha, A. P., Duncan, N. A., and Sen, A. (2023). Physiological oxygen conditions enhance the angiogenic properties of extracellular vesicles from human mesenchymal stem cells. *Stem Cells Res. Ther.* 14, 218. doi:10.1186/s13287-023-03439-9

Qureshi, A., Gulati, A., Adukia, V., Shah, A., and Mangwani, J. (2023). The influence of the site of rupture and gap distance in acute Achilles tendon rupture treated with functional rehabilitation. *Injury* 54, 1216–1221. doi:10.1016/j.injury.2023.02.020

Ragni, E., Orfei, C. P., Silini, A. R., Colombini, A., Vigano, M., Parolini, O., et al. (2020). miRNA reference genes in extracellular vesicles released from amniotic membrane-derived mesenchymal stromal cells. *Pharmaceutics* 12, 347. doi:10.3390/pharmaceutics12040347

Ragni, E., papait, A., Orfei, C. P., Silini, A. R., Colombini, A., Vigano, M., et al. (2021). Amniotic membrane-mesenchymal stromal cells secreted factors and extracellular vesicle-miRNAs: anti-inflammatory and regenerative features for musculoskeletal tissues. *Stem Cells Transl. Med.* 10, 1044–1062. doi:10.1002/sctm.20-0390

Rieber, J., Meier-Burgisser, G., Miescher, I., Weber, F. E., Wolint, P., Yao, Y., et al. (2023). Bioactive and elastic emulsion electrospun DegraPol tubes delivering IGF-1 for tendon rupture repair. *Int. J. Mol. Sci.* 24, 10272. doi:10.3390/ijms241210272

Saarensilta, A., Aufwerber, S., Silbernagel, K. G., and Ackermann, P. W. (2023a). Early tendon morphology as a biomarker of long-term patient outcomes after surgical repair of Achilles tendon rupture: a prospective cohort study. *Orthop. J. Sports Med.* 11, 23259671231205326. doi:10.1177/23259671231205326

Saarensilta, A., Chen, J., Reitzner, S., Hart, D. A., Ahmed, A., and Ackermann, P. W. (2023b). *Novel tissue biomarkers predict deep vein thrombosis and healing outcomes after Achilles tendon rupture*. Submitted.

Sarcinella, A., Femmino, S., and Brizzi, M. F. (2023). Extracellular vesicles: emergent and multiple sources in wound healing treatment. *Int. J. Med. Sci.* 24, 15709. doi:10.3390/ijms242115709

Stewman, C. G. (2023). Ultrasound-guided electroacupuncture treatment for rotator cuff tendinopathy: proposing an effective alternative to nonoperative medical treatments. *Med. Acupunct.* 35, 257–261. doi:10.1089/acu.2023.0042

Svedman, S., Juthberg, R., Edman, G., and Ackermann, P. W. (2018). Reduced time to surgery improves patient-reported outcome after Achilles tendon rupture. *Am. J. Sports Med.* 46, 2929–2934. doi:10.1177/0363546518793655

Van der Eng, D. M., Schepers, T., Goslings, J. C., and Schep, N. W. L. (2013). Rerupture rate after early weightbearing in operative versus conservative treatment of Achilles tendon ruptures: a meta-analysis. *J. Foot Ankle Surg.* 52, 622–628. doi:10.1053/j.jfas.2013.03.027

Wang, Y., and Li, J. (2023). Current progress in growth factors and extracellular vesicles in tendon healing. *Int. Wound J.* 20, 3871–3883. doi:10.1111/iwj.14261

Wiig, M., Dahlin, L. B., Friden, J., Hagberg, L., Larsen, S. E., Wiklund, K., et al. (2014). PXL01 in sodium hyaluronate for improvement of hand recovery after flexor tendon repair surgery: randomized controlled trial. *PLoS One* 9, e110735. doi:10.1371/journal.pone.0110735

Wu, X., Chen, J., Sun, W., Hart, D. A., Ackermann, P. W., and Ahmed, A. S. (2023). Network proteomic analysis identifies inter-alpha-trypsin inhibitor heavy chain 4 during early human Achilles tendon healing as a prognostic biomarker of good long-term outcomes. *Front. Immunol.* 14, 1191536. doi:10.3389/fimmu.2023.1191536

Xue, Y., Riva, N., Zhao, L., Shieh, J.-S., Chin, Y.-T., Gatt, A., et al. (2023). Recent advances of exosomes in soft tissue injuries in sports medicine: a critical review on biological and biomaterial applications. *J. Control Release* 364, 90–108. doi:10.1016/j.jconrel.2023.10.031

Yuan, Z., Yu, H., Long, H., Dai, Y., Shi, L., Zhao, J., et al. (2023). Stem cell applications and tenogenic differentiation strategies for tendon repair. *Stem Cells Int.* 2023, 1–15. doi:10.1155/2023/3656498

Zhang, Z., Li, Y., Zhang, T., Shi, M., Song, X., Yang, S., et al. (2021). Hepatocyte growth factor-induced tendon stem cell conditioned medium promotes healing of injured Achilles tendon. *Front. Cell Dev. Biol.* 9, 654084. doi:10.3389/fcell.2021.654084

Zou, J., Yang, W., Cui, W., Li, C., Ma, C., Ji, X., et al. (2023). Therapeutic potential and mechanisms of mesenchymalstem cell-derived exosomes as bioactive materials in tendon-bone healing. *J. Nanobiotechnology.* 21, 14. doi:10.1186/s12951-023-01778-6

Zulkifli, A., Ahmad, R. E., Krishnan, S., Kong, P., Nam, H. Y., and Kamarul, T. (2023). The potential mechanism of hypoxia-induced tenogenic differentiation of mesenchymal stem cell for tendon regeneration. *Tissue Cell* 82, 102075. doi:10.1016/j.tice.2023.102075

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Original Research
PUBLISHED 16 February 2024
DOI 10.3389/fbioe.2024.1308161

OPEN ACCESS

EDITED BY
Dai Fei Elmer Ker,
The Chinese University of Hong Kong, China

REVIEWED BY
Takashi Taguchi,
Western University of Health Sciences, United States
Burhan Gharaibeh,
University of Pittsburgh, United States

*CORRESPONDENCE
Brendan Y. Shi,
byshi@mednet.ucla.edu

RECEIVED 06 October 2023
ACCEPTED 29 January 2024
PUBLISHED 16 February 2024

CITATION
Shi BY, Sriram V, Wu SY, Huang D, Cheney A, Metzger MF, Sundberg O, Lyons KM, McKenna CE, Nishimura I and Kremen TJ (2024), Novel bisphosphonate-based cathepsin K-triggered compound targets the enthesis without impairing soft tissue-to-bone healing. *Front. Bioeng. Biotechnol.* 12:1308161. doi: 10.3389/fbioe.2024.1308161

Novel bisphosphonate-based cathepsin K-triggered compound targets the enthesis without impairing soft tissue-to-bone healing

Brendan Y. Shi[1]*, Varun Sriram[1], Shannon Y. Wu[1], Dave Huang[2], Alexis Cheney[1], Melodie F. Metzger[2], Oskar Sundberg[3], Karen M. Lyons[1,4], Charles E. McKenna[3], Ichiro Nishimura[5] and Thomas J. Kremen Jr[1]

[1]Department of Orthopaedic Surgery, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, United States, [2]Department of Orthopaedic Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, United States, [3]Department of Chemistry, University of Southern California, Los Angeles, CA, United States, [4]Department of Molecular, Cellular, and Developmental Biology, University of California at Los Angeles, Los Angeles, CA, United States, [5]Weintraub Center for Reconstructive Biotechnology, School of Dentistry, University of California at Los Angeles, Los Angeles, CA, United States

Background: Osteoadsorptive fluorogenic sentinel 3 (OFS-3) is a recently described compound that contains a bone-targeting bisphosphonate (BP) and cathepsin K (Ctsk)-triggered fluorescence signal. A prior study in a murine Achilles repair model demonstrated its effectiveness at targeting the site of tendon-to-bone repair, but the intrinsic effect of this novel bisphosphonate chaperone on tendon-to-bone healing has not been previously explored. We hypothesized that application of this bisphosphonate-fluorophore cargo conjugate would not affect the biomechanical properties or histologic appearance of tendon-bone repairs.

Materials and Methods: Right hindlimb Achilles tendon-to-bone repair was performed on 12-week old male mice. Animals were divided into 2 groups of 18 each: 1) Achilles repair with OFS-3 applied directly to the repair site prior to closure, and 2) Achilles repair with saline applied prior to closure. Repaired hindlimbs from 12 animals per group were harvested at 6 weeks for biomechanical analysis with a custom 3D-printed jig. At 4 and 6 weeks, repaired hindlimbs from the remaining animals were assessed histologically using H&E, immunohistochemistry (IHC) staining for the presence of Ctsk, and second harmonic generation (SHG) imaging to evaluate collagen fibers.

Results: At 6 weeks, there was no significant difference in failure load, stiffness, toughness, or displacement to failure between repaired hindlimbs that received OFS-3 *versus* saline. There was no difference in tissue healing on H&E or Ctsk staining on immunohistochemistry between animals that received OFS-3 *versus* saline. Finally, second harmonic generation imaging demonstrated no difference in collagen fiber parameters between the two groups.

Conclusion: OFS-3 did not significantly affect the biomechanical properties or histologic appearance of murine Achilles tendon-to-bone repairs. This study demonstrates that OFS-3 can target the site of tendon-to-bone repair without

causing intrinsic negative effects on healing. Further development of this drug delivery platform to target growth factors to the site of tendon-bone repair is warranted.

KEYWORDS

enthesis, biomechanics, growth factor delivery, rotator cuff repair, targeted delivery

1 Introduction

Roughly half of the 32 million musculoskeletal injuries that occur annually in the United States affect tendons or ligaments (Butler et al., 2004). Rotator cuff disease, specifically, has been shown to have a prevalence of 7%–10% in patients younger than 30 and a prevalence upwards of 30% in patients 60 and older (Teunis et al., 2014). As the elderly population continues to expand, the number of rotator cuff repairs performed per year continues to rise (Colvin et al., 2012; Yanik et al., 2021). Unfortunately, the reported rate of recurrent or persistent tears after repair ranges from 11% to 20% (Gerber et al., 2000; Slabaugh et al., 2010), with some reports as high as 94% (Galatz et al., 2004). Despite significant advances in arthroscopic technology and increased use of biomechanically superior transosseous equivalent techniques, recent studies have continued to report similar recurrent tear rates (Kim and Kim, 2016).

One major reason for rotator cuff repair failure is the inability of current surgical techniques to restore the tissue structure and biomechanical properties found at the native enthesis (Genin et al., 2009; Kanazawa et al., 2016; Derwin et al., 2018). Instead, rotator cuff tendons repaired to bone form poorly organized fibrovascular scar tissue that are compositionally deficient, lacking adequate organization of Type 1 collagen and fibrocartilage among other elements (Murray et al., 2007). Although growth factors including bone morphogenic proteins (BMPs) have been shown to improve the organization, fiber orientation, and biomechanical strength of tendon-to-bone repairs (Murray et al., 2007; Rodeo et al., 2007; Kovacevic et al., 2011; Pauly et al., 2012), the lack of targeted delivery options remains a significant barrier to wide spread use of growth factor-based therapies.

During surgical tendon-to-bone repair, local bone is mechanically disrupted, exposing hydroxyapatite (HAP) mineral matrix, and undergoes remodeling with localized osteoclastic bone resorption (Cole et al., 2016). Given their affinity for HAP, bisphosphonates (BP) are a logical option for targeting the repair site (Le et al., 2014). Osteoadsorptive Fluorogenic Sentinel 3 (OFS-3) is a recently described molecule (Richard et al., 2021) that contains a BP molecule conjugated to fluorochrome F) and quencher Q) molecules. The quencher, which inhibits emission of fluorescence when in close proximity to the fluorochrome, is linked to the BP-F parent molecule via a peptide sequence that is sensitive to the actions of the osteoclast-derived protease, cathepsin K (Ctsk). Thus, not only is OFS-3 targeted to hydroxyapatite minerals in bone, like all BPs, but release of OFS-3's coupled moieties is limited to areas with high osteoclast (OC) activity such as those found at the surgical site after tendon-to-bone repair (Kremen et al., 2023). Using a murine Achilles repair model, we recently demonstrated that OFS-3 can effectively target the site of tendon-to-bone repair whether applied locally at the time of surgery or administered via systemic injection after surgery (Kremen et al., 2023).

However, while OFS-3 represents a useful tactic for bringing coupled moieties to the site of bone remodeling, BPs potential inhibition of osteoclast activity (Hughes et al., 1995; Drake and Cremers, 2010) may have unintended effects separate from their role as a delivery mechanism. Prior studies have demonstrated that BPs can impair fracture healing (Savaridas et al., 2013), may reduce bone resorption at the tendon-bone interface (Thomopoulos et al., 2007), and may reduce the strength and stiffness of the tendon-bone interface (Hjorthaug et al., 2018). OFS-3 utilizes a modified pamidronate BP molecule designed to have significantly decreased biological activity due to changing the side chain amino group to an *N*-substituted amide. This alteration is expected to result in significantly lowered farnesyl pyrophosphate synthase (FPPS) activity (Tsoumpra et al., 2015; Sung et al., 2020; Okawa et al., 2022) both due to the amine/amide modification and the introduction of a sterically bulky amido substituent. However, the effect of OFS-3 on tendon-to-bone healing has not been explored previously.

This study aimed to evaluate the feasibility of using OFS-3 to target the site of tendon-bone repair without impairing the strength of repair or the biology of healing using a murine model of tendon-bone healing. We hypothesized that OFS-3 would not affect the biomechanical properties, histologic features or degree of Ctsk activity in tendon-to-bone repairs.

2 Materials and Methods

2.1 Molecule synthesis

OFS-3 was synthesized in the laboratory of Charles McKenna, PhD (Richard et al., 2021). Once obtained, it was suspended in sterile phosphate buffered saline at a pH of 7.4. As described by Richard et al. (Richard et al., 2021), OFS-3 contains a modified pamidronate (BP) molecule conjugated to a sulfo-cyanine 5 fluorochrome and BlackBerry Quencher 650 (Berry & Associates, Dexter, MI) which quenches any external fluorescence emitted from the sulfo-cyanine 5. However, since the quencher is conjugated to the BP molecule via a peptide with the Ctsk-sensitive sequence GHPGGPQG, in the presence of Ctsk, the peptide sequence is cleaved and the quencher is released, allowing fluorescence to be observed externally.

2.2 Surgical procedures

After approval by our institution's Animal Research Committee, thirty-six 12-week-old male C57BL/6 mice (Charles River Laboratories, Wilmington, MA) underwent right hindlimb surgery for either Achilles tendon-to-bone repair or sham surgery

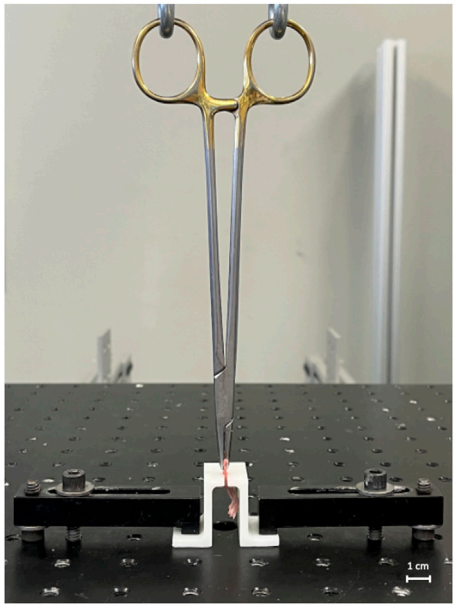


FIGURE 1
Biomechanical testing apparatus. The apparatus consists of a hydraulic testing machine and custom 3D-printed jig.

as previously described (Kremen et al., 2023). In brief, the skin on the lateral side of the right Achilles tendon was first incised sharply to expose the tendon. For animals receiving Achilles repair, 5–0 nylon suture was used to place a transverse stitch through the Achilles tendon and the posterior calcaneus. After capturing these tissues, the Achilles tendon was sharply transected at its calcaneus attachment and the bony footprint was decorticated with a dental burr. The tendon was then repaired to bone using the previously placed 5–0 nylon suture.

All animal procedures were performed under isoflurane anesthesia using standard aseptic technique. Animals were administered long-acting buprenorphine once immediately prior to the procedure and once more at 72 h post-operatively. After surgery, animals were monitored daily for signs of stress and to ensure adequate food intake and hydration.

2.3 Biomechanical assessment

Our biomechanical assessment experiments included 24 mice randomized into two groups of 12 animals. The Achilles repair treated with OFS-3 (AR + OFS3) group underwent Achilles tendon-to-bone repair followed by application of 5 μL of 1,000 nM OFS-3 via micro-pipet directly onto the tendon-bone junction at the repair site prior to skin closure. The Achilles repair (AR) group (Saline/Control group) underwent Achilles tendon-to-bone repair followed by application of 5 μL of sterile saline via micro-pipet directly onto the repaired bone-tendon junction prior to skin closure.

Six weeks after surgery, all animals were euthanized. Repaired hindlimbs were harvested and frozen. Specimens were taken out to thaw 1 h prior to testing. Once thawed, the tibia and fibula were removed and the soft tissue was dissected away, leaving the hindfoot and repaired Achilles tendon. To minimize disruption of the fibrotic scar formation at the repair site, the surgically placed suture was not removed.

The proximal end of the Achilles tendon was captured 3 mm (measured using digital calipers) from the tendon-bone junction using a needle driver that was attached to the actuator of a mechanical testing system (370.02 Bionix, MTS Systems Corp., Eden Prairie, MN). The calcaneus was secured to the frame of the testing machine via a custom 3D printed jig (Figure 1). Mounted specimens were loaded at a rate of 0.15 mm/s until failure while tensile force and displacement were continually recorded. While preconditioning is typically recommended when analyzing viscoelastic materials like tendons and ligaments, we deviated from these standard procedures to ensure consistency and specificity in our measurements as preconditioning could potentially alter the natural mechanical responses of this complex interface. This methodology is consistent with prior research studies analyzing the bone-tendon interface (Bell et al., 2015; Cong et al., 2018). Maximum failure load N), maximum displacement (mm), stiffness (N/mm), and toughness (Nmm) were determined from the resultant force-displacement curve.

Prior to testing repaired specimens, pilot testing on identically prepared unrepaired murine hindlimbs was performed, demonstrating that the described clamping system and loading protocol had high reproducibility and yielded low variance in all recorded outcomes. To evaluate the biomechanical contribution of the suture material itself, 5–0 nylon suture loops were also tested to failure with the same loading protocol.

2.4 Histologic analysis

Our histologic analysis included 12 animals randomized into two groups of 6. Similar to the biomechanical experiments, the AR + OFS3 group underwent Achilles repair followed by direct OFS-3 application, whereas the AR group (Saline/Control) underwent Achilles repair followed by saline application. Hindlimbs were harvested from 3 animals per group at 4 weeks and 6 weeks after surgery.

Each hindlimb sample was fixed in 4% paraformaldehyde for 3 days before being decalcified in formic acid for 2 days and embedded in paraffin. Sagittal sections 5 μm thick were generated with a microtome. Representative sections from 3 separate regions of the tendon-bone junction (medial, mid-sagittal and lateral) per mouse were selected for histologic analysis and underwent staining with hematoxylin and eosin (H&E). Sections immediately adjacent to the 3 regions selected for H&E underwent immunohistochemistry (IHC) staining for the presence of Ctsk using cathepsin K antibodies (Abcam, Cat: ab19027) at a dilution of 1:100 using standard protocols used in the translational pathology core at our institution as previously described (Littlewood-Evans et al., 1997). DAB (3, 3' diaminobenzidine) methodology was then used to visualize the degree of cathepsin K antibodies (Binch et al., 2020). Finally, representative sections from the mid-sagittal region of the tendon-bone junction were selected for second harmonic generation (SHG) imaging to assess collagen fiber orientation and size.

After H&E and IHC staining, images were acquired with the Keyence BZ-X800 microscope using a ×10 objective lens. The H&E-stained sagittal sections from both OFS-3 treated animals and saline control animals (3 sections per mouse) were then compared via

qualitative assessment of the anterior-to-posterior size of the reparative tissue. In addition, the degree of Ctsk staining was compared. Qualitative assessments were performed by two independent examiners trained in murine musculoskeletal histology and blinded to treatment group.

Next, the ratio between osteoclast (OC) surface area and total bone surface area was quantified using previously described methods (Lemmon et al., 2018). Brightfield images of stained tissue sections from 3 animals in each treatment group at 6 weeks after tendon-bone repair were acquired using the Leica SP8 MP-DIVE two-photon microscope (Leica Camera, Wetzlar, Germany). Images were collected using a 40x (NA = 1.25) oil immersion objective lens and 12-bit Leica DFC420 camera (pixel size of 0.162 microns) with a 300 m exposure time. A commercial AI-based image analysis software (Aivia 13.0, Leica Microsystems, Wetzlar, Germany) was used to quantify the OC/bone surface area ratio at the site of bone-tendon repair within each image. The pixel-classifier tool was used to generate OC and bone area confidence maps, first on relatively small regions within a single image. The classifier was then saved and applied on the full region of interest (ROI) of the training image, followed by unseen images captured under similar conditions. In all training scenarios, sub-optimally labelled regions were identified and corrected in an iterative fashion until satisfactory results were observed and agreed upon by 3 observers. As staining conditions varied, additional training regions taken from 5 different images were added to the training set. The AIVIA built-in 'Recipe' called 'Cell Count' was then used on the resultant masks to segment regions of high confidence and calculate the total area of the segmented objects for each mask.

SHG imaging of collagen fibers were obtained with the Leica SP8 microscope using a method adapted from Schlegel et al. (Schlegel et al., 2023). For 10x imaging, unstained sections were imaged using an excitation wavelength of 830 nm at ~580 mW (30% of maximum laser power) with backwards SHG signal collected in reflectance mode from 405 to 425 nm. Tile scans were taken using a ×10 dry objective lens and qualitatively assessed for the location of regions of interest. Once the regions of interest were identified, images of these regions were obtained using a ×40 water immersion objective lens (NA = 1.1) with an excitation wavelength of 830 nm at 400 mW (20% of maximum laser power). Tile scans were taken and cropped into regions of interest of ~150 × 150 microns. Three regions of interest per mouse in each treatment group were used for the analysis.

Collagen fiber alignment was assessed using the open-source, MATLAB-based software tools CurveAlign version 5.0 (University of Wisconsin-Madison, Madison, WI) and CT-FIRE version 3.0 (University of Wisconsin-Madison, Madison, WI) for curvelet transform-based fibrillar collagen quantification (Liu et al., 2017; Liu et al., 2020). Absolute angles measured between the fiber and horizontal axis (ranging from 0 to 180°) are converted to circular angles, and their mean value is divided by 2 to determine orientation. The length of the sum of orientation vectors divided by the total number of angles is then used as the alignment metric, with values ranging from 0 (not aligned) to 1 (perfectly aligned). Collagen fiber alignment values consisted of the alignment of each collagen fiber relative to its neighbors within 2 pixels, 4 pixels, 8 pixels, and 16 pixesl. The mean alignment value used for analysis was defined as the mean of each of these pixel distances for each fiber.

2.5 Statistical analysis

Statistical analysis of normally distributed histologic data including the collagen fiber assessments were performed by comparing the means from each treatment group using a random effects (mixed) analysis of variance model where region within each mouse was a random effect as well as within region measurement error (replicate error). OC/bone surface area ratio distribution did not follow the normal distribution and, thus, the p values were computed using the non-parametric Kruskal–Wallis one-way analysis of variance.

Biomechanical outcomes between groups were compared with one-way ANOVA and Tukey's *post hoc* test, with alpha set at 0.05. Stata 12 Software (StataCorp LLC, College Station, TX) was used for statistical analyses.

A prospective power analysis was performed using data from pilot testing on unrepaired murine hindlimbs (mean failure load 11N, standard deviation 1.6). Twelve animals per group were deemed necessary to detect a 2N difference in load to failure with a power of 0.80 (G*Power Version 3.1.9, Düsseldorf, Germany).

3 Results

3.1 Biomechanical assessment

There was no significant difference in ultimate failure load, maximum displacement, stiffness, or toughness between repaired hindlimbs that received local OFS-3 and those that received saline (Table 1). The mean failure load was 17.4 N in animals that received local OFS-3 after repair and 16.0 N in saline control animals ($p = 0.440$). Mean maximum displacement values for the Achilles repair (AR), Achilles repair plus OFS3 (AR + OFS3), and uninjured groups were 1.9 mm, 2.2 mm, and 1.9 mm, respectively. All tested specimens failed at the tendon/reparative tissue-bone junction (Figure 2).

Compared to the uninjured hindlimbs used in pilot studies, both groups of repaired hindlimbs demonstrated significantly greater failure load, toughness, and stiffness. Displacement was similar between repaired and uninjured hindlimbs.

Biomechanical testing of 5–0 suture loops in isolation demonstrated significantly lower load to failure (13.1 N), decreased stiffness (1.2 N/mm), decreased toughness (76.8 Nmm), and increased maximum displacement before failure (12.2 mm) compared to the repaired hindlimb groups ($p < 0.001$ for all comparisons). Based on our force-displacement data for the suture, at a displacement of 2 mm, the sutures exhibited a mean tensile force of less than 0.4N, substantially lower than the failure loads observed in our tissue samples.

3.2 Histologic analysis

Four weeks after surgery, animals in both groups (AR *versus* AR + OFS3) developed fibrotic tissue at the repair site with accumulation of fibroblast-appearing cells at the site of injury (Figure 3). Animals in both treatment groups had equivalent qualitative assessments of reparative tissue size and distributions of Ctsk-positive IHC staining throughout the calcaneus and Achilles stump, as noted by the distribution of brown DAB staining

TABLE 1 Biomechanical testing results after achilles repair. *p*-values comparing the two treatment groups are in bold.

	Experimental condition			*p*-values		
	AR (n = 12)	AR + OFS3 (n = 12)	Uninjured (n = 12)	**AR vs AR + OFS3**	Uninjured vs AR	Uninjured vs AR + OFS3
Failure Load N)	16.0 ± 2.9	17.4 ± 3.7	11.0 ± 1.5	**0.440**	<0.001	<0.001
Toughness (Nmm)	12.9 ± 4.3	16.8 ± 5.2	8.4 ± 2.1	**0.069**	0.027	<0.001
Stiffness (N/mm)	10.9 ± 2.3	12.0 ± 1.9	8.7 ± 1.8	**0.347**	0.036	0.001
Maximum displacement (mm)	1.9 ± 0.6	2.2 ± 0.6	1.9 ± 0.4	**0.335**	0.972	0.458

AR, achilles repair with saline; AR + OFS3 = Achilles repair with local OFS-3. Values given as mean ± standard deviation.

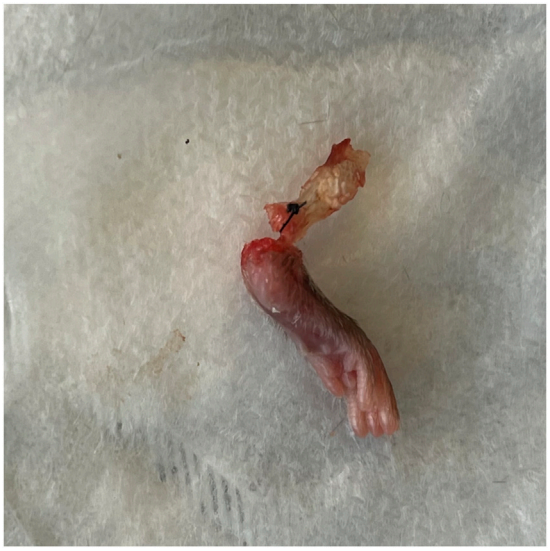


FIGURE 2
Representative specimen after load to failure. All specimens failed at the junction of the tendon/reparative tissue and bone.

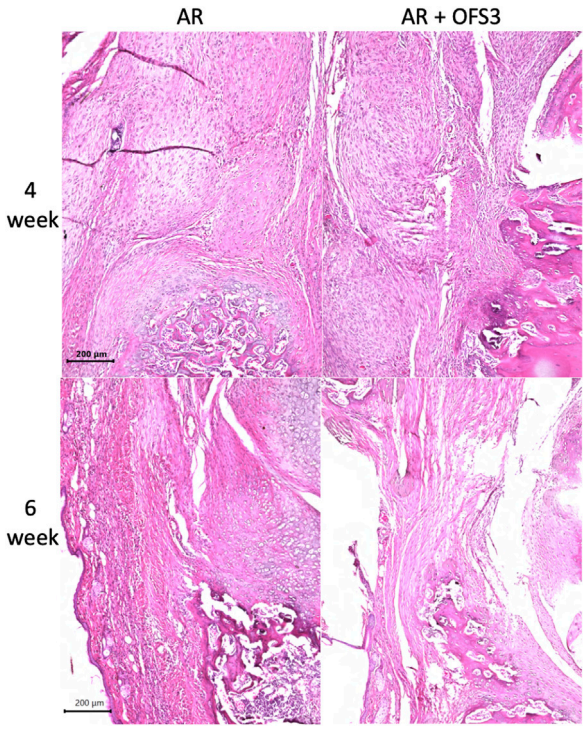


FIGURE 3
H + E results: Mouse hindfoot sagittal sections taken 4 and 6 weeks after Achilles tendon-to-bone repair followed by saline (AR) and Achilles tendon-to-bone repair followed by application of OFS-3 (AR + OFS-3), stained with H&E.

throughout each section (Figure 4). Of note, the reparative tissue at the repair site had a greater size in both the sagittal and coronal planes compared to the native tendon tissue at its insertion onto the calcaneus. Similar to our 4 weeks results, at 6 weeks after surgery, there was also no difference noted on qualitative assessments of the reparative tissue size, the fibrotic tissue appearance, or the intensity and distribution of Ctsk-positive IHC staining in animals that underwent Achilles repair followed by OFS-3 administration compared to saline control (Figure 4). Quantitative analysis demonstrated no statistically significant difference with regard to OC/bone area ratios between the AR and AR + OFS3 treatment groups (13.2% vs 21.5%, $p = 0.3827$) (Figure 5). For reference, the OC/bone area ratio in our uninjured specimens was approximately 6%.

Second harmonic generation imaging demonstrated disorganization of collagen at the repair site 6 weeks after tendon repair compared to native tendon (Figure 6; Figure 7). Quantitive analysis with CT-FIRE and CurveAlign demonstrated no significant difference in collagen fiber orientation angle ($p = 0.109$), length ($p = 0.841$), straightness ($p = 0.770$), or width ($p = 0.941$) between treatment groups (Figure 8). Collagen fiber alignment analysis demonstrated a significant mean difference between the AR and AR + OFS3 groups (mean 0.653 in AR, mean 0.569 in AR + OFS3, $p = 0.0247$). However, analysis of the components of variance demonstrated that 94% of the variation was due to replicate variability (SD = 0.185) where fiber to fiber variance was much larger than the variation between different regions of the mouse tendon repair sites or between different mice in each treatment group (mean difference of 0.084 is smaller than the replicate variability standard deviation of 0.185). Thus, the statistically significant mean difference in fiber alignment may not be clinically significant and may not be due to OFS-3 treatment.

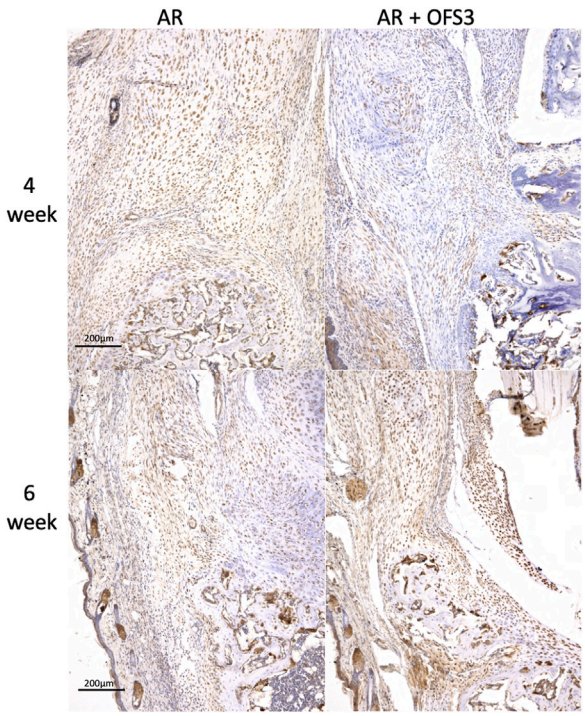


FIGURE 4
IHC results: Mouse hindfoot sagittal sections taken 4 and 6 weeks after Achilles tendon-to-bone repair followed by saline (AR) or OFS-3 (AR + OFS-3) treatment, stained with immunohistochemistry for Ctsk. Anti-Ctsk antibodies are stained brown while blue stain reflects residual hematoxylin.

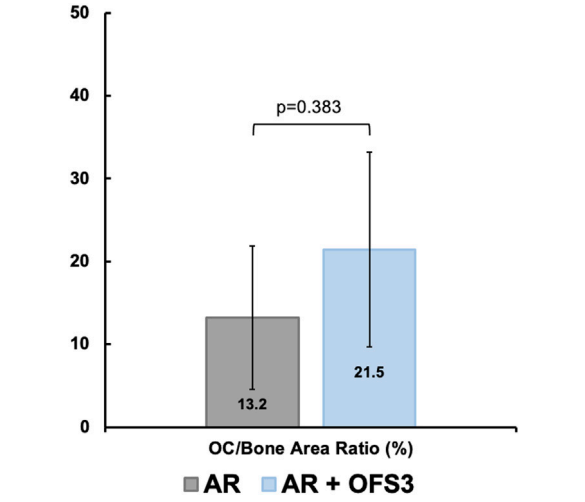


FIGURE 5
OC/total bone surface area ratio between AR and AR + OFS-3 groups.

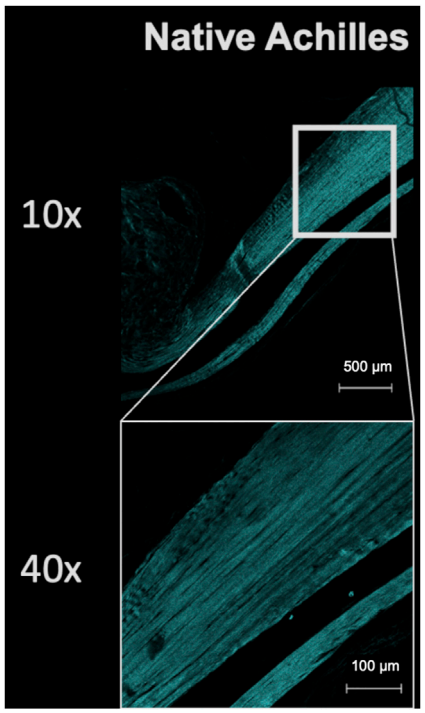


FIGURE 6
Second harmonic imaging of uninjured native Achilles. Collagen is displayed as cyan.

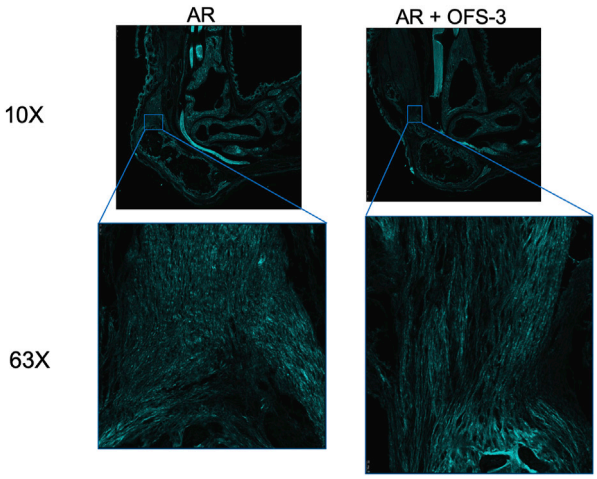


FIGURE 7
Second harmonic imaging of repaired specimens 6 weeks after Achilles tendon-to-bone repair followed by saline (AR) or OFS-3 (AR + OFS-3). Collagen is displayed as cyan.

4 Discussion

In this study, we used a murine Achilles repair model to determine the feasibility of using OFS-3 to target the site of tendon-bone repair without impairing the strength of repair or the biology of healing. We found that the addition of OFS-3 to tendon-to-bone repairs did not significantly affect the histological appearance of tendon healing and did not impact the biomechanical properties of the repaired tissue.

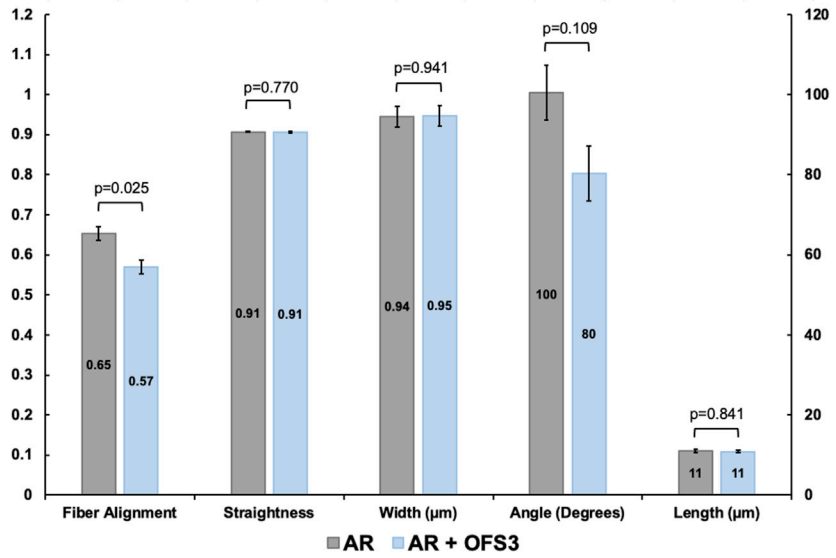


FIGURE 8
Collagen fiber dimensions and alignment between groups. Values for length and width are given in μm and angles in degrees. Straightness is unitless. For alignment, a value of 1 indicates all fibers are aligned in one direction and a value of 0 indicates fibers are not aligned.

Our previously published *in vivo* imaging results demonstrated that bisphosphonate-based compounds reliably target the site of tendon-bone repair, and that release of coupled moieties can be controlled through a Ctsk-sensitive mechanism dependent on local osteoclast present at the repair site (Kremen et al., 2023). Fluorescent signal at the repair site remained elevated for over 2 weeks and was equivalent between animals that received OFS-3 locally or parenterally, illustrating the possibility of targeted yet delayed administration of the therapeutic agent beyond the time of the surgical procedure (Kremen et al., 2023). In the current study, we additionally report that application of OFS-3 does not significantly affect the histologic appearance, collagen alignment, or biomechanical strength of tendon-to-bone repair. These findings suggest that this BP-targeted Ctsk-coupled delivery scheme has minimal negative biologic consequences, justifying further development of this platform as a potential method for delivering bioactive agents such as BMP-2 directly to the site of tendon-to-bone repair through percutaneous or intravenous injection at time points beyond the time of surgery.

While previous studies have explored the use of bisphosphonates in targeting bone for the treatment of cancer or inflammatory bone disease (Uludag and Yang, 2002; Cole et al., 2016; Farrell et al., 2018), studies investigating the role of bisphosphonates in soft tissue-to-bone healing have focused on the effects of osteoclast inhibition on repair strength. Cadet et al. found that zoledronate improved bone density in a rat supraspinatus tear model (Cadet et al., 2010), while Thomopoulos et al. found that alendronate both prevented bone loss and improved load to failure in canine flexor tendon repairs (Thomopoulos et al., 2007). Conversely, Hjorthaug et al. found that the addition of zoledronate to a murine Achilles repair model significantly impaired the ultimate failure load and stiffness of the repaired tendon at 3 and 6 weeks after surgery (Hjorthaug et al., 2018). One of the reasons for the disparate findings between the current study and prior literature may be the choice of bisphosphonate. While the class of nitrogen-containing BPs (e.g., alendronate, zoledronate, pamidronate) exhibit anti-resorptive abilities due to their inhibition of FPPS, other bisphosphonates can be engineered to have minimal to no FPPS inhibition (Sung et al., 2020) while still maintaining their affinity for hydroxyapatite minerals, as is the case with OFS-3 (Hokugo et al., 2019; Okawa et al., 2022). Furthermore, BPs conjugated to other molecules, like OFS-3, may demonstrate even further attenuated antiresorptive effects compared to BPs used in isolation (Sun et al., 2016).

The addition of OFS-3 was not found to affect the histologic appearance, collagen organization, or progression of healing after Achilles tendon transection and repair. In both groups, a thick fibrotic callus developed at the site of the tendon transection by 4 weeks post-operatively, connecting the tendon stump to its bony footprint. At 6 weeks, the fibrous scar tissue demonstrated decreased cellularity and increased alignment of collagen fibers in the direction of stress, signifying unimpeded progression to the regenerative phase of tendon-to-bone healing in both groups (Bunker et al., 2014). At 6 weeks, we additionally found no significant difference in collagen angle, length, straightness, or width between animals that received OFS-3 or saline after Achilles repair. Finally, the distribution of Ctsk-positive IHC staining and the OC/bone area ratio was not significantly different between the two groups. The fact that treatment with OFS-3 after Achilles repair did not cause decreased OC surface area or decreased Ctsk levels (a proxy for osteoclast activity) suggests that OFS-3 does not significantly inhibit osteoclast activity. These findings are consistent to those reported by Hjorthaug et al. (Hjorthaug et al., 2018), who demonstrated that systemic administration of zoledronic acid in rats that underwent Achilles tendon-to-bone repair did not affect the size or organization of callus formation. Overall, while there remains a lack of consensus regarding the effect of clinically available osteoclast-inhibiting bisphosphonates on tendon-to-bone healing,

our findings suggest that OFS-3, a molecule containing a BP with minimal, if any, FPPS inhibition, does not significantly impair tendon-to-bone healing.

Both groups of repaired hindlimbs demonstrated significantly higher failure load, toughness, and stiffness compared to the uninjured native hindlimbs among our study animals. As cross-sectional area is directly proportional to stiffness (K [stiffness] = E [Young's Modulus] * Area/Length), the increased stiffness after repair is likely due to the increased size (area) and cellularity of the fibrotic reparative tissue. During dissection, it was noted that the fibrotic reparative tissue was notably larger in size (width and thickness) than native tendon tissue at its insertion onto the calcaneus. Silva et al. compared the biomechanical properties of native canine flexor digitorum tendons with those transected at their bony insertion, and found that the injured tendons demonstrated increased cellularity, thickness, and ultimate failure load at 21 days compared to native, uninjured tendons (Silva et al., 2004). Repaired tendons were also stiffer, exhibiting decreased displacement to failure compared to native tendons (Silva et al., 2004). Other animal studies have also reported that the scar tissue generated during the proliferative stage of Achilles tendon-to-bone healing results in increased failure load and stiffness relative to the native tendon insertion (Hibino et al., 2007; Hjorthaug et al., 2015). It is important to note that assessing stiffness parameters alone may not be the ideal metric for translating biomechanical findings to the *in vivo* condition. Shah et al. found repaired rat supraspinatus tendons to be thicker, yet weaker than native tendon tissue after cyclic loading and load-to-failure (Shah et al., 2017). Freedman et al. performed full thickness and partial (50%) width mid-substance Achilles transections in a murine model and found that although stiffness only decreased by 25%, the number of cycles to failure decreased by nearly 37-fold (Freedman et al., 2014). While their partial width tear model is expected to have different biomechanical findings to our tendon-bone repair model, Freedman's findings demonstrate that small changes in stiffness may be associated with markedly different performance with cyclic loading. Thus, increased load to failure seen in repaired tendons may not translate to better performance with cyclic loading or when exposed to physiologic loading conditions.

Suture was not removed from repaired hindlimbs prior to our biomechanical testing due to two key reasons. First, clinically non-absorbable suture material is routinely implanted and maintained after surgical repair of tendons in humans. Thus, maintaining the suture in our specimens more accurate reflects a real-world protocol and could be considered more translationally relevant. Second, as noted above, healing tendon-bone repairs are associated with exuberant fibrotic reparative tissue (Silva et al., 2004). This reparative tissue often encases the suture material and attempting to remove the suture material risks damaging the tissue integrity at the repair site. To help determine the effect of retained sutures, we conducted an additional experiment to assess 5–0 nylon suture material in isolation and found that these suture loops had far lower load to failure and stiffness compared to the repaired tissue specimens. Since the hindlimb samples were around 10 times stiffer than the suture loops in isolation, each hindlimb specimen failed well before the maximum displacement of the suture loops. Therefore, the presence of the suture during mechanical testing is unlikely to have significantly affected our results, particularly in terms of the failure load of the healed tendon-bone repairs.

In summary, this study demonstrates that a bisphosphonate-based targeting and cathepsin K-coupled system can effectively deliver molecular cargo to the site of tendon-to-bone repair with minimal effect on the surrounding tissue in a mouse model of Achilles tendon-bone repair. Future studies are needed to evaluate whether this platform can effectively deliver bioactive agents such as BMP-2 or TGF-β.

4.1 Limitations

This study has several limitations. First, Achilles tendon transection is a sharp injury of healthy tissue, which may not accurately recapitulate tendon-bone repair in torn human diseased tendon tissue. Thus, the biomechanical findings in our murine Achilles tendon repair model among a relatively small number of male quadruped animals may not be generalizable to common clinical tendon injuries such as distal biceps tendon tears, pectoralis major tendon tears, or rotator cuff tendon tears. Second, the suture material at the repair site was not removed prior to biomechanical testing. Although we have discussed our rationale for this previously and our biomechanical testing of the suture material itself supports our rationale, this element of the study design can still be viewed as a limitation. Furthermore, although we noted that the reparative tissue at the site of tendon-bone repair was greater in size in both the sagittal and coronal planes, the cross-sectional area of this region was not quantified. As a result, normalization of force and displacement relative to cross-sectional area was not performed when evaluating the mechanical properties of our repaired hindlimbs. This is a limitation of this study. However, our primary aim was to study the mechanical behavior at the bone-tendon interface, an area characterized by the coexistence of heterogeneous tissue types and architecture with distinct mechanical properties. Given this heterogeneity, we believe that presenting unnormalized force and displacement data provides a more accurate representation of the mechanical behavior at this interface. Notably, this methodology is consistent with prior studies analyzing the bone-tendon interface which also did not normalize by cross-sectional area (Bell et al., 2015; Cong et al., 2018). This study also did not quantify the effect of OFS-3 treatment on local gene expression in the repaired tissue. While gene expression analysis of repaired tissue with and without OFS-3 administration may be informative and represents a potential area of future study, this study primarily aimed to assess whether the addition of OFS-3 would lead to worse tendon-bone healing strength and altered biomechanical properties. In addition, only one dose of this BP-based molecule delivered at one time point was evaluated in this preliminary study. Different doses and additional administrations may affect the histologic and biomechanical testing results. Also noteworthy, our findings are specific to OFS-3, a novel modified pamidronate-based BP molecule. Different bisphosphonates can have significantly different degrees of osteoclast inhibition (Hokugo et al., 2019; Okawa et al., 2022), which may have alternative effects on outcome measures. Finally, this investigation is an early-phase translational investigation of a method for targeting molecules to the site of tendon-bone repair.

The efficacy of this approach as a growth factor delivery strategy that may enhance soft tissue-to-bone healing has not yet been demonstrated.

4.2 Conclusion

OFS-3 did not significantly affect the biomechanical properties or histologic appearance of murine Achilles tendon-to-bone repairs. This study shows that a BP-based Ctsk-coupled target-and-release drug delivery strategy can be executed in a manner that does not affect the biomechanical integrity or histologic organization of tendon-to-bone repairs.

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 approved by UCLA Institutional Animal Care and Use Committee (Animal Research Committee). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

BS: Funding acquisition, Investigation, Methodology, Project administration, Writing–original draft. VS: Data curation, Formal Analysis, Methodology, Software, Visualization, Writing–original draft. SW: Investigation, Methodology, Writing–original draft. DH: Data curation, Formal Analysis, Methodology, Writing–review and editing. AC: Data curation, Methodology, Visualization, Writing–review and editing. MM: Data curation, Formal Analysis, Investigation, Methodology, Writing–review and editing. OS: Investigation, Methodology, Resources, Writing–review and editing. KL: Conceptualization, Supervision, Writing–review and editing. CM: Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Writing–review and editing. IN: Conceptualization, Resources, Supervision, Writing–review and editing. TK: Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing–original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. BS was aided by a grant from the Orthopaedic Research and Education Foundation with funding provided by the Ira Roschelle Family Foundation. In addition, this research was supported in part by the HH LEE Research Program and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under the Ruth L. Kirschstein National Research Service Award Number T32AR059033. Research support for TK, Jr is provided by US Department of Veterans Affairs grant number IK2BX005199 and the American Society of Bone and Mineral Research. Research support for CM is provided by the USC Bridge Institute. Research support for the Advanced Light Microscopy/Spectroscopy Laboratory and Leica Microsystems Center of Excellence at the California NanoSystems Institute is provided by the NIH Shared Instrumentation Grant S10OD025017 and NSF Major Research Instrumentation grant CHE-0722519.

Acknowledgments

The authors thank Jeffrey Gornbein, DrPH, from the UCLA Department of Biomathematics for his assistance with statistical analysis, and Haley Marks, PhD for her assistance with image acquisition and analysis. SHG imaging, collagen fiber analysis, and quantification of the OC/bone surface areas was performed at the Advanced Light Microscopy/Spectroscopy Laboratory and Leica Microsystems Center of Excellence at the California NanoSystems Institute at the University of California, Los Angeles.

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.

Publisher's note

References

Bell, R., Taub, P., Cagle, P., Flatow, E. L., and Andarawis-Puri, N. (2015). Development of a mouse model of supraspinatus tendon insertion site healing. *J. Orthop. Res. Jan.* 33 (1), 25–32. doi:10.1002/jor.22727

Binch, A., Snuggs, J., and Le Maitre, C. L. (2020). Immunohistochemical analysis of protein expression in formalin fixed paraffin embedded human intervertebral disc tissues. *JOR Spine* 3 (3), e1098. doi:10.1002/jsp2.1098

Bunker, D. L., Ilie, V., Ilie, V., and Nicklin, S. (2014). Tendon to bone healing and its implications for surgery. *Muscles Ligaments Tendons J. Jul* 4 (3), 343–350. doi:10.32098/mltj.03.2014.13

Butler, D. L., Juncosa, N., and Dressler, M. R. (2004). Functional efficacy of tendon repair processes. *Annu. Rev. Biomed. Eng.* 6, 303–329. doi:10.1146/annurev.bioeng.6.040803.140240

Cadet, E. R., Vorys, G. C., Rahman, R., Park, S., Gardner, T. R., Lee, F. Y., et al. (2010). Improving bone density at the rotator cuff footprint increases supraspinatus tendon failure stress in a rat model. *J. Orthop. Res. Mar.* 28 (3), 308–314. doi:10.1002/jor.20972

Cole, L. E., Vargo-Gogola, T., and Roeder, R. K. (2016). Targeted delivery to bone and mineral deposits using bisphosphonate ligands. *Adv. Drug Deliv. Rev.* 99 (Pt A), 12–27. doi:10.1016/j.addr.2015.10.005

Colvin, A. C., Egorova, N., Harrison, A. K., Moskowitz, A., and Flatow, E. L. (2012). National trends in rotator cuff repair. *J. Bone Jt. Surg. Am.* 94 (3), 227–233. doi:10.2106/JBJS.J.00739

Cong, G. T., Lebaschi, A. H., Camp, C. L., Carballo, C. B., Nakagawa, Y., Wada, S., et al. (2018). Evaluating the role of subacromial impingement in rotator cuff tendinopathy: development and analysis of a novel murine model. *J. Orthop. Res. Oct.* 36 (10), 2780–2788. doi:10.1002/jor.24026

Derwin, K. A., Galatz, L. M., Ratcliffe, A., and Thomopoulos, S. (2018). Enthesis repair: challenges and opportunities for effective tendon-to-bone healing. *J. Bone Jt. Surg. Am.* 100 (16), e109. doi:10.2106/JBJS.18.00200

Drake, M. T., and Cremers, S. C. (2010). Bisphosphonate therapeutics in bone disease: the hard and soft data on osteoclast inhibition. *Mol. Interv. Jun* 10 (3), 141–152. doi:10.1124/mi.10.3.5

Farrell, K. B., Karpeisky, A., Thamm, D. H., and Zinnen, S. (2018). Bisphosphonate conjugation for bone specific drug targeting. *Bone Rep. Dec* 9, 47–60. doi:10.1016/j.bonr.2018.06.007

Freedman, B. R., Sarver, J. J., Buckley, M. R., Voleti, P. B., and Soslowsky, L. J. (2014). Biomechanical and structural response of healing Achilles tendon to fatigue loading following acute injury. *J. Biomech. Jun 27* 47 (9), 2028–2034. doi:10.1016/j.jbiomech.2013.10.054

Galatz, L. M., Ball, C. M., Teefey, S. A., Middleton, W. D., and Yamaguchi, K. (2004). The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J. Bone Jt. Surg. Am. Feb* 86 (2), 219–224. doi:10.2106/00004623-200402000-00002

Genin, G. M., Kent, A., Birman, V., Wopenka, B., Pasteris, J. D., Marquez, P. J., et al. (2009). Functional grading of mineral and collagen in the attachment of tendon to bone. *Biophys. J.* 97 (4), 976–985. doi:10.1016/j.bpj.2009.05.043

Gerber, C., Fuchs, B., and Hodler, J. (2000). The results of repair of massive tears of the rotator cuff. *J. Bone Jt. Surg. Am. Apr* 82 (4), 505–515. doi:10.2106/00004623-200004000-00006

Hibino, N., Hamada, Y., Sairyo, K., Yukata, K., Sano, T., and Yasui, N. (2007). Callus formation during healing of the repaired tendon-bone junction. A rat experimental model. *J. Bone Jt. Surg. Br. Nov.* 89 (11), 1539–1544. doi:10.1302/0301-620X.89B11.19847

Hjorthaug, G. A., Madsen, J. E., Nordsletten, L., Reinholt, F. P., Steen, H., and Dimmen, S. (2015). Tendon to bone tunnel healing--a study on the time-dependent changes in biomechanics, bone remodeling, and histology in a rat model. *J. Orthop. Res. Feb* 33 (2), 216–223. doi:10.1002/jor.22756

Hjorthaug, G. A., Soreide, E., Nordsletten, L., Madsen, J. E., Reinholt, F. P., Niratisairak, S., et al. (2018). Negative effect of zoledronic acid on tendon-to-bone healing. *Acta Orthop. Jun* 89 (3), 360–366. doi:10.1080/17453674.2018.1440189

Hokugo, A., Kanayama, K., Sun, S., Morinaga, K., Sun, Y., Wu, Q., et al. (2019). Rescue bisphosphonate treatment of alveolar bone improves extraction socket healing and reduces osteonecrosis in zoledronate-treated mice. *Bone. Jun* 123, 115–128. doi:10.1016/j.bone.2019.03.027

Hughes, D. E., Wright, K. R., Uy, H. L., Sasaki, A., Yoneda, T., Roodman, D. G., et al. (1995). Bisphosphonates promote apoptosis in murine osteoclasts *in vitro* and *in vivo*. *J. Bone Min. Res. Oct.* 10 (10), 1478–1487. doi:10.1002/jbmr.5650101008

Kanazawa, T., Gotoh, M., Ohta, K., Honda, H., Ohzono, H., Shimokobe, H., et al. (2016). Histomorphometric and ultrastructural analysis of the tendon-bone interface after rotator cuff repair in a rat model. *Sci. Rep.* 6, 33800. doi:10.1038/srep33800

Kim, I. B., and Kim, M. W. (2016). Risk factors for retear after arthroscopic repair of full-thickness rotator cuff tears using the suture Bridge technique: classification system. *Arthrosc. Nov.* 32 (11), 2191–2200. doi:10.1016/j.arthro.2016.03.012

Kovacevic, D., Fox, A. J., Bedi, A., Ying, L., Deng, X. H., Warren, R. F., et al. (2011). Calcium-phosphate matrix with or without TGF-β_3Improves tendon-bone healing after rotator cuff repair. *Am. J. Sports Med.* 39 (4), 811–819. doi:10.1177/0363546511399378

Kremen, T. J., Shi, B. Y., Wu, S. Y., Sundberg, O., Sriram, V., Kim, W., et al. (2023). Biologically-coupled bisphosphonate chaperones effectively deliver molecules to the site of soft tissue-bone healing. *J. Orthop. Res. Apr* 41, 2250–2260. doi:10.1002/jor.25579

Le, B. T., Wu, X. L., Lam, P. H., and Murrell, G. A. (2014). Factors predicting rotator cuff retears: an analysis of 1000 consecutive rotator cuff repairs. *Am. J. Sports Med.* 42 (5), 1134–1142. doi:10.1177/0363546514525336

Lemmon, E. A., Locke, R. C., Szostek, A. K., Ganji, E., and Killian, M. L. (2018). Partial-width injuries of the rat rotator cuff heal with fibrosis. *Connect. Tissue Res. Sep.* 59 (5), 437–446. doi:10.1080/03008207.2018.1485666

Littlewood-Evans, A., Kokubo, T., Ishibashi, O., Inaoka, T., Wlodarski, B., Gallagher, J., et al. (1997). Localization of cathepsin K in human osteoclasts by *in situ* hybridization and immunohistochemistry. *Bone. Feb* 20 (2), 81–86. doi:10.1016/s8756-3282(96)00351-1

Liu, Y., Keikhosravi, A., Mehta, G. S., Drifka, C. R., and Eliceiri, K. W. (2017). Methods for quantifying fibrillar collagen alignment. *Methods Mol. Biol.* 1627, 429–451. doi:10.1007/978-1-4939-7113-8_28

Liu, Y., Keikhosravi, A., Pehlke, C. A., Bredfeldt, J. S., Dutson, M., Liu, H., et al. (2020). Fibrillar collagen quantification with curvelet transform based computational methods. *Front. Bioeng. Biotechnol.* 8, 198. doi:10.3389/fbioe.2020.00198

Murray, D. H., Kubiak, E. N., Jazrawi, L. M., Araghi, A., Kummer, F., Loebenberg, M. I., et al. (2007). The effect of cartilage-derived morphogenetic protein 2 on initial healing of a rotator cuff defect in a rat model. *J. Shoulder Elb. Surg. Mar-Apr* 16 (2), 251–254. doi:10.1016/j.jse.2006.07.002

Okawa, H., Kondo, T., Hokugo, A., Cherian, P., Campagna, J. J., Lentini, N. A., et al. (2022). Mechanism of bisphosphonate-related osteonecrosis of the jaw (BRONJ) revealed by targeted removal of legacy bisphosphonate from jawbone using competing inert hydroxymethylene diphosphonate. *Elife* 11, e76207. doi:10.7554/eLife.76207

Pauly, S., Klatte, F., Strobel, C., Schmidmaier, G., Greiner, S., Scheibel, M., et al. (2012). BMP-2 and BMP-7 affect human rotator cuff tendon cells *in vitro*. *J. Shoulder Elb. Surg.* 21 (4), 464–473. doi:10.1016/j.jse.2011.01.015

Richard, E. T., Morinaga, K., Zheng, Y., Sundberg, O., Hokugo, A., Hui, K., et al. (2021). Design and synthesis of cathepsin-K-activated osteoadsorptive fluorogenic sentinel (OFS) probes for detecting early osteoclastic bone resorption in a multiple myeloma mouse model. *Bioconjug Chem.* 32 (5), 916–927. doi:10.1021/acs.bioconjchem.1c00036

Rodeo, S. A., Potter, H. G., Kawamura, S., Turner, A. S., Kim, H. J., and Atkinson, B. L. (2007). Biologic augmentation of rotator cuff tendon-healing with use of a mixture of osteoinductive growth factors. *J. Bone Jt. Surg. Am. Nov.* 89 (11), 2485–2497. doi:10.2106/JBJS.C.01627

Savaridas, T., Wallace, R. J., Salter, D. M., and Simpson, A. H. (2013). Do bisphosphonates inhibit direct fracture healing? a laboratory investigation using an animal model. *Bone Jt. J. Sep.* 95-B (9), 1263–1268. doi:10.1302/0301-620X.95B9.31562

Schlegel, P., Yan, K., Upadhyaya, S., Buyens, W., Wong, K., Chen, A., et al. (2023). Tissue-engineered vocal fold replacement in swine: methods for functional and structural analysis. *PLoS One* 18 (4), e0284135. doi:10.1371/journal.pone.0284135

Shah, S. A., Kormpakis, I., Havlioglu, N., Ominsky, M. S., Galatz, L. M., and Thomopoulos, S. (2017). Sclerostin antibody treatment enhances rotator cuff tendon-to-bone healing in an animal model. *J. Bone Jt. Surg. Am.* 99 (10), 855–864. doi:10.2106/JBJS.16.01019

Silva, M. J., Ritty, T. M., Ditsios, K., Burns, M. E., Boyer, M. I., and Gelberman, R. H. (2004). Tendon injury response: assessment of biomechanical properties, tissue morphology and viability following flexor digitorum profundus tendon transection. *J. Orthop. Res. Sep.* 22 (5), 990–997. doi:10.1016/j.orthres.2004.01.004

Slabaugh, M. A., Nho, S. J., Grumet, R. C., Wilson, J. B., Seroyer, S. T., Frank, R. M., et al. (2010). Does the literature confirm superior clinical results in radiographically healed rotator cuffs after rotator cuff repair? Arthroscopy. *Mar* 26 (3), 393–403. doi:10.1016/j.arthro.2009.07.023

Sun, S., Blazewska, K. M., Kadina, A. P., Kashemirov, B. A., Duan, X., Triffitt, J. T., et al. (2016). Fluorescent bisphosphonate and carboxyphosphonate probes: a versatile imaging toolkit for applications in bone biology and biomedicine. *Bioconjug Chem. Feb 17* 27 (2), 329–340. doi:10.1021/acs.bioconjchem.5b00369

Sung, C. M., Kim, R. J., Hah, Y. S., Gwark, J. Y., and Park, H. B. (2020). *In vitro* effects of alendronate on fibroblasts of the human rotator cuff tendon. *BMC Musculoskelet. Disord.* 21 (1), 19. doi:10.1186/s12891-019-3014-1

Teunis, T., Lubberts, B., Reilly, B. T., and Ring, D. (2014). A systematic review and pooled analysis of the prevalence of rotator cuff disease with increasing age. *J. Shoulder Elb. Surg.* 23 (12), 1913–1921. doi:10.1016/j.jse.2014.08.001

Thomopoulos, S., Matsuzaki, H., Zaegel, M., Gelberman, R. H., and Silva, M. J. (2007). Alendronate prevents bone loss and improves tendon-to-bone repair strength in a canine model. *J. Orthop. Res. Apr* 25 (4), 473–479. doi:10.1002/jor.20293

Tsoumpra, M. K., Muniz, J. R., Barnett, B. L., Kwaasi, A. A., Pilka, E. S., Kavanagh, K. L., et al. (2015). The inhibition of human farnesyl pyrophosphate synthase by nitrogen-containing bisphosphonates. Elucidating the role of active site threonine 201 and tyrosine 204 residues using enzyme mutants. *Bone. Dec* 81, 478–486. doi:10.1016/j.bone.2015.08.020

Uludag, H., and Yang, J. (2002). Targeting systemically administered proteins to bone by bisphosphonate conjugation. *Biotechnol. Prog.* 18 (3), 604–611. doi:10.1021/bp0200447

Yanik, E. L., Chamberlain, A. M., and Keener, J. D. (2021). Trends in rotator cuff repair rates and comorbidity burden among commercially insured patients younger than the age of 65 years, United States 2007-2016. *JSES Rev. Rep. Tech. Nov.* 1 (4), 309–316. doi:10.1016/j.xrrt.2021.06.009

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Original Research
PUBLISHED 17 April 2024
DOI 10.3389/fbioe.2024.1374352

OPEN ACCESS

EDITED BY
Cheemeng Tan,
University of California, Davis, United States

REVIEWED BY
Takashi Taguchi,
Western University of Health Sciences, United States
Hans-Peter Wiesinger,
Paracelsus Medical University, Austria

*CORRESPONDENCE
Jian Xing,
190423831@qq.com
Wenhua Huang,
orthobiomech@163.com
Yanbing Li,
hnlybup001@163.com
Dongliang Zhao,
zdl768@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 22 January 2024
ACCEPTED 01 April 2024
PUBLISHED 17 April 2024

CITATION
Liu X, Deng Y, Liang Z, Qiao D, Zhang W, Wang M, Li F, Liu J, Wu Y, Chen G, Liu Y, Tan W, Xing J, Huang W, Zhao D and Li Y (2024), The alteration of the structure and macroscopic mechanical response of porcine patellar tendon by elastase digestion.
Front. Bioeng. Biotechnol. 12:1374352.
doi: 10.3389/fbioe.2024.1374352

The alteration of the structure and macroscopic mechanical response of porcine patellar tendon by elastase digestion

Xiaoyun Liu[1,2†], Yuping Deng[1,2,3†], Zeyu Liang[1†], Dan Qiao[4†], Wentian Zhang[1,5], Mian Wang[1,2,6], Feifei Li[1], Jiannan Liu[1], Yaobing Wu[1], Guangxin Chen[7], Yan Liu[8], Wenchang Tan[2], Jian Xing[7]*, Wenhua Huang[1,3,9]*, Dongliang Zhao[2]* and Yanbing Li[1]*

[1]National Key Discipline of Human Anatomy, Guangdong Provincial Key Laboratory of Medical Biomechanics, Guangdong Engineering Research Center for Translation of Medical 3D Printing Application, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China, [2]Institute of Biomedical Engineering, Shenzhen Bay Laboratory, Peking University Shenzhen Graduate School, Shenzhen, China, [3]Department of Orthopedics and Traumatology, Integrated Hospital of Traditional Chinese Medicine, Southern Medical University, Guangzhou, China, [4]Department of Pathology, Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou, Zhejiang, China, [5]The School of Basic Medical Sciences, Fujian Medical University, Fujian, China, [6]Department of Orthopaedics, Pingshan General Hospital of Southern Medical University, Shenzhen, China, [7]Medical Image College, Mudanjiang Medical University, Mudanjiang, Heilongjiang, China, [8]Department of Anatomy, Gannan Healthcare Vocational College, Ganzhou, China, [9]Guangdong Medical Innovation Platform for Translation of 3D Printing Application, The Third Affiliated Hospital of Southern Medical University, Guangzhou, China

Background: The treatment of patellar tendon injury has always been an unsolved problem, and mechanical characterization is very important for its repair and reconstruction. Elastin is a contributor to mechanics, but it is not clear how it affects the elasticity, viscoelastic properties, and structure of patellar tendon.

Methods: The patellar tendons from six fresh adult experimental pigs were used in this study and they were made into 77 samples. The patellar tendon was specifically degraded by elastase, and the regional mechanical response and structural changes were investigated by: (1) Based on the previous study of elastase treatment conditions, the biochemical quantification of collagen, glycosaminoglycan and total protein was carried out; (2) The patellar tendon was divided into the proximal, central, and distal regions, and then the axial tensile test and stress relaxation test were performed before and after phosphate-buffered saline (PBS) or elastase treatment; (3) The dynamic constitutive model was established by the obtained mechanical data; (4) The structural relationship between elastin and collagen fibers was analyzed by two-photon microscopy and histology.

Results: There was no statistical difference in mechanics between patellar tendon regions. Compared with those before elastase treatment, the low tensile modulus decreased by 75%–80%, the high tensile modulus decreased by 38%–47%, and the transition strain was prolonged after treatment. For viscoelastic behavior, the stress relaxation increased, the initial slope increased by 55%, the saturation slope increased by 44%, and the transition time increased by 25% after enzyme treatment. Elastin degradation made the collagen fibers of patellar tendon become disordered and looser, and the fiber wavelength increased significantly.

Conclusion: The results of this study show that elastin plays an important role in the mechanical properties and fiber structure stability of patellar tendon, which supplements the structure-function relationship information of patellar tendon. The established constitutive model is of great significance to the prediction, repair and replacement of patellar tendon injury. In addition, human patellar tendon has a higher elastin content, so the results of this study can provide supporting information on the natural properties of tendon elastin degradation and guide the development of artificial patellar tendon biomaterials.

KEYWORDS

patellar tendon, elastin, nonlinearity, stress relaxation, two-photon imaging

1 Introduction

Patellar tendon injury is usually caused by repeated high loads, which often happens to athletes who need to repeat the maximal jumps (basketball or volleyball) (Reinking, 2016). As many as 50% excellent basketball and volleyball players suffer from patellar tendon injury (Burton, 2022). However, the operation or repair strategy for the patellar tendon has a high re-injury or rupture rate, limiting their efficiency in restoring the original structure and function of the patellar tendon. Tissue engineering of the tendons is considered a novel strategy for repair and regeneration. The structure-function relationship of tissues remains a key goal in tissue engineering (Wang et al., 2018). Biomaterials must have mechanical properties similar to those of the natural tissue structure, vital in stress transmission and load-bearing in the early stage of patellar tendon regeneration (Zhao et al., 2020; Russo et al., 2022). Therefore, an in-depth understanding of the microstructure and biomechanical properties of the patellar tendon tissue aid in understanding the pathogenesis of injury, developing surgical and reconstructive effects.

Research has shown that a few proteins or non-protein components combine in complex tissue structures, affecting the nonlinear and stress relaxation behaviors of materials (Zitnay and Weiss, 2018). The diversity of tendon components and their complex structures, such as collagen fibers, elastic fibers, and glycosaminoglycans (GAGs), are crucial for the normal mechanical function of the tendon. Clinical research has shown that elastic fibers are affected by hereditary diseases such as Marfan syndrome and skin laxity, causing joint relaxation which damages the mechanical integrity of the tendons and connective tissues near the joints (Zhang et al., 2022; Krarup et al., 2023). Elastic fibers degenerate during aging, possibly causing a loss of anti-fatigue ability and increasing the risk of tendon injury in older adults (Eekhoff et al., 2023). As a dense connective tissue, elastin in the patellar tendon can combine with molecules, such as decorin and dimers, affecting the mechanical properties of the tendon (Beach et al., 2022). Research has shown that elastic fibers provide elasticity that can restore the tissue to its original state after mechanical deformation and that elastin fibers store elastic energy, protecting collagen fibers from impact loads (Fazaeli et al., 2020).

The elastin content and function of different species and types of tendons are different, and the elastin content of human tendons is the highest, compared with pigs, cows and mice (Eekhoff et al., 2023). Before entering the experimental study of human tendon injury repair, it is generally verified on animal models. It is considered that porcine tendon is a qualified substitute for biomechanical research of human tendon repair *in vitro* (Burgio et al., 2023), and porcine patellar tendon is also a medium commonly used in tissue engineering to verify that biological scaffold (Wang et al., 2018) and hydrogel (Freedman et al., 2022a) promote tendon repair and regeneration. Studies show that the elastic protein content of energy storage tendon is more than that of position tendon (Godinho et al., 2017). Patellar tendon, as one of the energy storage tendons, needs greater ductility and elasticity when walking or exercising (Thorpe et al., 2016). The patellar tendon is an anisotropic nonlinear viscoelastic material, and its stress-strain curve exhibits typical nonlinearity (Mohamed et al., 2020). The stress relaxation test exhibited typical time-dependent behavior. Previous studies used elastase to treat pig tricuspid valve anterior lobe, pig thoracic aorta and human upper eyelid, so as to explore the influence of elastin on them (Ugradar et al., 2020; De Moudt et al., 2021; Salinas et al., 2022). Hence, enzymatic degradation is a recent focus to study the relationship between elastin and biomechanics. The correlation between chemical composition and biomechanical properties of bovine patellar tendon shows that elastin content may predict the mechanical properties of patellar tendon, such as Young's modulus and stiffness (Ristaniemi et al., 2020). However, the mechanical analysis of patellar tendon after specific degradation of elastin has not been studied. Elastin is an essential component affecting the viscoelastic properties of the tissue extracellular matrix and is crucial for tissue ductility (Urbanczyk et al., 2020). Although elastase treatment significantly affects the hysteresis experiment of tissue (Godinho et al., 2021), the existing research has not completely clarified the effect of elastin on the stress relaxation characteristics of viscoelastic tissue. The proximal patellar tendon is considered a common injury site (Pearson and Hussain, 2014), but the influence of elastin on the mechanical properties and structure of its region has not been better explored. Thus, it is necessary to completely investigate elastin's role in the mechanical response of patellar tendon in axial tension and stress relaxation and its influence on structure.

Therefore, this study aimed to characterize the effects of elastin degradation on the structure, quasi-static tensile material properties, and viscoelastic properties of different porcine patellar tendon regions. The mechanical behavior of patellar tendon under the change of elastin fiber content and distribution was quantitatively analyzed by uniaxial stretching. Furthermore, establishing a dynamic constitutive model of patellar tendon before and after elastin degradation. The numerical calculations can be used to optimize the surgical patellar tendon reconstruction technology and select grafts, and guide the development of biomaterial scaffolds. Histological analysis and two-photon imaging were used to study the effect of elastin degradation on the microstructure of patellar tendon.

2 Materials and methods

2.1 Sample preparation

The patellar tendons from six fresh adult experimental pigs (Bama Xiao Xiang pig, weighing 35–50 kg, approximately 12-months old, mixed sex) were dissected (Mariano et al., 2023), and the specimens were free from disease and injury, as approved by the Ethics Committee of Shenzhen Bay Laboratory. The left and right patellar tendons were obtained from each pig, and the difference between them were not discussed in this study. Then the patellar tendons were wrapped with phosphate-buffered saline (PBS)-soaked gauze, and stored frozen at −20 °C (Maeda et al., 2021) until needed. Except the damaged samples, 77 samples were used in this study (Supplementary Table S1).

2.2 Elastase digestion

Based on previous studies (Liu et al., 2023), the samples were divided into two groups: 5 U/mL elastase, PBS. The two groups both contained 1χPBS and 0.1 mg/mL soybean trypsin inhibitor (SBTI) solution. And the samples in the two group were incubated for 8 h at room temperature. To further confirm the feasibility of this treatment protocol, the samples were washed thrice in PBS for subsequent biochemical analysis.

2.3 Biochemical analysis

Collagen, GAGs, and total protein contents were quantified in the patellar tendons incubated with elastase solution or PBS buffer. Collagen was quantified through hydroxyproline detection using a hydroxyproline detection kit (Solarbio, BC0255) according to the manufacturer's instructions (n = 18, n = 3 for each). Hydroxyproline is the iconic amino acid of collagen, accounting for approximately 13.5% of collagen. The total collagen content is 7.5-fold higher than that of hydroxyproline (Stoilov et al., 2018). The GAGs were quantitatively analyzed according to the instructions of the GAGs Test Kit (Biocolor, B1000). Ninhydrin colorimetry was used to quantitatively analyze the total protein content. Compared to the amino acid standard, the total protein content can be indirectly quantified (Starcher, 2001).

2.4 Mechanical test

The patellar tendons were taken out of the refrigerator at −20 °C and placed in 1χPBS buffer for 30–60 min at room temperature to completely thaw (Buján et al., 2000; Lee and Eliott, 2017; Darrieutort-Laffite et al., 2023; Solis-Cordova et al., 2023). Then they were evenly divided into three parts along the direction from the femur to the tibia (n = 9, proximal, central, and distal; Figure 1A (Rigozzi et al., 2009). The thickness of the patellar tendon prepared using cryomicrotome was approximately 550 μm, and the actual thickness of the patellar tendon was measured using a thickness gauge. Subsequently, the patellar tendon was divided into two test samples with dimensions of approximately 4.5χ20 mm (for fixation), and the actual width was measured using a Vernier caliper to calculate the cross-sectional area (Figure 1A). Finally, a 3D printed auxiliary loading device of 10 mm was used to assist in the loading of the test sample.

This study involved repeated mechanical tests; therefore, finding a repeatable mechanical protocol was essential to reduce the influence of the stretching times on the results. The results of repeated mechanical tests showed that the second and third tensile or stress relaxation mechanical data differed insignificantly, indicating that their tests were repetitive (Supplementary Figures S1A–M). Thus, the mechanical data of the second stretch was selected as the mechanical property baseline of the patellar tendon before incubation and used for subsequent mechanical data analysis. The samples were tested by tensile testing machine (CARE Measurement and Control Co. Ltd.), and they were gripped by custom-built clamps (Figure 1B). During the mechanical test, the samples were soaked in 1χPBS solution to keep hydrated. A tensile test and stress-relaxation test were performed according to the protocol (Figure 1C): After the mechanical sensor returned to zero, the sample was preloaded with 0.01 N to remove slack, preconditioned with 10 triangular wave cycles from 0.01 N to 0.02 N at a speed of 0.01 N/s and a frequency of 0.25 Hz to stabilize the sample (Yamamoto et al., 1999; Vafek et al., 2018). Then return to the preloaded 0.01N (0.01N was the preload. When the sample ended 10 cycles, the equipment made it stay at the position of 0.02N, so it needed to return to the preload state, that is, to 0.01N), and record the distance from clamp to clamp at this moment as the initial length L_0 of the sample (Eskandari et al., 2018). Then, the sample was uniaxial loaded to 10% deformation at the speed of 1%/s (Lee and Eliott, 2017; Smith et al., 2019) to evaluate the elastic characteristics of patellar tendon (Henninger et al., 2015). After reaching 10% deformation, it was kept for 300 s to study the viscoelastic stress relaxation response (Castile et al., 2016; Eskandari et al., 2018). This completed the first mechanical test. After standing for 1 min, the sample was returned to its initial length (recorded in the first test) and the second stretching-relaxation was repeated. Subsequently, the clamps were removed from the mechanical machine, along with the samples. Two test samples from the same region were randomly divided into PBS and elastase group (Figure 1A). They were soaked in 0.1 mg/mL SBTI solution for 15 min. And then the control group was treated with PBS solution (containing 0.1 mg/mL SBTI solution), while the treated group was incubated with 5 U/mL elastase solution, both of which were incubated at room temperature for 8 h. After incubation, the samples were washed three times with PBS. Then the clamps with test samples were placed on a stretching machine. The same initial length recorded in the first mechanical test was loaded, and then the sample was retested with the same mechanical protocol. The mechanical baseline of each group before incubation was used as a control.

2.5 Constitutive model

2.5.1 Hyperelastic constitutive model

Based on the mechanical test results of this study, the nonlinear constitutive equation was used to describe the strain energy density

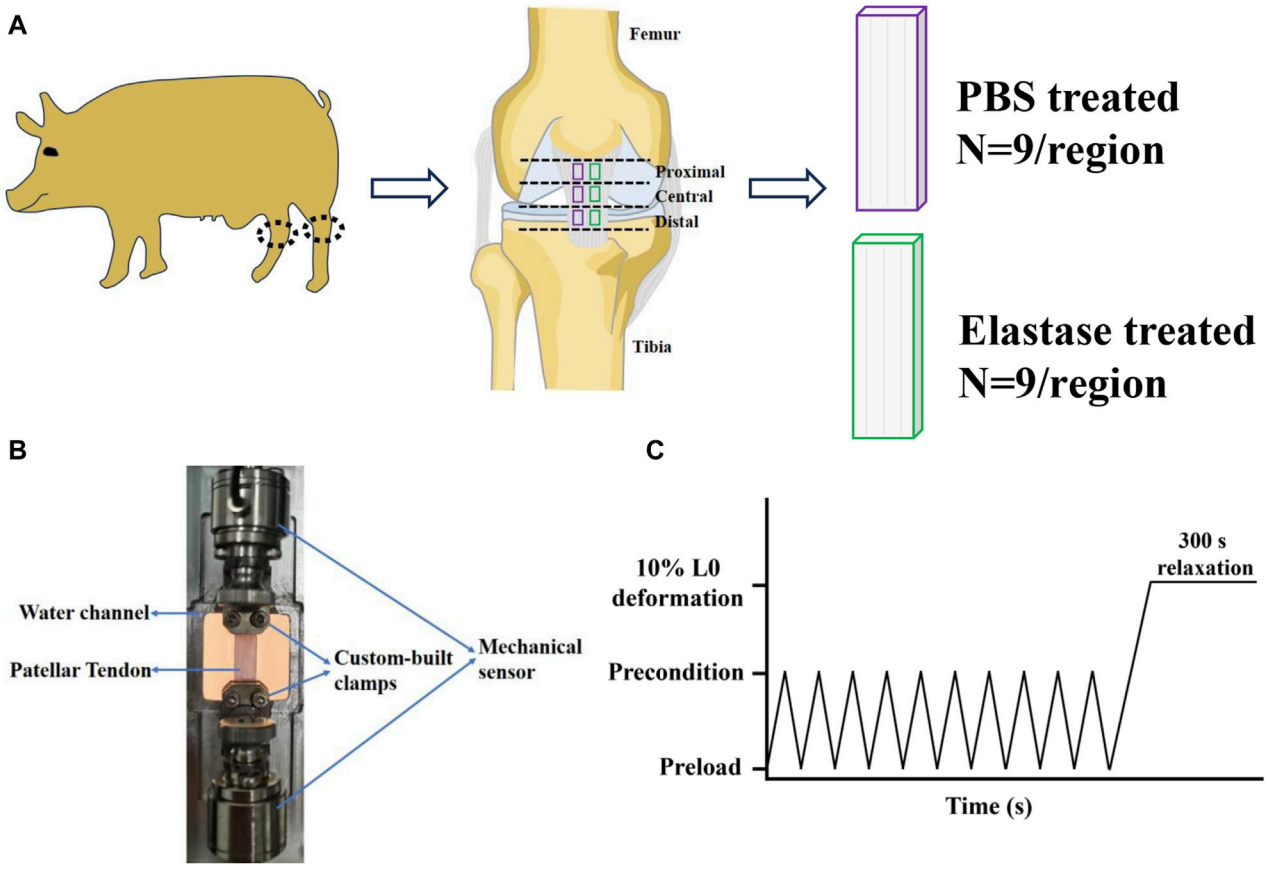


FIGURE 1
Sample preparation and mechanical test of patellar tendon. **(A)** Division of patellar tendon region and preparation of grouped samples. The black dotted frame is the knee joint. PBS is phosphate-buffered saline. **(B)** Experimental device for uniaxial mechanical testing of the patellar tendon sample. **(C)** Mechanical test protocol.

function of patellar tendon deformation. The constitutive models commonly used to represent tendons were Yeoh model (Zumbrunn et al., 2018), Ogden model (Bajuri et al., 2016), Fung-model (Ngwangwa et al., 2022), *etc.* As the arrangement of fibers and bundles is approximately unidirectional, tendon is considered as a transversely isotropic material (Böl et al., 2015). In the transversely isotropic constitutive model, Yeoh model has the fastest convergence speed and the most stable performance in all initial parameter estimation (Ngwangwa and Nemavhola, 2021), and it is one of the solutions for rapid mechanical response modeling of tendons (Ekiert et al., 2021). Therefore, assuming that the patellar tendon is incompressible, transversely isotropic, and hyperelastic, Yeoh model was used to establish the material properties of the patellar tendon and simulate their tensile mechanical changes before and after elastin degradation. The strain energy function of Yeoh model can be expressed as (Ngwangwa and Nemavhola, 2021):

$$W = \sum_{i=1}^{N} C_{i0} (I_1 - 3)^i \quad (1)$$

For incompressible materials, the typical parameters were expressed as:

$$W = C_{10} (I_1 - 3) + C_{20} (I_1 - 3)^2 + C_{30} (I_1 - 3)^3 \quad (2)$$

where W is the strain energy density function, N = 3, I is a nonzero natural number, and I_1 is an invariant. C_{10}, C_{20} and C_{30} are material parameters indicating the stiffness of the material and were obtained by fitting the experimental data.

2.5.2 Viscoelastic constitutive model

Prony series is a model of viscoelastic materials commonly used in engineering, which can be used to better simulate the time-dependent behavior of materials (Bose et al., 2020; Park et al., 2023). Therefore, the viscoelasticity of the patellar tendon was characterized by a second-order Prony series (Shearer, et al., 2020), and the stress-time data from the stress relaxation experiment were analyzed. Young's modulus E was obtained from the stress relaxation experiment data. Assuming that the patellar tendon was an incompressible material, the Poisson's ratio was set to a constant, that is, ν = 0.5 (Mihai and Goriely, 2017), and the shear modulus G was calculated. The formula is as follows (Maritz et al., 2021):

$$\sigma = \frac{F}{CSA} \quad (3)$$

$$\varepsilon = \frac{\Delta L}{L_0} \quad (4)$$

$$E = \frac{\sigma}{\varepsilon} \tag{5}$$

$$G = \frac{E}{2(1+\nu)} \tag{6}$$

where σ is stress, ε is strain, F is load, and CSA is cross-sectional area; L_0 is initial length, and ΔL is length variation.

The formula for the shear modulus of the relaxation effect of the Prony coefficient is as follows (Pan et al., 2022):

$$G(t) = G_\infty + \sum_{i=1}^{N} G_i e^{\frac{-t}{\tau_i}} = G_0\left(\alpha_\infty + \sum_{i=1}^{N} \alpha_i . e^{\frac{-t}{\tau_i}}\right) \tag{7}$$

$$\alpha_\infty = 1 - \sum_{i=1}^{N} \alpha_i \tag{8}$$

where G_0 is the instantaneous shear modulus, G(t) is the shear modulus of relaxation effect, t is time, N is the number of Prony series, α_i is the material parameter of correlation modulus, and τ_i is the material parameter of relaxation time.

2.6 Histological analysis

The patellar tendons incubated with PBS or elastase were fixed in 4% paraformaldehyde for 48 h after mechanical testing, and embedded in paraffin after dehydration. Three paraffin longitudinal sections with a thickness of 5 μm were obtained in the middle of the patellar tendon continuously, and Verhoeff's Van Gieson (VVG) staining was performed (n = 3), exhibiting collagen fibers in red and elastin fibers in black. Standard images representing collagen and elastic fibers were selected for analysis. Six areas were selected in the film, and ImageJ software was used to calculate the wavelength of the collagen fibers. The wavelength was defined as the distance between two consecutive bending peaks. The wavelength of six consecutive peaks was calculated, and the average value was used for statistical analysis.

A fresh experimental porcine patellar tendon was obtained, and the patellar tendon was divided into three regions according to mechanical test standards: proximal, central, and distal. The fixation of patellar tendon was the same as VVG staining. Structural differences among the three patellar tendon regions were identified using Movat's staining. This staining revealed collagen fibers (yellow), smooth muscle cells (red), proteoglycans or matrix (blue-green), elastic fibers or nuclei (purple to black), and foam cells (purple).

2.7 Two-photon microscopy

Two photon microscopy (Olympus, FVMPE-RS) with 40χwater lens (NA 0.8) was used to collect the microstructure of patellar tendon and to analyze the effect of elastin degradation on collagen fiber. A central patellar tendon was made to the dimensions consistent with the mechanical test and divided into two samples, which were randomly divided into PBS group and elastase treatment group (Figure 1A). The clamps for mechanical test were fixed in a 60 mm Petri dish with glue and gripped fresh samples, which were at the same horizontal line without stress. The samples were imaged in pure water, and the collagen fibers were visualized by second harmonic generation (SHG, excitation: 840nm; emission: 410–460 nm) (Eekhoff et al., 2020). Randomly select a field of view. Images were acquired with a resolution of 4096 × 4096 pixels, and sampling speed was 2.0 us/pixel. Once imaging was completed, 12 mL of PBS or 5 U/mL elastase solution was added to the dish, respectively, and the samples were incubated for 8 h at room temperature. *In-situ* second harmonic imaging was performed on the treated sample again. Since it was difficult to find the region consistent with the pre-treated imaging, the imaging region was randomly selected for the treated samples.

2.8 Data analysis

The tensile stress and strain were calculated using Eqs 3, 4, respectively. The slopes of the low- and high-strain linear regions on the stress-strain curve were obtained by bidirectional curve fitting and were recorded as low (E^{LT}) and high (E^{HT}) tensile moduli, respectively (Herbert et al., 2016; Song et al., 2022). The intersection of the two tangents defined a transition point, with the abscissa of the transition strain and the ordinate of the transition stress, and the intersection of the tangent of the high-strain linear region and the *X*-axis was recorded as the ductility index ε *. A data analysis diagram is shown in Figure 2A (Pineda-Castillo et al., 2022).

In the stress-relaxation part, the stress was normalized. The peak stress was the maximum stress after stretching to 10% deformation, the equilibrium stress was the steady stress after stress relaxation for 300 s, and the relaxation percentage was the relative change value after relaxation for 300 s. On the stress-time curve, the initial slope dR^1/dt was the linear fitting of the 5 s data, and the saturation slope dR^2/dt was the linear fitting of the last 50 data points. The intersection of the two tangents was used as an index of the transition time, which was used to quantitatively evaluate the shape of the stress-relaxation curve. The data analysis diagram is shown in Figure 2B (Duginski et al., 2020).

2.9 Statistical analysis

The statistical analysis software GraphPad Prism nine was used to sort and statistically analyze the data. All data were expressed as mean ± standard deviation, and Shapiro–Wilk test was used to test the normality of all the data. If the experimental data conformed to the normal distribution, the basic data and mechanical baseline of the different patellar tendon areas were compared using a single-factor analysis of variance combined with a *post hoc* Bonferroni's test for multiple comparisons. The remaining biochemical indices and wavelengths of the PBS treatment and elastase incubation groups were analyzed using a two-sample *t*-test. If it did not conform to the normal distribution, the Kruskal–Wallis test was used, and a *post hoc* Dunnett's test was used for multiple comparisons. According to the normality of the data, a paired *t*-test was used to evaluate the mechanical properties of samples incubated with elastase or PBS before and after treatment; otherwise, the Wilcoxon signed-rank test was used for paired samples. For all analyses, $^*p < 0.05$ was considered significant.

3 Results

3.1 Baseline data comparison

There was no significant difference in the cross-sectional area and initial length of the mechanical test samples of different groups

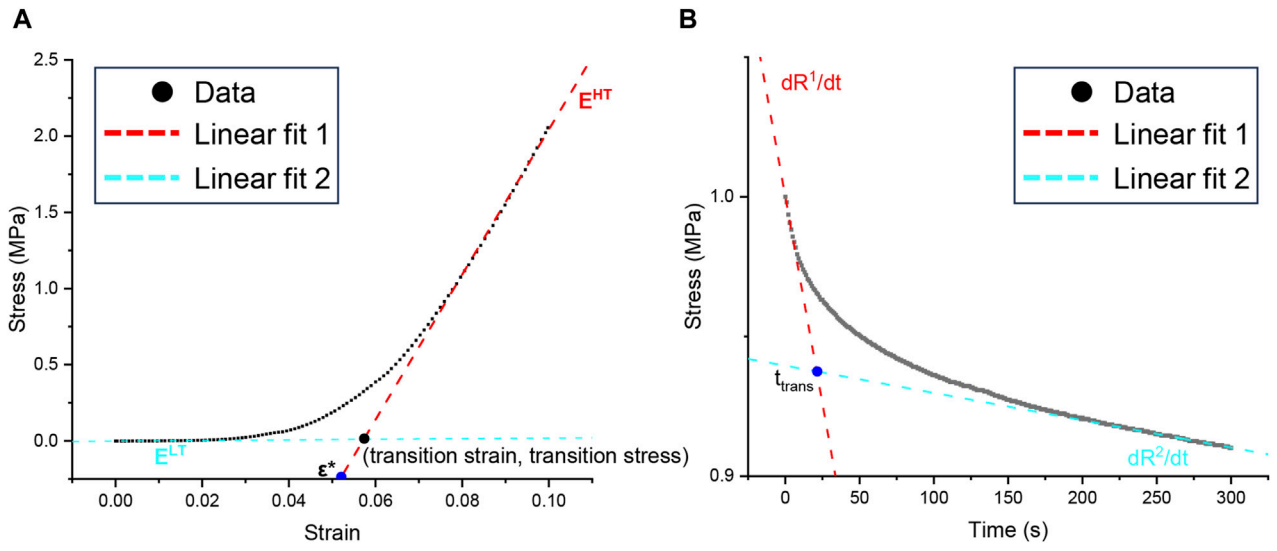


FIGURE 2
Graphic analysis of mechanical data. **(A)** Parameters quantified from uniaxial tension tests, including the low-tension modulus E^{LT}, the high-tension modulus E^{HT}, the index of extensibility ε^*, the transition strain, and the transition stress. **(B)** Parameters quantified from stress relaxation data, including the initial slopes dR^1/dt, the saturated slopes dR^2/dt, and the transition time t_{trans}.

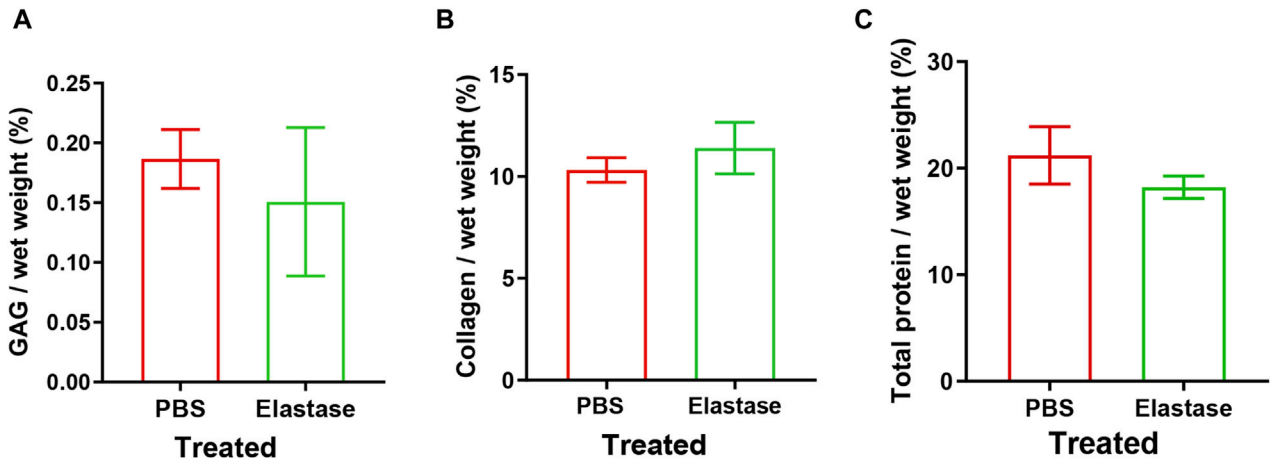


FIGURE 3
Biochemical indexes of patellar tendon incubated with 5 U/mL elastase or PBS solution. PBS is phosphate-buffered saline. **(A–C)** The content of sGAG, hydroxyproline, and total protein remaining after PBS or elastase treatment, respectively.

(Supplementary Figures S2A, B), which indicated that different samples could be used for comparison.

3.2 Biochemical results

Compared with the PBS control group, the GAGs and total protein contents in the enzyme treatment group decreased; but no significant effect was observed (Figures 3A, C). Similarly, elastase treatment insignificantly affected collagen (Figure 3B). Therefore, it was shown that incubation with elastase had no significant effect on other biochemical indices possibly related to mechanics.

3.3 Elastin degradation significantly affected the tensile mechanical properties of patellar tendon

The stress at the proximal patellar tendon before treatment was greater than that at the central and distal tendons; however, the central and distal patellar tendons coincided (Figure 4A). The results in Figure 4B differed from those in Figure 4A, and the mechanics of the patellar tendon area were that the central was greater than the proximal, and the proximal was greater than the distal. In all three regions, the stress before elastase treatment was not statistically different from that before PBS treatment ($p > 0.05$). In the patellar tendon regions, the stress after treatment was appeared to be lower

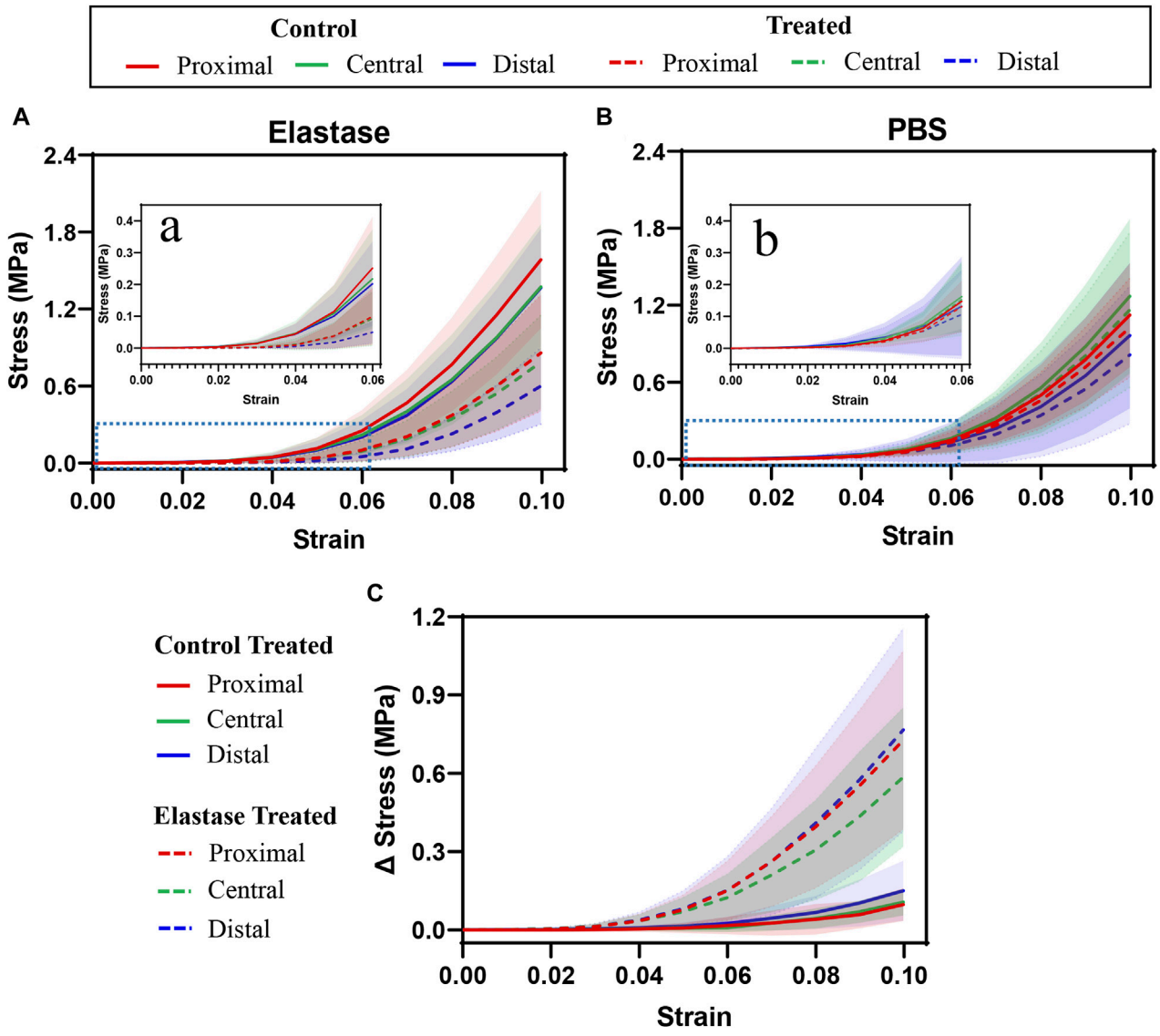


FIGURE 4
Tensile stress-strain curves of three different regions of patellar tendon treated with PBS or elastase. The control group contained the experimental data of each group before treatment. PBS is phosphate-buffered saline. **(A,B)** Mechanical curves of 5 U/mL elastase or PBS solution before and after incubation are shown. **(C)** Difference before and after treatment with PBS or 5 U/mL elastase. **(A,B)** Enlarged view of the blue dotted line in Figure **(A)** and **(B)**. Shaded area: standard deviation.

than that before treatment (elastase group: proximal $p < 0.002$, central $p < 0.027$, and distal $p < 0.08$; PBS group: proximal $p < 0.281$, central $p < 0.055$, and distal $p < 0.009$) (Figures 4A, B); however, compared with the PBS control group, the stress of the patellar tendon after elastase incubation was significantly reduced (proximal $p < 0.004$, central $p < 0.003$, and distal $p < 0.004$) (Figure 4C). The toe area of the patellar tendon after elastase treatment was longer than that before treatment, and it was stressed with an increase in fixture displacement after 4% strain (Figure 4A). However, this trend was not observed in the PBS treatment group; the toe region was similar before and after treatment, and the tissue was stressed at 4% (Figure 4B).

Although the moduli of the patellar tendon regions differed before treatment, these differences were statistically insignificant. The low tensile modulus of the distal patellar tendon before treatment was significantly higher than that of the proximal patellar tendon ($p = 0.015$) (Figure 5A). After elastin degradation in the three patellar tendon regions, the low tensile modulus decreased significantly (75%, 77%, and 80% in the proximal, central, and distal, respectively; all $p < 0.0001$) (Figure 5A), whereas the high tensile modulus decreased significantly (40%, 38%, and 47% in the proximal, central, and distal, respectively; $p < 0.0001$, $p = 0.0039$, $p < 0.0001$, respectively) (Figure 5B), and the stiffness of the tissue decreased. Although a trend of tissue stiffness reduction was found in the PBS group, it was insignificant in all regions, and the reduction was smaller than that in the enzyme group (Figures 5A, B). In addition, the ductility index among the regions before treatment differed insignificantly. Only the distal patellar tendon significantly increased in ductility index (by 16%) after enzymatic digestion of elastin ($p = 0.0039$) (Figure 5C).

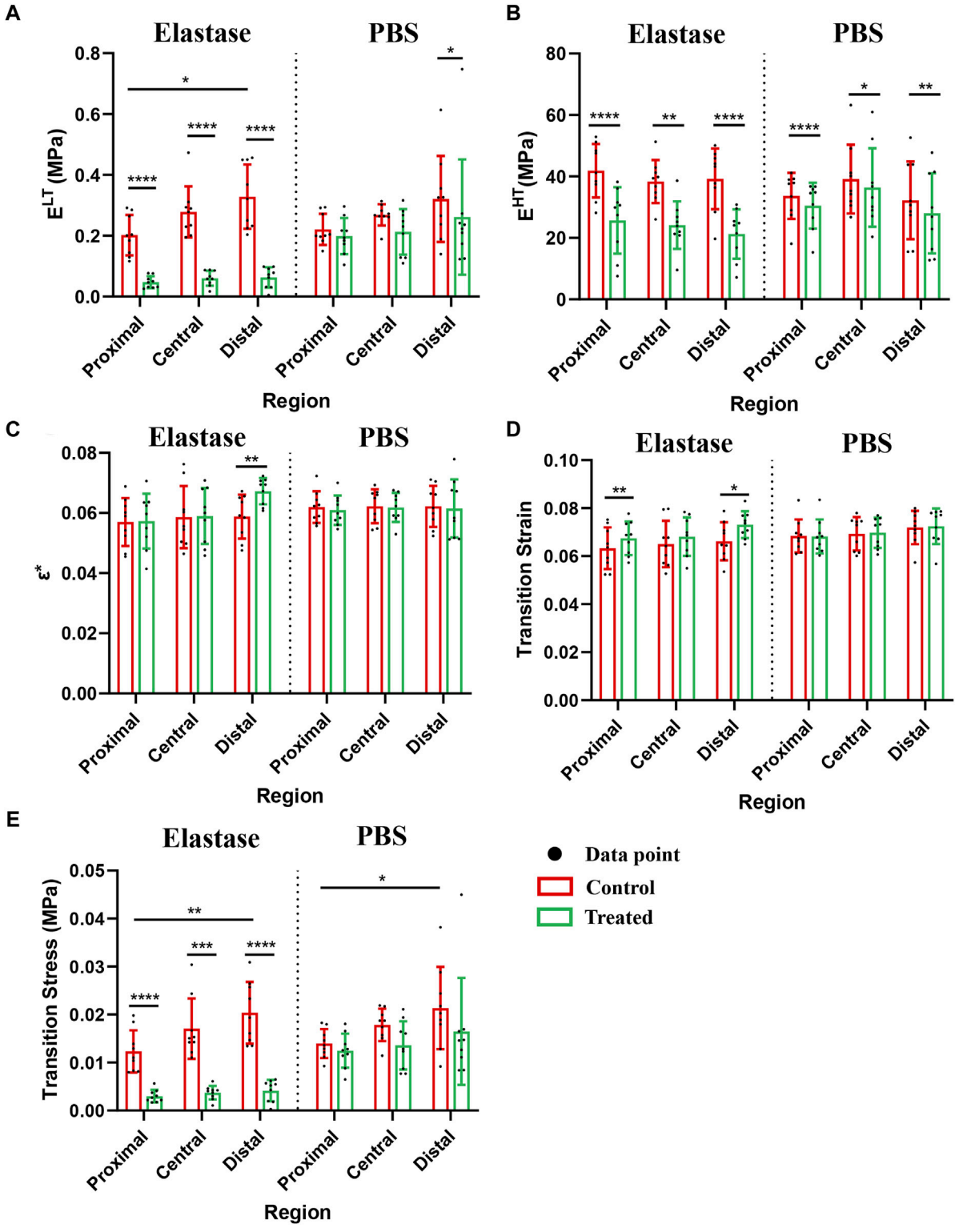


FIGURE 5
Mechanical properties of patellar tendon before and after treatment with three different regions. The control group contained the experimental data of each group before treatment. PBS is phosphate-buffered saline. **(A–E)** Comparison of the low-tension modulus, high-tension modulus, index of extensibility, transition strain, and transition stress before and after treatment with 5 U/mL elastase or PBS, respectively. * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, **** for $p < 0.0001$.

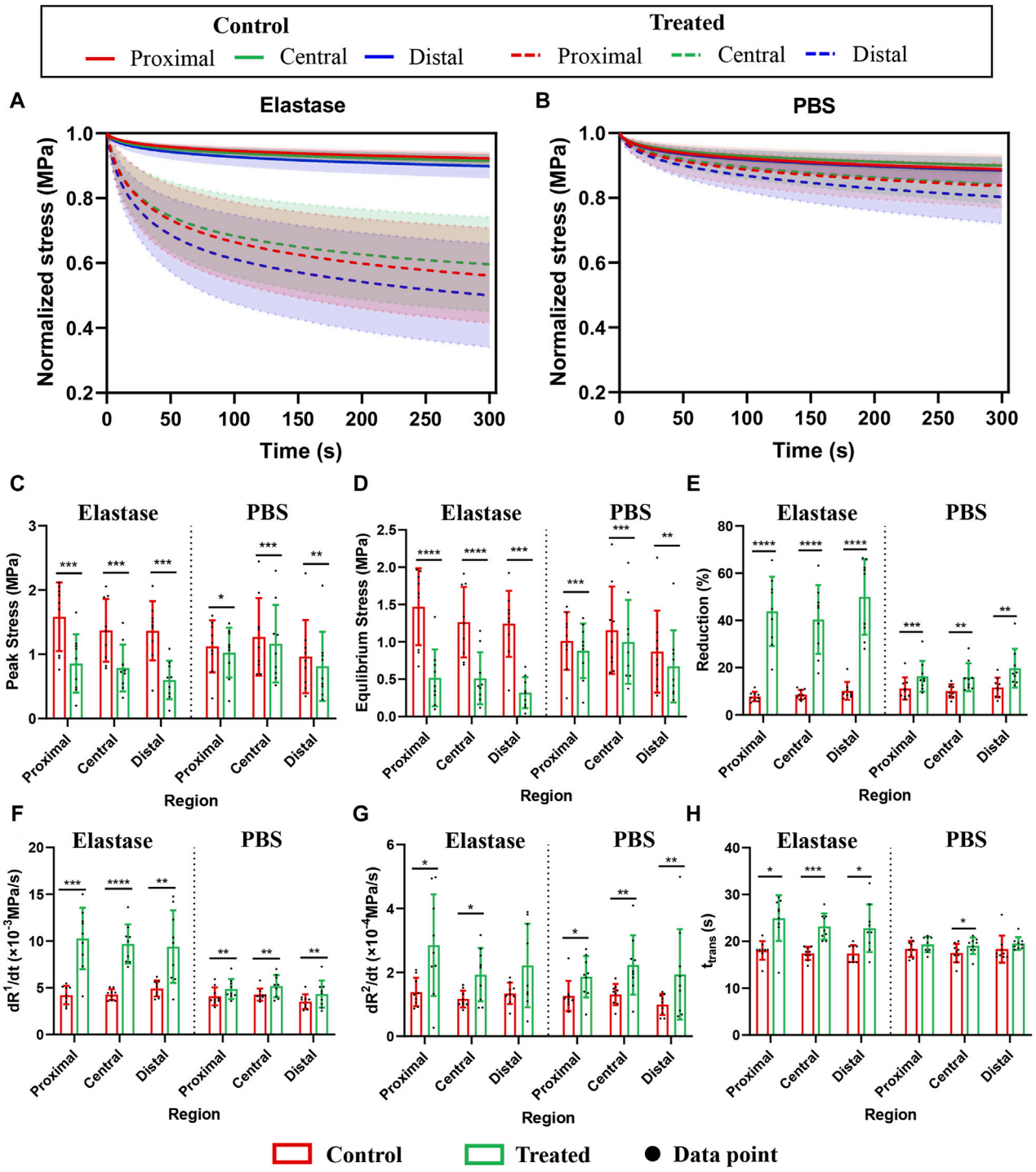


FIGURE 6
Stress relaxation properties of three different regions of the patellar tendon before and after treatment. The control group contained the experimental data of each group before treatment. PBS is phosphate-buffered saline. **(A)** Normalized stress-time curves before and after incubation with 5 U/mL elastase. **(B)** Normalized stress-time curves before and after PBS treatment. **(C–H)** Comparison of peak stress, equilibrium stress, relaxation percentage, initial slope, saturation slope, and transition time before and after treatment with 5 U/mL elastase or PBS, respectively. * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, **** for $p < 0.0001$.

After elastase treatment, the transition strain increased, particularly in the proximal and distal patellar tendons ($p = 0.0039$, $p = 0.0359$, respectively, Figure 5 D). The transition strain between the patellar tendon regions differed insignificantly; however, the transition stress at the distal patellar tendon was greater than that at the proximal region ($p = 0.0207$, $p = 0.0293$) (Figure 5E). Similarly, the transition stress decreased significantly after elastin degradation in different patellar tendon regions (75%,

77%, and 79% in the proximal, central, and distal, respectively; $p < 0.0001$, $p = 0.0001$, $p < 0.0001$, respectively) (Figure 5E). Although a downward trend was observed in the PBS treatment group, this difference was statistically insignificant (Figure 5E).

3.4 Effect of elastin degradation on stress relaxation mechanical properties of the patellar tendon

Significant stress relaxation was observed in all patellar tendon regions, and they had similar stress (Figure 6). Compared with the PBS control group, elastin showed a more evident downward trend after degradation, and the stress reduction value was concurrently greater (Figures 6A, B).

The quantitative performance of stress relaxation in the patellar tendon regions before treatment differed insignificantly; however, after enzyme treatment, the peak stress was significantly reduced (47%, 43%, and 56% in the proximal, central, and distal, respectively; $p = 0.0002$, $p = 0.0002$, $p = 0.0004$, respectively) (Figure 6C), and the equilibrium stress was also significantly reduced (66%, 62%, and 74% in the proximal, central, and distal, respectively; $p < 0.0001$, $p < 0.0001$, $p = 0.0001$, respectively) (Figure 6D). However, compared with the PBS group, the range of stress reduction after elastin degradation was larger (Figures 6C, D). The relaxation percentage of the enzyme treatment group reached 40% in the three regions, higher than that of the PBS group (Figure 6E).

Furthermore, the initial slope, saturation slope, or transition time among the three regions of the patellar tendon regions differed insignificantly (Figures 6F–H). The patellar tendons in different regions showed an increasing trend after treatment in the PBS control and enzyme treatment groups. In the enzyme treatment group, the initial slope increased significantly (59%, 56%, and 48% in the proximal, central, and distal, respectively; $p = 0.0002$, $p < 0.0001$, $p = 0.0044$, respectively) (Figure 6F), the saturation slope increased significantly (51%, 40%, and 39% in the proximal, central, and distal, respectively; $p = 0.0159$ $p = 0.0106$, $p = 0.0572$, respectively) (Figure 6G), and the transition time was also increased significantly (28%, 25%, and 24% in the proximal, central, and distal, respectively; $p = 0.0126$, $p = 0.0004$, $p = 0.0186$, respectively) (Figure 6H). But the changes of the three mechanical indexes in PBS treatment group were small (Figures 6F–H). As the differences among the patellar tendon regions were insignificant, the data before and after processing of the three regions were summed, and the average value was calculated. The initial slopes of the enzyme group before and after the control: 4.454 ± 0.81 and −9.79 ± 3.08, respectively; The saturation slopes: 1.297 ± 0.35 and −2.332 ± 1.24, respectively; The transition time: 17.62 ± 1.727 and 23.65 ± 4.253, respectively.

3.5 Constitutive modeling

3.5.1 Hyperelastic constitutive model under uniaxial tensile

In this study, Yeoh's hyperelastic constitutive model was used to fit the collected stress-strain data. The goodness-of-fit R^2 and NRMSE (Ngwangwa et al., 2022) showed an ideal fitting effect (Supplementary Figures S3A, B). The R^2 values were above 0.99 and NRMSE was less than 0.10, indicating that the fitting effect was significant. Based on this constitutive model, the stiffness parameters C_{10}, C_{20}, C_{30} were calculated before and after elastin degradation in the three patellar tendon regions (Table 1 and 2). In different regions, elastin degradation greatly reduced the stiffness of the material. However, the stiffness of the material in the PBS group fluctuated slightly after treatment.

3.5.2 Viscoelastic constitutive model under stress relaxation

The Prony series were used to fit the stress-relaxation data of the patellar tendon. It showed a better fitting effect of the experimental data (Supplementary Figures S3C, D). The goodness-of-fit R^2 and NRMSE were also used to measure the fitting effect, where the data R^2 values were above 0.99 and NRMSE was close to 0, indicating that the fitting effect was significant. The fitting parameters before and after elastin degradation in the three different areas of the patellar tendon were calculated (Table 3 and 4). In different regions, it was found that the instantaneous modulus of elastin decreased after degradation, but the material parameters α increased, τ_1 increased, but τ_2 decreased. In the PBS control group, the instantaneous modulus decreased, and the material parameters α increased, with a range smaller than that in the enzyme treatment group, and τ increased.

3.6 Histological analysis

The patellar tendon was evaluated using VVG staining, and the black, slender elastic fibers in the patellar tendon were closely arranged along the red collagen fibers (Figure 7A). After incubation with elastase, elastin was degraded, inducing morphological changes in the collagen fibers (Figure 7A). The tissue became loose, and the wavelength of the collagen curl increased, which caused fiber straightening (Figure 7B).

Histological evaluation of Movat's staining revealed that the three patellar tendon regions had similar microstructures, all comprising collagen fibers, cells, proteoglycans, and elastin fibers, and the inter bundle matrices between the collagen fiber bundles were evident (Figure 7C). Similarly, slender black elastin fibers could also be found in all patellar tendon areas, which were closely arranged along yellow collagen fibers.

3.7 SHG imaging of patellar tendon

The two-photon image of patellar tendon showed the characteristic structure of collagen fibers treated with PBS or elastase (Figure 8). Samples were imaged directly without special liquid fixation, and collagen fibers were displayed in green. In the samples before PBS or enzyme treatment, it was found that collagen fibers had periodic curl and were closely recruited by slender fiber bundles (Supplementary Figures S4A, B). However, compared with the structure before treatment, the collagen fibers in PBS control group remained tightly packed, and the integrity of collagen fibers was not damaged by elastin degradation (Figure 8). It was generally found that the degradation of elastin made collagen fibers looser and

TABLE 1 Changes of tensile fitting parameters of patellar tendon before or after 5 U/mL elastase treatment.

Region	Control					Elastase treated				
	C_{10} (MPa)	C_{20} (MPa)	C_{30} (MPa)	R^2	NRMSE	C_{10} (MPa)	C_{20} (MPa)	C_{30} (MPa)	R^2	NRMSE
Proximal	−0.23 ± 0.20	44.87 ± 40.01	290.10 ± 563.88	0.99	0.05	−0.13 ± 0.16	16.13 ± 23.30	358.51 ± 326.99	0.99	0.06
Central	−0.14 ± 0.21	34.66 ± 39.49	314.03 ± 527.61	0.99	0.04	−0.11 ± 0.12	14.61 ± 21.37	319.26 ± 293.00	0.99	0.07
Distal	−0.13 ± 0.17	30.49 ± 32.06	412.80 ± 494.13	0.99	0.05	−0.04 ± 0.07	3.32 ± 8.75	417.95 ± 191.58	0.99	0.09

TABLE 2 Changes of tensile fitting parameters of patellar tendon before or after PBS treatment.

Region	Control					PBS treated				
	C_{10} (MPa)	C_{20} (MPa)	C_{30} (MPa)	R^2	NRMSE	C_{10} (MPa)	C_{20} (MPa)	C_{30} (MPa)	R^2	NRMSE
Proximal	−0.13 ± 0.14	22.18 ± 22.80	414.71 ± 274.09	0.99	0.05	−0.13 ± 0.13	20.90 ± 19.88	374.23 ± 219.02	0.99	0.06
Central	−0.11 ± 0.23	22.39 ± 31.41	522.74 ± 232.99	0.99	0.04	−0.12 ± 0.18	21.65 ± 27.97	460.31 ± 231.87	0.99	0.05
Distal	−0.00 ± 0.09	11.89 ± 31.59	473.75 ± 427.80	0.99	0.05	−0.02 ± 0.08	10.09 ± 28.81	406.65 ± 409.71	0.99	0.05

the distance between fibers relatively longer, which led to that the enzyme treatment group consisted of fewer fibers than the PBS group in the same size images.

4 Discussion

In this study, biomechanics and optical imaging were combined to explore the mechanical properties and structural changes of the same tissue sample in three different regions of patellar tendon before and after elastin degradation, which evaluated the contribution of elastin to the mechanical integrity and fiber structure arrangement of patellar tendon. The Movat's staining results of the three regions suggest that their mechanical properties differed insignificantly, possibly owing to their similar structures. Mechanical characterization showed that the degradation of elastin reduced tensile stress, modulus and transition strain. In the stress relaxation experiment, the influence of elastin on the viscoelastic behavior of the patellar tendon was better understood. After elastin degradation, the initial and saturation slopes increased, the transition time became longer, the relaxation percentage increased, and the stress reduction amplitude increased. Optical imaging results showed that elastin degradation reduced the degree of fiber recruitment of patellar tendon. These results aid in understanding the relationship between patellar tendon diseases, microstructure, and mechanics.

Two-photon imaging and histological staining revealed the spatial arrangement of elastin and collagen fibers in patellar tendon. The decrease of collagen fiber fluctuation caused by elastin degradation could lead to mechanical changes, which better explained the mechanical contribution of fiber structure to the patellar tendon. Soft tissue shows a typical nonlinear stress-strain curve under a tensile load, which has four typical regions: low stress-strain toe linear elastic state, highly nonlinear transition state, linear elastic state, and finally, yield and failure of the structure (Herbert et al., 2016; Li et al., 2019). This study highlighted the contribution of elastin to the biomechanical nonlinear mechanical behavior of patellar tendon. After enzyme treatment, the low stress-strain region of the patellar tendon was prolonged, stress exertion began after 4% strain, and the transition strain and ductility index increased, indicating that elastin degradation significantly affected the mechanical properties of the toe region. When performing two-photon imaging, the clamp distance before or after sample treatment was the same, and the phenomenon found in the imaging process could be considered as the effect of elastase treatment. This imaging method was similar to the mechanical test of samples, that is, the initial length was consistent before and after treatment. After enzyme treatment, the fiber arrangement of the sample was loose and the curling distance was prolonged, which led to that the treated sample had no stress at the same initial length, thus explaining the lengthening of the toe region in the nonlinear region. The morphological results of patellar tendon showed that there was a certain interaction between elastin and collagen fiber. The interaction between them was destroyed, which caused the damage of mechanical properties, and might indicated that elastin played a bearing role in toe region (Chow et al., 2013). It is said that insufficient elastin changes the recruitment of collagen fibers, causing tissue elongation, and the straightness of collagen fibers leads to a delay in the ability of tissues to bear loads (Grant et al., 2015; Fazaeli et al., 2020). After elastin degradation, the patellar tendon is in an extended state without a preload, similar to the phenomenon where the joint relaxes (Eekhoff et al., 2023), and the natural tissue becomes more malleable, severely harming the human body. In this study, the low and high moduli after enzyme treatment were significantly reduced, which was similar to a study on bronchus (Mariano et al., 2023). It emphasizes the contribution of elastin to the low-stress mechanical behavior and tissue elasticity. The decrease in the high tensile modulus may be caused by the failure of collagen fibers to fully dominate the load owing to tissue elongation.

The patellar tendon is a viscoelastic material, and stress exhibits time-dependent behavior, causing a small amount of energy to

TABLE 3 Fitting parameters of second-order Prony series before or after 5 U/mL elastase treatment of the patellar tendon.

Region	Control							Elastase treated						
	G_0 (MPa)	α_1 (MPa)	τ_1 (s)	α_2 (MPa)	τ_2 (s)	R^2	NRMSE	G_0 (MPa)	α_1 (MPa)	τ_1 (s)	α_2 (MPa)	τ_2 (s)	R^2	NRMSE
Proximal	5.28 ± 1.78	0.03 ± 0.01	14.21 ± 1.11	0.06 ± 0.02	195.31 ± 18.18	0.99	0.00	2.85 ± 1.52	0.19 ± 0.09	16.57 ± 4.54	0.28 ± 0.10	146.11 ± 39.61	0.99	0.00
Central	4.57 ± 1.63	0.03 ± 0.01	13.44 ± 1.49	0.06 ± 0.01	181.87 ± 38.91	0.99	0.00	2.61 ± 1.21	0.18 ± 0.08	15.57 ± 2.63	0.24 ± 0.08	135.98 ± 26.03	0.99	0.00
Distal	4.55 ± 1.54	0.03 ± 0.01	12.59 ± 1.71	0.07 ± 0.03	167.86 ± 13.88	0.99	0.00	1.98 ± 0.99	0.24 ± 0.12	16.41 ± 4.41	0.32 ± 0.11	204.17 ± 163.53	0.99	0.00

TABLE 4 Fitting parameters of second-order Prony series before or after PBS treatment of the patellar tendon.

Region	Control							Elastase treated						
	G_0 (MPa)	α_1 (MPa)	τ_1 (s)	α_2 (MPa)	τ_2 (s)	R^2	NRMSE	G_0 (MPa)	α_1 (MPa)	τ_1 (s)	α_2 (MPa)	τ_2 (s)	R^2	NRMSE
Proximal	3.74 ± 1.35	0.04 ± 0.02	13.47 ± 1.53	0.09 ± 0.03	188.08 ± 18.60	0.99	0.00	3.41 ± 1.30	0.06 ± 0.03	14.82 ± 1.93	0.14 ± 0.05	218.25 ± 63.48	0.99	0.00
Central	4.22 ± 2.02	0.03 ± 0.01	12.94 ± 2.19	0.08 ± 0.03	186.47 ± 30.42	0.99	0.00	3.86 ± 2.01	0.05 ± 0.02	15.49 ± 1.98	0.15 ± 0.07	236.35 ± 58.32	0.99	0.00
Distal	3.21 ± 1.89	0.04 ± 0.02	13.88 ± 1.84	0.09 ± 0.03	188.37 ± 28.07	0.99	0.00	2.79 ± 1.79	0.06 ± 0.02	14.94 ± 1.09	0.18 ± 0.09	224.20 ± 72.87	0.99	0.00

10.3389/fbioe.2024.1374352

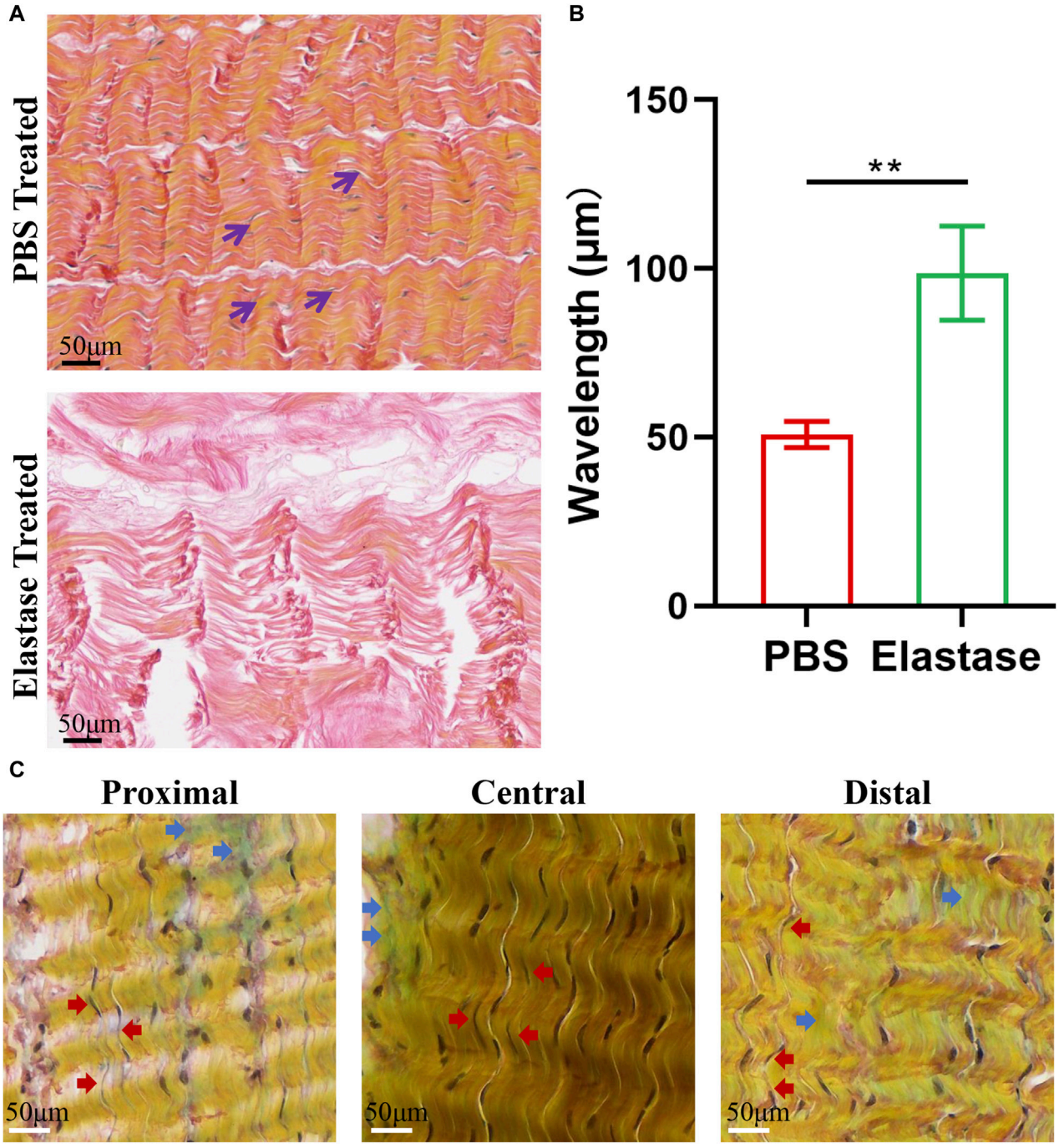


FIGURE 7
Typical histological staining of porcine patellar tendon. PBS is phosphate-buffered saline. **(A)** The structural diagrams of PBS treatment or 5 U/mL elastase treatment in VVG staining. After elastase treatment, the black elastic fibers disappeared, and the red collagen fibers became longer. **(B)** The wavelength of patellar tendon fibers treated with elastase increased. Black arrows denote elastic fibers. **(C)** Movat's staining structure diagram of three regions of patellar tendon. Blue arrows indicated blue-green proteoglycans, and red arrows indicated black elastic fibers. Similar composition structures were observed in three regions, and black elastic fibers could be seen to be arranged along yellow collagen fibers in each region.

dissipate, and the change in viscoelasticity affects the progress of the disease (Chaudhuri et al., 2020). In a stress relaxation experiment of elastin degradation, enzyme treatment significantly affected viscoelastic properties, and the percentage of total stress attenuation decreased significantly, providing experimental information for understanding how patellar tendons are affected by extracellular matrix composition changes and time-dependent behavior (Ross et al., 2021). The increase in the initial slope showed that tissue stress decreased faster after enzyme treatment, and the increase in saturation slope showed that the ability of tissue lacking elastin to reach an equilibrium state was relatively reduced; the increase in transition time representing relaxed shape also showed

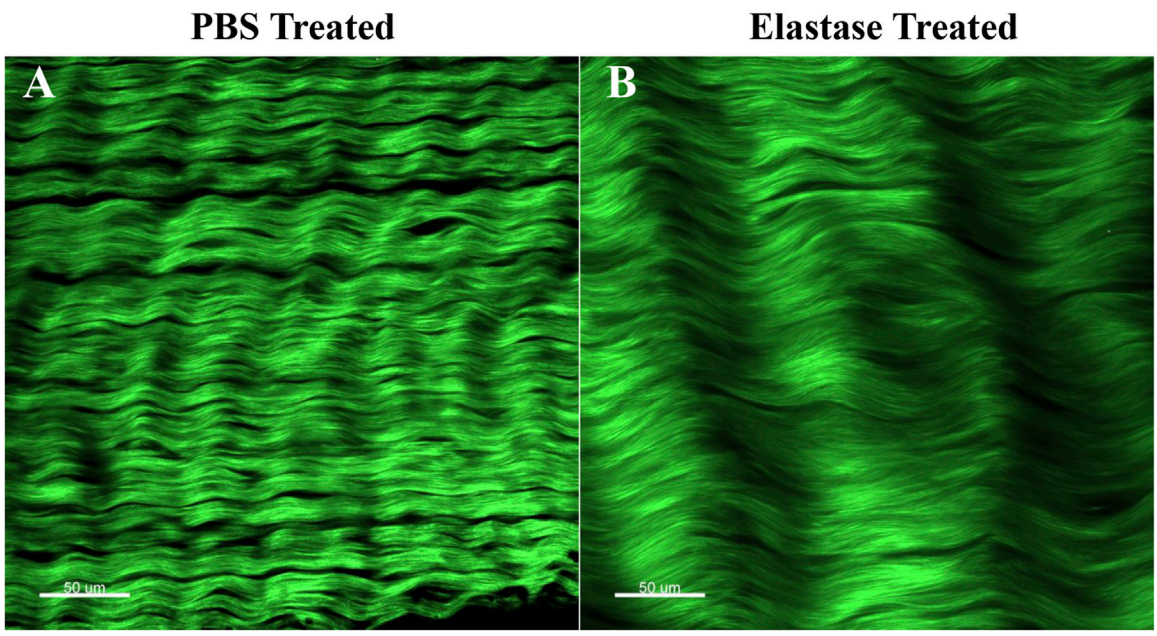


FIGURE 8
Two-photon imaging of patellar tendon. Green represented collagen fibers. PBS is phosphate-buffered saline. **(A)** Representative image after PBS treatment. **(B)** Representative image after elastase treatment.

this point. These results indicate that microstructural elastin affects the viscoelastic properties of the patellar tendon, and changes in its content affect tissue homeostasis (Huang et al., 2019). The exact origin of the viscoelastic behavior of the tendon is unclear; however, stress relaxation results from the interaction between the matrix and fluid, which changes with time in the material (Screen et al., 2013). It is believed that stress relaxation may be caused by the relaxation of collagen fibers (Maritz et al., 2021) and that the degradation of elastin causes an arrangement change in the fiber structure, inducing a change in the stress relaxation phenomenon. This possibility is consistent with the histological results found in this study. Stress relaxation mechanisms can even affect tissue morphology and tumorigenesis (Elosegui-Artola et al., 2023). Viscoelasticity is an essential parameter in the design of tissue engineering and regenerative medicine materials, and its characteristics significantly influence cell behavior (Obuchowicz et al., 2019; Freedman et al., 2022b). Elastin should be added in the future manufacturing of biomaterials to make them more in line with the natural tissue structure and mechanical environment (Schmelzer et al., 2020).

Understanding the biomechanical characteristics of soft tissue is very important for the development of calculation model, which can provide more physical insights (Jan et al., 2022). Soft tissue is a nonlinear, heterogeneous and anisotropic material, but there is no unified constitutive model to describe the properties of the material in previous studies. For example, the compressive Neo-Hookean strain-energy was used to describe the mechanical response of bronchi (Eskandari et al., 2019). Study on the effect of GAGs on the recruitment of aortic collagen fibers based on structural-based constitutive model (Mattson et al., 2019). A new strain energy function based on the geometric arrangement of fibrils and Holzapfel-Gasser-Ogden model were used to compare and analyze the mechanical behavior of ligaments and tendons (Shearer, 2015). At the same time, many studies assume that soft tissue is an incompressible hyperelastic and isotropic material, and use Ogden model, Yeoh model or other models to describe the stress-strain relationship of skin and tendon structure (Cheng and Gan, 2008; Remache et al., 2018). Compared with previous studies, this study adopted a simplified Yeoh model, which was proved to be the most suitable for fitting hyperelastic and transversely isotropic tendons (Liber-Kneć and Łagan, 2020). In the existing research, there are few studies on directly obtaining the mechanical data of tendon elastin before and after degradation and fitting the constitutive model at the same time. The constitutive model of this study was established in an ideal state, and it was fitted by Yeoh model, a hyperelastic constitutive model. R^2 and NRMSE showed that the fitting effect of this model was great and suitable for this study. It deduced the material parameters of the constitutive model of the patellar tendon before and after elastin degradation. Similarly, elastin degradation reportedly decreases material stiffness. However, some models based on structure (distribution of fiber networks) can also be used to predict soft tissue responses (Henninger et al., 2019). Soft tissue is a viscoelastic material. Many studies have also proposed a viscoelastic model to simulate stress relaxation behavior, such as quasi-linear viscoelastic (QLV) model (Duenwald et al., 2010). Since the theory of this model is linear viscosity hypothesis, it cannot fully describe the nonlinear viscoelastic behavior of various biological soft tissues (Shetye et al., 2014). However, Prony series is widely used and proved to be a constitutive equation that can effectively express the viscoelasticity of materials (Grega et al., 2020; Shearer et al., 2020; Li et al., 2021; Morrison et al., 2023). In this study, R^2 and NRMSE obtained by fitting the experimental data of stress relaxation of patellar tendon before and after treatment showed that Prony series had a good fitting effect and was suitable for this study. In the

material parameters of the viscoelastic constitutive model of the three patellar tendon regions before and after elastin degradation, it was found that after enzyme treatment, the patellar tendon degraded by elastase exhibited greater stress attenuation and a longer relaxation time. Compared with the untreated tissue, elastin degradation increased the material parameter α, indicating that the lack of elastin causes the patellar tendon to exhibit elastic behavior (Ross et al., 2021). The mechanical constitutive models established in this study are helpful to better understand the incidence and prevention of patellar tendon diseases, because they can simulate the biomechanical behavior of tissues and their components from the phenomenon (Khayyeri et al., 2016).

It showed that the stress of porcine ligament was reduced by shear test and transverse tension after elastase treatment (Henninger et al., 2015). The degradation of elastin leads to a significant decrease in the viscoelasticity of the interfascicular matrix of horse energy storage tendons, but it does not affect the fascicle mechanics (Godinho et al., 2021). However, the compression test of tendon after enzyme treatment was not found. It may be because tendons are more influenced by uniaxial direction (that is, fiber direction) *in vivo*, and axial tension is a conventional mechanical test method (Ristaniemi et al., 2021; Lake et al., 2023). Through uniaxial stretching, it is concluded that elastin has made a more significant contribution to the patellar tendon as an energy storage tendon. There may be some differences in the mechanical properties of different species and different types of tendons, which may be used to explain different research results. Previous studies have also used elastin knockout mice to reduce elastin content, and the results of this method are inconsistent with those of this study (Eekhoff et al., 2017). However, using elastase to break down elastin within the tissue is completely different from the elastin knockout mice. This inconsistency can be attributed to the differences in their development conditions. Elastin in transgenic mice does not develop normally, and there is a compensation mechanism, which may lead to the result that it is not entirely due to the decrease of elastin (Eekhoff et al., 2017; Eekhoff et al., 2021). Because of the lack of elastin, the structural relationship and interaction between elastin and collagen fiber cannot be determined. However, the patellar tendon of pigs treated with elastase developed normally, and the effect of selectively degrading elastin on the structure and function of normal tendon can be studied. This is more suitable for guiding the repair and reconstruction of patellar tendon injury and designing biomaterials.

During the evolution of tendon lesions, collagen fibers were found to relax and curl unevenly (Wu et al., 2020; Mohindra et al., 2022), which was consistent with the morphological changes in collagen fibers caused by elastin degradation in this study. It provided evidence for tendon lesions caused by elastin degradation. Without the protection of elastin, collagen fibers were easily damaged and needed to be repaired for a long time, and long-term accumulation would lead to tissue damage or fracture (Naya and Takanari, 2023). In tendon tissue engineering, replacement and regeneration are challenging because the scaffold must ensure sufficient hierarchical structure and mechanical properties to bear the load. To guide cell proliferation and growth, the scaffold should provide a fiber network imitating the microstructural arrangement of collagen and elastic fibers in the extracellular matrix of the tendon (Bianchi et al., 2021). In addition, understanding the relationship between these microstructures and mechanics is helpful for tendon reconstruction so that the reconstructed graft can better fit the microstructure of the natural tendon structure (Smith et al., 2019). This study found that elastin plays an important role in the elasticity and viscoelasticity of patellar tendon, which was not discussed in depth in the previous biomechanical research of patellar tendon, and it also led to the lack of biomaterial properties at present. At present, there are collagen-based biomaterials (Yuan et al., 2021; Maeda, et al., 2022) and novel knitted scaffold made of microfiber/nanofiber core–sheath yarns (Cai, et al., 2020) used in tendon tissue engineering. However, these biomaterials cannot completely copy the structural and mechanical properties of the original tendon. Therefore, in order to design biomaterials that are more in line with natural tissues (such as artificial patellar tendon), elastin should be added. That is to say, when making different proportions of collagen-based biomaterials, different proportions of elastin should be added to make the mechanical properties of the materials better simulate the tissue, and these materials should have the spatial relationship between elastin and collagen fibers. The scaffold can be coated with elastin by electrospinning and bioprinting, and the hydrogel structure of elastin can be made.

This study has some limitations. First, the sample size is small in the study, although this number has been recognized by some studies (Fang and Lake, 2016; Fazaeli et al., 2020), it will also have some limitations on the results. Second, patellar tendon is an anisotropic material. The constitutive model established in this study assumed that it was transversely isotropic and divided the experimental data of tension and stress relaxation into two parts for fitting (Khayyeri et al., 2016; Remache et al., 2018). The transversely isotropic model cannot fully represent the real properties of materials. Future research should be devoted to establishing a constitutive model that conforms to the nonlinearity, anisotropy, and viscoelasticity of the patellar tendon and includes microstructures such as elastin and collagen fiber to obtain more real material parameters. Third, the samples of this study were from animals, and human samples should be selected to further verify the conclusions of this study so that the research results can be used in clinical practice. Fourth, only uniaxial tensile test was carried out in this study, but the mechanical environment of patellar tendon in the body is complex. Exploring the directional mechanical effect of elastase therapy can be an avenue for future studies. Finally, only the role of elastin was considered, we should consider the effect of collagen fiber and its degradation in the future. To further improve the patellar tendon model, it should be combined with imaging technology and materials science to further observe the connection components and their functions between elastin and collagen fibers in patellar tendon (Durgam et al., 2020; Sallehuddin et al., 2022).

In summary, this study provides an insight into the previously uninvestigated effects of elastin on the mechanical properties and fiber structure of different regions of patellar tendon. Our results showed that there was no evident regional mechanical difference in the patellar tendon under load. The specific degradation of patellar tendon elastin changed the structural arrangement of collagen fibers and affected the elastic mechanical behavior and viscoelastic properties of the tissues. Through imaging technology and biomechanical experiments, the microstructure-function relationship of patellar tendon was proved. The results benefit

the model speculation of elastin fibers in normal, pathological, and injured connective tissues and guide the development of patellar tendon materials in tissue engineering and obtaining ideal properties.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by the Ethics Committee of Shenzhen Bay Laboratory. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

XL: Writing–review and editing, Writing–original draft. YD: Methodology, Data curation, Writing–review and editing. ZL: Methodology, Writing–review and editing. DQ: Writing–review and editing. WZ: Writing–review and editing. MW: Methodology, Data curation, Writing–review and editing. FL: Writing–review and editing. JL: Writing–review and editing. YW: Investigation, Writing–review and editing. GC: Writing–review and editing. YL: Writing–review and editing. WT: Methodology, Writing–review and editing. JX: Writing–review and editing. WH: Funding acquisition, Writing–review and editing. DZ: Writing–review and editing, Methodology, Funding acquisition. YL: Funding acquisition, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Science and Technology Planning Project of Guangdong Province (2018B090944002). National Key R&D Program of China (2022YFF1202600). China Postdoctoral Science Foundation (2022M711533). Medical Science and Technology Research Fund of Guangdong Province (A2023129). Postdoctoral Program of International Training Program for Young Talents of Guangdong Province. National Key R&D Program of China (2022YFB4600600). National Natural Science Foundation of China (32271181). National Natural Science Foundation of China (12202017). Guangdong Basic and Applied Basic Research Foundation (2020B1515120001). Shenzhen Science and Technology Program (JCYJ20210324130401005). Shenzhen Medical Research Fund (SMRF A2303037). Starting Grants of Shenzhen Bay Laboratory (QH30003).

Acknowledgments

We would appreciate the participation of each participant.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2024.1374352/full#supplementary-material

References

Bajuri, M. N., Isaksson, H., Eliasson, P., and Thompson, M. S. (2016). A hyperelastic fibre-reinforced continuum model of healing tendons with distributed collagen fibre orientations. *Biomech. Model. Mechanobiol.* 15, 1457–1466. doi:10.1007/s10237-016-0774-5

Beach, Z. M., Bonilla, K. A., Dekhne, M. S., Sun, M., Adams, T. H., Adams, S. M., et al. (2022). Biglycan has a major role in maintenance of mature tendon mechanics. *J. Orthop. Res.* 40, 2546–2556. doi:10.1002/jor.25299

Bianchi, E., Ruggeri, M., Rossi, S., Vigani, B., Miele, D., Bonferoni, M. C., et al. (2021). Innovative strategies in tendon tissue engineering. *Pharmaceutics* 13, 89. doi:10.3390/pharmaceutics13010089

Böl, M., Ehret, A. E., Leichsenring, K., and Ernst, M. (2015). Tissue-scale anisotropy and compressibility of tendon in semi-confined compression tests. *J. Biomech.* 48, 1092–1098. doi:10.1016/j.jbiomech.2015.01.024

Bose, S., Li, S., Mele, E., and Silberschmidt, V. V. (2020). Dry vs. wet: properties and performance of collagen films. Part II. Cyclic and time-dependent behaviours. *J. Mech. Behav. Biomed. Mater.* 112, 104040. doi:10.1016/j.jmbbm.2020.104040

Buján, J., Pascual, G., García-Honduvilla, N., Gimeno, M. J., Jurado, F., Carrera-San, M. A., et al. (2000). Rapid thawing increases the fragility of the cryopreserved arterial wall. *Eur. J. Vasc. Endovasc. Surg.* 20, 13–20. doi:10.1053/ejvs.2000.1090

Burgio, V., Casari, S., Milizia, M., Sanna, F., Spezia, G., Civera, M., et al. (2023). Mechanical properties of animal ligaments: a review and comparative study for the identification of the most suitable human ligament surrogates. *Biomech. Model. Mechanobiol.* 22, 1645–1683. doi:10.1007/s10237-023-01718-1

Burton, I. (2022). Interventions for prevention and in-season management of patellar tendinopathy in athletes: a scoping review. *Phys. Ther. Sport* 55, 80–89. doi:10.1016/j.ptsp.2022.03.002

Cai, J., Xie, X., Li, D., Wang, L., Jiang, J., Mo, X., et al. (2020). A novel knitted scaffold made of microfiber/nanofiber core-sheath yarns for tendon tissue engineering. *Biomater. Sci.* 8, 4413–4425. doi:10.1039/d0bm00816h

Castile, R. M., Skelley, N. W., Babaei, B., Brophy, R. H., and Lake, S. P. (2016). Microstructural properties and mechanics vary between bundles of the human anterior

cruciate ligament during stress-relaxation. *J. Biomech.* 49, 87–93. doi:10.1016/j.jbiomech.2015.11.016

Chaudhuri, O., Cooper-White, J., Janmey, P. A., Mooney, D. J., and Shenoy, V. B. (2020). Effects of extracellular matrix viscoelasticity on cellular behaviour. *Nature* 584, 535–546. doi:10.1038/s41586-020-2612-2

Cheng, T., and Gan, R. Z. (2008). Experimental measurement and modeling analysis on mechanical properties of tensor tympani tendon. *Med. Eng. Phys.* 30, 358–366. doi:10.1016/j.medengphy.2007.04.005

Chow, M. J., Mondonedo, J. R., Johnson, V. M., and Zhang, Y. (2013). Progressive structural and biomechanical changes in elastin degraded aorta. *Biomech. Model. Mechanobiol.* 12, 361–372. doi:10.1007/s10237-012-0404-9

Darrieutort-Laffite, C., Beach, Z. M., Weiss, S. N., Eekhoff, J. D., and Soslowsky, L. J. (2023). Knockdown of biglycan reveals an important role in maintenance of structural and mechanical properties during tendon aging. *J. Orthop. Res.* 41, 2287–2294. doi:10.1002/jor.25536

De Moudt, S., Leloup, A., and Fransen, P. (2021). Aortic stiffness hysteresis in isolated mouse aortic segments is intensified by contractile stimuli, attenuated by age, and reversed by elastin degradation. *Front. Physiol.* 12, 723972. doi:10.3389/fphys.2021.723972

Duenwald, S. E., Vanderby, R., Jr., and Lakes, R. S. (2010). Stress relaxation and recovery in tendon and ligament: experiment and modeling. *Biorheology* 47, 1–14. doi:10.3233/BIR-2010-0559

Duginski, G. A., Ross, C. J., Laurence, D. W., Johns, C. H., and Lee, C. H. (2020). An investigation of the effect of freezing storage on the biaxial mechanical properties of excised porcine tricuspid valve anterior leaflets. *J. Mech. Behav. Biomed. Mater.* 101, 103438. doi:10.1016/j.jmbbm.2019.103438

Durgam, S., Singh, B., Cole, S. L., Brokken, M. T., and Stewart, M. (2020). Quantitative assessment of tendon hierarchical structure by combined second harmonic generation and immunofluorescence microscopy, *Tissue Eng. Part C Methods.* 26, 253–262. doi:10.1089/ten.TEC.2020.0032

Eekhoff, J. D., Abraham, J. A., Schott, H. R., Solon, L. F., Ulloa, G. E., Zellers, J. A., et al. (2023). Fascicular elastin within tendon contributes to the magnitude and modulus gradient of the elastic stress response across tendon type and species. *Acta. Biomater.* 163, 91–105. doi:10.1016/j.actbio.2022.03.025

Eekhoff, J. D., Fang, F., Kahan, L. G., Espinosa, G., Cocciolone, A. J., Wagenseil, J. E., et al. (2017). Functionally distinct tendons from elastin haploinsufficient mice exhibit mild stiffening and tendon-specific structural alteration. *J. Biomech. Eng.* 139, 1110031–1110039. doi:10.1115/1.4037932

Eekhoff, J. D., Steenbock, H., Berke, I. M., Brinckmann, J., Yanagisawa, H., Wagenseil, J. E., et al. (2020). Dysregulated assembly of elastic fibers in fibulin-5 knockout mice results in a tendon-specific increase in elastic modulus. *J. Mech. Behav. Biomed. Mater.* 113, 104134. doi:10.1016/j.jmbbm.2020.104134

Ekiert, M., Tomaszewski, K. A., and Mlyniec, A. (2021). The differences in viscoelastic properties of subtendons result from the anatomical tripartite structure of human Achilles tendon - *ex vivo* experimental study and modeling. *Acta. Biomater.* 125, 138–153. doi:10.1016/j.actbio.2021.02.041

Elosegui-Artola, A., Gupta, A., Najibi, A. J., Seo, B. R., Garry, R., Tringides, C. M., et al. (2023). Matrix viscoelasticity controls spatiotemporal tissue organization. *Nat. Mater* 22, 117–127. doi:10.1038/s41563-022-01400-4

Eskandari, M., Arvayo, A. L., and Levenston, M. E. (2018). Mechanical properties of the airway tree: heterogeneous and anisotropic pseudoelastic and viscoelastic tissue responses. *J. Appl. Physiol. (1985)* 125, 878–888. doi:10.1152/japplphysiol.00090.2018

Eskandari, M., Nordgren, T. M., and O'Connell, G. D. (2019). Mechanics of pulmonary airways: linking structure to function through constitutive modeling, biochemistry, and histology. *Acta. Biomater.* 97, 513–523. doi:10.1016/j.actbio.2019.07.020

Fang, F., and Lake, S. P. (2016). Multiscale mechanical integrity of human supraspinatus tendon in shear after elastin depletion. *J. Mech. Behav. Biomed. Mater.* 63, 443–455. doi:10.1016/j.jmbbm.2016.06.032

Fazaeli, S., Mirahmadi, F., Everts, V., Smit, T. H., Koolstra, J. H., and Ghazanfari, S. (2020). Alteration of structural and mechanical properties of the temporomandibular joint disc following elastase digestion. *J. Biomed. Mater. Res. B. Appl. Biomater.* 108, 3228–3240. doi:10.1002/jbm.b.34660

Freedman, B. R., Knecht, R. S., Tinguely, Y., Eskibozkurt, G. E., Wang, C. S., and Mooney, D. J. (2022a). Aging and matrix viscoelasticity affect multiscale tendon properties and tendon derived cell behavior. *Acta. Biomater.* 143, 63–71. doi:10.1016/j.actbio.2022.03.006

Freedman, B. R., Kuttler, A., Beckmann, N., Nam, S., Kent, D., Schuleit, M., et al. (2022b). Enhanced tendon healing by a tough hydrogel with an adhesive side and high drug-loading capacity. *Nat. Biomed. Eng.* 6, 1167–1179. doi:10.1038/s41551-021-00810-0

Godinho, M. S., Thorpe, C. T., Greenwald, S. E., and Screen, H. R. C. (2021). Elastase treatment of tendon specifically impacts the mechanical properties of the interfascicular matrix. *Acta. Biomater.* 123, 187–196. doi:10.1016/j.actbio.2021.01.030

Godinho, M. S. C., Thorpe, C. T., Greenwald, S. E., and Screen, H. R. C. (2017). Elastin is localised to the interfascicular matrix of energy storing tendons and becomes increasingly disorganised with ageing. *Sci. Rep.* 7, 9713. doi:10.1038/s41598-017-09995-4

Grant, T. M., Yapp, C., Chen, Q., Czernuszka, J. T., and Thompson, M. S. (2015). The mechanical, structural, and compositional changes of tendon exposed to elastase. *Ann. Biomed. Eng.* 43, 2477–2486. doi:10.1007/s10439-015-1308-5

Grega, K. L., Segall, R. N., Vaidya, A. J., Fu, C., and Wheatley, B. B. (2020). Anisotropic and viscoelastic tensile mechanical properties of aponeurosis: experimentation, modeling, and tissue microstructure. *J. Mech. Behav. Biomed. Mater.* 110, 103889. doi:10.1016/j.jmbbm.2020.103889

Henninger, H. B., Ellis, B. J., Scott, S. A., and Weiss, J. A. (2019). Contributions of elastic fibers, collagen, and extracellular matrix to the multiaxial mechanics of ligament. *J. Mech. Behav. Biomed. Mater.* 99, 118–126. doi:10.1016/j.jmbbm.2019.07.018

Henninger, H. B., Valdez, W. R., Scott, S. A., and Weiss, J. A. (2015). Elastin governs the mechanical response of medial collateral ligament under shear and transverse tensile loading. *Acta. Biomater.* 25, 304–312. doi:10.1016/j.actbio.2015.07.011

Herbert, A., Brown, C., Rooney, P., Kearney, J., Ingham, E., and Fisher, J. (2016). Bi-linear mechanical property determination of acellular human patellar tendon grafts for use in anterior cruciate ligament replacement. *J. Biomech.* 49, 1607–1612. doi:10.1016/j.jbiomech.2016.03.041

Huang, D., Huang, Y., Xiao, Y., Yang, X., Lin, H., Feng, G., et al. (2019). Viscoelasticity in natural tissues and engineered scaffolds for tissue reconstruction. *Acta Biomater.* 97, 74–92. doi:10.1016/j.actbio.2019.08.013

Jan, Y. K., Major, M. J., Pu, F., and Sonenblum, S. E. (2022). Editorial: soft tissue biomechanics in wound healing and prevention. *Front. Bioeng. Biotechnol.* 10, 897860. doi:10.3389/fbioe.2022.897860

Khayyeri, H., Longo, G., Gustafsson, A., and Isaksson, H. (2016). Comparison of structural anisotropic soft tissue models for simulating Achilles tendon tensile behaviour. *J. Mech. Behav. Biomed. Mater.* 61, 431–443. doi:10.1016/j.jmbbm.2016.04.007

Krarup, N. T., Hvidbjerg, M., Zaremba, T., Sommerlund, M., and Christensen, M. K. (2023). Autosomal dominant cutis laxa and critical stenosis of the left main coronary artery in a 21-year-old female with an intronic mutation in the elastin gene. *Am. J. Med. Genet. A* 191, 1059–1064. doi:10.1002/ajmg.a.63095

Lake, S. P., Snedeker, J. G., Wang, V. M., Awad, H., Screen, H. R. C., and Thomopoulos, S. (2023). Guidelines for *ex vivo* mechanical testing of tendon. *J. Orthop. Res.* 41, 2105–2113. doi:10.1002/jor.25647

Lee, A. H., and Elliott, D. M. (2017). Freezing does not alter multiscale tendon mechanics and damage mechanisms in tension. *Ann. N. Y. Acad. Sci.* 1409, 85–94. doi:10.1111/nyas.13460

Li, R. L., Russ, J., Paschalides, C., Ferrari, G., Waisman, H., Kysar, J. W., et al. (2019). Mechanical considerations for polymeric heart valve development: biomechanics, materials, design and manufacturing. *Biomaterials* 225, 119493. doi:10.1016/j.biomaterials.2019.119493

Li, W., Shepherd, D. E. T., and Espino, D. M. (2021). Investigation of the compressive viscoelastic properties of brain tissue under time and frequency dependent loading conditions. *Ann. Biomed. Eng.* 49, 3737–3747. doi:10.1007/s10439-021-02866-0

Liber-Kneć, A., and Łagan, S. (2020). "Experimental and constitutive approaches for a study of mechanical properties of animal tendons," in *Current trends in biomedical engineering and bioimages analysis. Advances in intelligent systems and computing.* Editors J. Korbicz, R. Maniewski, K. Patan, and M. Kowal (Cham: Springer), 1033. PCBEE 2019. doi:10.1007/978-3-030-29885-2_26

Liu, X., Deng, Y., Li, F., Zhao, D., Yang, Y., Huang, T., et al. (2023). Effect of elastin degradation of patellar tendon on the quasi-static tensile mechanical properties. *Chin. J. Tissue Eng. Res.* 27, 2831–2836. doi:10.12307/2023.317

Maeda, E., Kawamura, R., Suzuki, T., and Matsumoto, T. (2022). Rapid fabrication of tendon-like collagen gel via simultaneous fibre alignment and intermolecular cross-linking under mechanical loading. *Biomed. Mater* 17, 045018. doi:10.1088/1748-605X/ac7305

Maeda, E., Kuroyanagi, K., and Matsumoto, T. (2021). Microscopic characterisation of local strain field in healing tissue in the central third defect of mouse patellar tendon at early-phase of healing. *J. Mech. Behav. Biomed. Mater.* 123, 104702. doi:10.1016/j.jmbbm.2021.104702

Mariano, C. A., Sattari, S., Ramirez, G. O., and Eskandari, M. (2023). Effects of tissue degradation by collagenase and elastase on the biaxial mechanics of porcine airways. *Respir. Res.* 24, 105. doi:10.1186/s12931-023-02376-8

Maritz, J., Agustoni, G., Dragnevski, K., Bordas, S. P. A., and Barrera, O. (2021). The functionally grading elastic and viscoelastic properties of the body region of the knee meniscus. *Ann. Biomed. Eng.* 49, 2421–2429. doi:10.1007/s10439-021-02792-1

Mattson, J. M., Wang, Y., and Zhang, Y. (2019). Contributions of glycosaminoglycans to collagen fiber recruitment in constitutive modeling of arterial mechanics. *J. Biomech.* 82, 211–219. doi:10.1016/j.jbiomech.2018.10.031

Mihai, L. A., and Goriely, A. (2017). How to characterize a nonlinear elastic material? A review on nonlinear constitutive parameters in isotropic finite elasticity. *Proc. Math. Phys. Eng. Sci.* 473, 20170607. doi:10.1098/rspa.2017.0607

Mohamed, A. A., Jan, Y. K., Rice, I. M., Pu, F., and Cheng, C. K. (2020). "Biomechanics of orthopedic rehabilitation," in *Frontiers in orthopaedic biomechanics*. Editors C. K. Cheng and S. L. Y. Woo (Singapore: Springer). doi:10.1007/978-981-15-3159-0_14

Mohindra, R., Mohindra, R., Agrawal, D. K., and Thankam, F. G. (2022). Bioactive extracellular matrix fragments in tendon repair. *Cell. Tissue. Res.* 390, 131–140. doi:10.1007/s00441-022-03684-z

Morrison, O., Destrade, M., and Tripathi, B. B. (2023). An atlas of the heterogeneous viscoelastic brain with local power-law attenuation synthesized using Prony-series. *Acta. Biomater.* 27 (23), S1742–S7061. doi:10.1016/j.actbio.2023.07.040

Naya, Y., and Takanari, H. (2023). Elastin is responsible for the rigidity of the ligament under shear and rotational stress: a mathematical simulation study. *J. Orthop. Surg. Res.* 18, 310. doi:10.1186/s13018-023-03794-6

Ngwangwa, H., Pandelani, T., Msibi, M., Mabuda, I., Semakane, L., and Nemavhola, F. (2022). Biomechanical analysis of sheep oesophagus subjected to biaxial testing including hyperelastic constitutive model fitting. *Heliyon* 8, e09312. doi:10.1016/j.heliyon.2022.e09312

Ngwangwa, H. M., and Nemavhola, F. (2021). Evaluating computational performances of hyperelastic models on supraspinatus tendon uniaxial tensile test data. *J. Comput. Appl. Math.* 52, 27–43. doi:10.22059/jcamech.2020.310491.559

Obuchowicz, R., Ekiert, M., Kohut, P., Holak, K., Ambrozinski, L., Tomaszewski, K. A., et al. (2019). Interfascicular matrix-mediated transverse deformation and sliding of discontinuous tendon subcomponents control the viscoelasticity and failure of tendons. *J. Mech. Behav. Biomed. Mater.* 97, 238–246. doi:10.1016/j.jmbbm.2019.05.027

Pan, G., Chen, M., Wang, Y., Zhang, J., Liu, L., Zhang, L., et al. (2022). Hyper-pseudo-viscoelastic model and parameter identification for describing tensile recovery stress-strain responses of rubber components in TBR. *Polym. (Basel)* 15, 76. doi:10.3390/polym15010076

Park, S., Chien, A. L., Brown, I. D., and Chen, J. (2023). Characterizing viscoelastic properties of human melanoma tissue using Prony series. *Front. Bioeng. Biotechnol.* 11, 1162880. doi:10.3389/fbioe.2023.1162880

Pearson, S. J., and Hussain, S. R. (2014). Region-specific tendon properties and patellar tendinopathy: a wider understanding. *Sports. Med.* 44, 1101–1112. doi:10.1007/s40279-014-0201-y

Pineda-Castillo, S. A., Aparicio-Ruiz, S., Burns, M. M., Laurence, D. W., Bradshaw, E., Gu, T., et al. (2022). Linking the region-specific tissue microstructure to the biaxial mechanical properties of the porcine left anterior descending artery. *Acta Biomater.* 150, 295–309. doi:10.1016/j.actbio.2022.07.036

Reinking, M. F. (2016). Current concepts in the treatment of patellar tendinopathy. *Int. J. Sports Phys. Ther.* 11, 854–866.

Remache, D., Caliez, M., Gratton, M., and Dos Santos, S. S. (2018). The effects of cyclic tensile and stress-relaxation tests on porcine skin. *J. Mech. Behav. Biomed. Mater.* 77, 242–249. doi:10.1016/j.jmbbm.2017.09.009

Rigozzi, S., Müller, R., and Snedeker, J. G. (2009). Local strain measurement reveals a varied regional dependence of tensile tendon mechanics on glycosaminoglycan content. *J. Biomech.* 42, 1547–1552. doi:10.1016/j.jbiomech.2009.03.031

Ristaniemi, A., Torniainen, J., Paakkonen, T., Stenroth, L., Finnilä, M. A. J., Tanska, P., et al. (2021). Biomechanical, biochemical, and near infrared spectral data of bovine knee ligaments and patellar tendon. *Data. Brief.* 36, 106976. doi:10.1016/j.dib.2021.106976

Ristaniemi, A., Torniainen, J., Stenroth, L., Finnilä, M. A. J., Paakkonen, T., Töyräs, J., et al. (2020). Comparison of water, hydroxyproline, uronic acid and elastin contents of bovine knee ligaments and patellar tendon and their relationships with biomechanical properties. *J. Mech. Behav. Biomed. Mater.* 104, 103639. doi:10.1016/j.jmbbm.2020.103639

Ross, C. J., Laurence, D. W., Echols, A. L., Babu, A. R., Gu, T., Duginski, G. A., et al. (2021). Effects of enzyme-based removal of collagen and elastin constituents on the biaxial mechanical responses of porcine atrioventricular heart valve anterior leaflets. *Acta. Biomater.* 135, 425–440. doi:10.1016/j.actbio.2021.08.043

Russo, V., El Khatib, M., Prencipe, G., Mauro, A., Di Giacinto, O., Haidar-Montes, A. A., et al. (2022). Tendon 3D scaffolds establish a tailored microenvironment instructing paracrine mediated regenerative amniotic epithelial stem cells potential. *Biomedicines* 10, 2578. doi:10.3390/biomedicines10102578

Salinas, S. D., Farra, Y. M., Amini Khoiy, K. K., Houston, J., Lee, C. H., Bellini, C., et al. (2022). The role of elastin on the mechanical properties of the anterior leaflet in porcine tricuspid valves. *PLOS ONE* 17, e0267131. doi:10.1371/journal.pone.0267131

Sallehuddin, N., Md Fadilah, N. I., Hwei, N. M., Wen, A. P. Y., Yusop, S. M., Rajab, N. F., et al. (2022). Characterization and cytocompatibility of collagen-gelatin-elastin (CollaGee) acellular skin substitute towards human dermal fibroblasts: *in vitro* assessment. *Biomedicines* 10, 1327. doi:10.3390/biomedicines10061327

Schmelzer, C. E. H., Hedtke, T., and Heinz, A. (2020). Unique molecular networks: formation and role of elastin cross-links. *IUBMB Life* 72, 842–854. doi:10.1002/iub.2213

Screen, H. R., Toorani, S., and Shelton, J. C. (2013). Microstructural stress relaxation mechanics in functionally different tendons. *Med. Eng. Phys.* 35, 96–102. doi:10.1016/j.medengphy.2012.04.004

Shearer, T. (2015). A new strain energy function for the hyperelastic modelling of ligaments and tendons based on fascicle microstructure. *J. Biomech.* 48, 290–297. doi:10.1016/j.jbiomech.2014.11.031

Shearer, T., Parnell, W. J., Lynch, B., Screen, H. R. C., and David Abrahams, I. (2020). A recruitment model of tendon viscoelasticity that incorporates fibril creep and explains strain-dependent relaxation. *J. Biomech. Eng.* 142, 071003. doi:10.1115/1.4045662

Shetye, S. S., Troyer, K. L., Streijger, F., Lee, J. H., Kwon, B. K., Cripton, P. A., et al. (2014). Nonlinear viscoelastic characterization of the porcine spinal cord. *Acta Biomater.* 10, 792–797. doi:10.1016/j.actbio.2013.10.038

Smith, M. V., Castile, R. M., Brophy, R. H., Dewan, A., Bernholt, D., and Lake, S. P. (2019). Mechanical properties and microstructural collagen alignment of the ulnar collateral ligament during dynamic loading. *Am. J. Sports Med.* 47, 151–157. doi:10.1177/0363546518812416

Solis-Cordova, J., Edwards, J. H., Fermor, H. L., Riches, P., Brockett, C. L., and Herbert, A. (2023). Characterisation of native and decellularised porcine tendon under tension and compression: a closer look at glycosaminoglycan contribution to tendon mechanics. *J. Mech. Behav. Biomed. Mater.* 139, 105671. doi:10.1016/j.jmbbm.2023.105671

Song, Y., Wu, D., Shen, M., Wang, L., Wang, C., Cai, Y., et al. (2022). Measuring human corneal stromal biomechanical properties using tensile testing combined with optical coherence tomography. *Front. Bioeng. Biotechnol.* 10, 882392. doi:10.3389/fbioe.2022.882392

Starcher, B. (2001). A Ninhydrin-based assay to quantitate the total protein content of tissue samples. *Anal. Biochem.* 292, 125–129. doi:10.1006/abio.2001.5050

Stoilov, I., Starcher, B. C., Mecham, R. P., and Broekelmann, T. J. (2018). Measurement of elastin, collagen, and total protein levels in tissues. *Methods. Cell. Biol.* 143, 133–146. doi:10.1016/bs.mcb.2017.08.008

Thorpe, C. T., Karunaseelan, K. J., Ng Chieng, H. J., Riley, G. P., Birch, H. L., Clegg, P. D., et al. (2016). Distribution of proteins within different compartments of tendon varies according to tendon type. *J. Anat.* 229, 450–458. doi:10.1111/joa.12485

Ugradar, S., Karlin, J., Le, A., Park, J., and Goldberg, R. A. (2020). Photochemical collagen cross-linking reverses elastase-induced mechanical degradation of upper eyelid tarsus. *Ophthal. Plast. Reconstr. Surg.* 36, 562–565. doi:10.1097/IOP.0000000000001635

Urbanczyk, M., Layland, S. L., and Schenke-Layland, K. (2020). The role of extracellular matrix in biomechanics and its impact on bioengineering of cells and 3D tissues. *Matrix Biol.* 85–86, 1–14. doi:10.1016/j.matbio.2019.11.005

Vafek, E. C., Plate, J. F., Friedman, E., Mannava, S., Scott, A. T., and Danelson, K. A. (2018). The effect of strain and age on the mechanical properties of rat Achilles tendons. *Muscles Ligaments Tendons J.* 7, 548–553. doi:10.11138/mltj/2017.7.3.548

Wang, Z., Lee, W. J., Koh, B. T. H., Hong, M., Wang, W., Lim, P. N., et al. (2018). Functional regeneration of tendons using scaffolds with physical anisotropy engineered via microarchitectural manipulation. *Sci. Adv.* 4, eaat4537. doi:10.1126/sciadv.Aat4537

Wu, Y. T., Wu, Y. T., Huang, T. C., Su, F. C., Jou, I. M., and Wu, C. C. (2020). Sequential inflammation model for Achilles tendinopathy by elastin degradation with treadmill exercise. *J. Orthop. Transl.* 23, 113–121. doi:10.1016/j.jot.2020.03.004

Yamamoto, E., Hayashi, K., and Yamamoto, N. (1999). Mechanical properties of collagen fascicles from stress-shielded patellar tendons in the rabbit. *Clin. Biomech.* 14, 418–425. doi:10.1016/s0268-0033(99)00006-6

Yuan, H., Li, X., Lee, M. S., Zhang, Z., Li, B., Xuan, H., et al. (2021). Collagen and chondroitin sulfate functionalized bioinspired fibers for tendon tissue engineering application. *Int. J. Biol. Macromol.* 170, 248–260. doi:10.1016/j.ijbiomac.2020.12.152

Zhang, R. M., Tiedemann, K., Muthu, M. L., Dinesh, N. E. H., Komarova, S., Ramkhelawon, B., et al. (2022). Fibrillin-1-regulated miR-122 has a critical role in thoracic aortic aneurysm formation. *Cell. Mol. Life Sci.* 79, 314. doi:10.1007/s00018-022-04337-8

Zhao, D., Niu, P., Sun, X., Yin, Z., Tan, W., and Huo, Y. (2020). Mechanical difference of left ventricle between rabbits of myocardial infarction and hypertrophy. *J. Biomech.* 111, 110021. doi:10.1016/j.jbiomech.2020.110021

Zitnay, J. L., and Weiss, J. A. (2018). Load transfer, damage, and failure in ligaments and tendons. *J. Orthop. Res.* 36, 3093–3104. doi:10.1002/jor.24134

Zumbrunn, T., Patel, R., Duffy, M. P., Rubash, H. E., Malchau, H., Freiberg, A. A., et al. (2018). Cadaver-specific models for finite-element analysis of iliopsoas impingement in dual-mobility hip implants. *J. Arthroplasty.* 33, 3574–3580. doi:10.1016/j.arth.2018.06.029

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Review
PUBLISHED 09 May 2024
DOI 10.3389/fbioe.2024.1379773

OPEN ACCESS

EDITED BY
Sarah Harriet Cartmell,
The University of Manchester, United Kingdom

REVIEWED BY
Mohammad El Khatib,
University of Teramo, Italy
Vasyl Pastukh,
University of Pittsburgh, United States

*CORRESPONDENCE
Carlos Julio Peniche Silva,
c.penichesilva@maastrichtuniversity.nl

RECEIVED 31 January 2024
ACCEPTED 15 April 2024
PUBLISHED 09 May 2024

CITATION
Peniche Silva CJ, Balmayor ER and van Griensven M (2024), Reprogramming tendon healing: a guide to novel molecular tools.
Front. Bioeng. Biotechnol. 12:1379773.
doi: 10.3389/fbioe.2024.1379773

Reprogramming tendon healing: a guide to novel molecular tools

Carlos Julio Peniche Silva[1]*, Elizabeth R. Balmayor[2] and Martijn van Griensven[1]

[1]Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands, [2]Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany

Tendons are a frequent site of injury, which greatly impairs the movement and locomotion of patients. Regrettably, injuries at the tendon frequently require surgical intervention, which leads to a long path to recovery. Moreover, the healing of tendons often involves the formation of scar tissue at the site of injury with poor mechanical properties and prone to re-injury. Tissue engineering carries the promise of better and more effective solutions to the improper healing of tendons. Lately, the field of regenerative medicine has seen a significant increase in the focus on the potential use of non-coding RNAs (e.g., siRNAs, miRNAs, and lncRNAs) as molecular tools for tendon tissue engineering. This class of molecules is being investigated due to their ability to act as epigenetic regulators of gene expression and protein production. Thus, providing a molecular instrument to fine-tune, reprogram, and modulate the processes of tendon differentiation, healing, and regeneration. This review focuses particularly on the latest advances involving the use of siRNAs, miRNAs, and lncRNAs in tendon tissue engineering applications.

KEYWORDS

tendon, siRNA, miRNA, lncRNA, RNAi, mRNA silencing

1 Introduction

Tendons are a crucial component of the musculoskeletal system, allowing for the movement and stabilization of joints. Tendons sustain tensile loads and dissipate the stress generated by muscle contraction and joint movement. The general tendon morphology consists of a highly specialized extracellular matrix made up of proteoglycans, a high content of water, and anisotropically aligned collagen fibers. Around 80% of the dry weight of tendons comes from collagen, which organizes into hierarchical structures of fibrillar networks aligned in the direction of loading (Buckley et al., 2013; Chartier et al., 2021).

The most abundant type of collagen found in tendons is the fibril-forming collagen type I. Collagen type I is organized into microfibrils and fibrils, granting tendons its natural mechanical durability and strength. Collagen type II and type III are also found in tendons but in lower amounts. Collagen type II is mostly concentrated at the tendon-to-bone insertion site (i.e., enthesis) while collagen type III is always associated with collagen type I. Collagen type III forms thinner fibrils than collagen type I. These fibrils are typically more disorganized and are mechanically weaker than those formed by collagen type I. However, they play a key role in the healing and pathogenesis of tendons. During the initial stages of healing, the content of randomly oriented collagen type III fibers increases at the wound site forming a fibrous scar tissue. Later, this tissue is replaced by a stronger and better-aligned network of collagen type I (Buckley et al., 2013; Chartier et al., 2021). Unfortunately, this

remodeling phase often fails to completely regenerate the uninjured morphology of the tendon, and the collagen type III-rich scar tissue remains (Nguyen and Hsu, 2020; Shen et al., 2022). Thus, this weakens the tissue and increases the chances of recurrent rupture. The poor healing that is often seen after tendon injuries constitutes a challenge that tissue engineers working on tendon regeneration are trying to address by combining biomaterial design, cells, and bioactive molecules.

The field of biomaterials for tendon tissue engineering applications is extensive (Li et al., 2022; Xue et al., 2022; Huang et al., 2023). It comprises the use of natural and/or synthetic biomaterials in a wide range of combinations and designs to provide the best possible substitute to the native tissue during the process of healing while promoting *de novo* tissue regeneration. Some of the most used natural polymers for tendon regeneration applications are collagen, silk fibroin, chitosan, and fibrin (Dietrich et al., 2015; Yan et al., 2017; Sarıkaya and Gümüşderelioğlu, 2021). Natural polymers are praised for their biocompatibility and biodegradability. Alternatively, synthetic biomaterials such as poly-Ɛ-caprolactone, poly(lactic acid), poly(glycolic acid), or poly(lactic-co-glycolic acid) have also gained significant attention due to their tunable mechanical properties (Sensini et al., 2019; Kempfert et al., 2022; Uyanik et al., 2022).

The development of tendon mimetic constructs usually combines the design of structures that mimic the morphology found in healthy tendons and the use of mesenchymal stem cells (MSCs) or tendon progenitor stem cells (TPSCs). MSCs and TPSCs have the potential to respond to the morphological cues provided by the tendon mimetic structures to differentiate towards a tenogenic lineage. Thus, they can promote the formation of tissue-engineered tendon-like tissue (Font Tellado et al., 2017; Pardo et al., 2022).

In our consideration, the latest advances based on the strategies for the obtention of tendon-mimetic cell-laden constructs for tendon tissue engineering have been extensively reviewed (Lim et al., 2019; Ruiz-Alonso et al., 2021; Huang et al., 2023). Instead, the present review is inspired by the increasing evidence supporting the role of bioactive molecules as candidates to aid and promote tendon differentiation, regeneration, and healing. In particular, we will focus on the non-coding RNA-mediated transcriptional and post-transcriptional regulation of gene expression in the context of tendon healing and regeneration. More specifically, on the potential use of short interference RNA (siRNA), microRNA (miRNA), and long non-coding RNA (lncRNA), as molecular tools for reprograming or fine-tuning the processes of inflammation, scarring, and tissue regeneration in tendon healing.

2 Non-coding RNAs and tendon tissue engineering

Non-coding RNAs (ncRNA) are a heterogeneous group of RNA transcripts that do not translate into protein. Instead, they are implicated in a myriad of other cellular processes, most notably genome organization and regulation of gene expression (Kapranov et al., 2007; Nemeth et al., 2023). Over 70% of the human genome encodes for ncRNAs, and several classes of ncRNAs have been identified. This includes circular RNAs (circRNA), ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), Piwi-interacting RNAs (piRNAs), siRNAs, lncRNAs, and miRNAs (Uszczynska-Ratajczak et al., 2018; Nemeth et al., 2023). The discovery of the mechanisms of RNA interference (RNAi) via ncRNAs as mediators of gene silencing, allowed for the development of novel therapeutic strategies to treat human diseases (Fire et al., 1998; Pal et al., 2005; Felekkis and Deltas, 2006; Oh and Park, 2009). This led to the first-in-human trial of an RNAi therapeutic in cancer patients via silencing of VEGF and kinesis spindle protein (KSP) (Tabernero et al., 2013).

Some of the best-studied ncRNAs are siRNAs, miRNAs, and lncRNAs. They are recognized as key regulators in many biological processes and have been associated with various human diseases. As such, a multitude of synthetic siRNA-based therapies as well as miRNA and lncRNA-based therapies are currently under investigation (Beg et al., 2017; Colpaert and Calore, 2019; Jin et al., 2021; Cerqueira et al., 2022; DiStefano and Gerhard, 2022; Iacomino, 2023).

For many years, researchers have focused on the potential role of ncRNAs in those human diseases that account for the highest mortality worldwide, including cancer, neurodegenerative diseases, and infectious diseases (Nemeth et al., 2023). However, more recently, tissue engineers have dived into the intricate world of ncRNA as a potential source of promising therapeutic tools that could lead to important breakthroughs in the fields of regenerative medicine and tissue engineering. Hereunder, we will summarize and discuss the latest advances in relationship with the potential use of lncRNA, siRNA, and miRNA-based therapy in tendon-tissue engineering.

2.1 siRNAs

Small interfering RNAs (siRNAs) are a class of double-stranded RNA molecules, typically between 21 and 23 nucleotides in length that play a crucial role in the regulation of gene expression. They owe their name to their ability to mediate in a process known as RNA interference (RNAi), a natural mechanism that controls the activity of genes by promoting the degradation of mRNA (Ipsaro and Joshua-Tor, 2015; Lam et al., 2015).siRNAs are the result of the processing of double-stranded RNA molecules (dsRNA) by the RNase III-like enzyme Dicer. These dsRNAs can be directly transcribed by the cells although they are often thought of as exogenous dsRNAs that might come from infecting pathogens as well as being artificially introduced into the cell via transfection vectors (Oh and Park, 2009). Once a dsRNA is processed into siRNAs, it can interact with the RNA-induced silencing complex (RISC) to target for degradation those mRNA molecules to which the siRNA guide strand is fully complementary. Thus allowing for a highly specific gene-silencing effect (Lam et al., 2015).

Such mechanism of action has been exploited to specifically target disease-related genes, most commonly in the context of illnesses like cancer as well as to fight infectious pathogens (Geisbert et al., 2006; Zamora et al., 2011; Sakurai et al., 2014; Chen Y. et al., 2015). Nevertheless, the field of regenerative medicine is rapidly expanding and recently has begun to explore novel tissue engineering applications based on RNAi.

Early studies involving siRNAs and tendons were mostly focused on the use of siRNA-mediated knockdowns to identify

novel molecules relevant to the development, homeostasis, and normal function of tendons (Richardson et al., 2007; Tiwari et al., 2015; Gargano et al., 2021). Some examples include the identification of PIN1 (Peptidylprolyl Cis/Trans Isomerase, NIMA-Interacting 1) as a senescence inducer of tendon stem/progenitor cells (TSPC) (Chen L. et al., 2015). Additionally, RNAi knockdown of the transcription factor protein P65 revealed that P65 promoted fibrogenic and proinflammatory activity in tendon fibroblasts (Chen et al., 2017). Similarly, siRNA-targeting of the activated transcription factor 6 (ATF-6) revealed an antifibrotic role for ATF-6 in TGβ-1 pretreated fibroblasts from the Achilles tendon in a rat model (Yao et al., 2019). Moreover, by performing a tendon cell-specific RNAi screening, Tiwari et al. reported 19 novel molecules with enzymatic function or known to be involved in transcription activity, cell adhesion, protein folding, and intracellular transport functions in the context of the myotendinous junction of *Drosophila* (Tiwari et al., 2015).

The previous examples not only contributed to revealing the fundamental biological functions of the molecules in question but also pointed to potential therapeutic applications for the siRNA-mediated modulation of tendon fibrosis and healing. Thus, RNAi has been increasingly explored as a therapeutic candidate to improve tendon healing. For instance, Liao et al. reported the use of a collagen III-targeting siRNA to suppress the expression of collagen type III in tenocytes cultured in the presence of TGFβ-1. Thus, showing a proof of concept where a siRNA-based approach could potentially serve as treatment for the prevention of fibrosis by regulating collagen type III production in tendon-related disorders (Liao et al., 2020).

Another study aimed at the improvement of tendon healing, investigated the role of the small collagen fibrils in tendon repair, more specifically collagen type V. Collagen type V is typically increased during healing and plays an important role in fibrillogenesis (Lu et al., 2011). Lu et al. demonstrated that *COLV*-siRNA-engineered tenocytes displayed better tendon regeneration capabilities by promoting the formation of larger collagen fibrils achieving improved tendon contour and morphology. However, this study concluded that the ratio between collagen type V and collagen type I should be carefully monitored as the full knockdown of *COLV* hinders the formation of normal collagen fibrils. Hence, RNAi could be used to modulate the expression of *COLV* to achieve the desired balance of collagen type I and type V production necessary for the effective regeneration of healthy tendon tissue while minimizing the occurrence of fibrosis (Lu et al., 2011).

The combination of siRNA-based therapeutic approaches with the use of biomaterials for tendon tissue engineering has proven to enhance the potency of RNAi in tendon healing applications. Cai et al. developed a self-healing hydrogel encapsulating *SMAD3*-siRNA as an antiadhesion barrier to prevent tendon fibrosis and improve tendon healing *in vivo*. The self-healing capabilities of the hydrogel allowed for an attenuated inflammation of the injured tendon as a consequence of the reduction of the shear stress between the hydrogel-wrapped injured tendon and the peritendinous tissue. Moreover, the *SMAD3*-siRNA reduced the expression levels of *SMAD3*, leading to a decrease in the activation of the *TGF-β1/SMAD3* pathway and the consequent reduction in fibroblast proliferation and collagen type III production (Cai et al., 2022).

Despite the growing interest in the potential tissue-engineering applications for siRNA and RNAi technology in general, practical limitations to their use in the clinic are still to be overcome (Ali Zaidi et al., 2023). siRNAs can be degraded by endosomal nucleases or remain trapped indefinitely in non-functional stress granules or other cytoplasmatic bodies, which would greatly affect their efficacy (LeCher et al., 2017; Wang et al., 2021). Additionally, siRNA entrapment can also occur in the extracellular space, where proteins from the serum could form a non-functional protein-siRNA complex, thus, hindering the siRNA therapeutic effect (Ali Zaidi et al., 2023).

2.2 miRNAs

MicroRNAs, also known as miRNAs, are naturally occurring, short-non codding RNAs usually between 19 and 25 nucleotides in length. They are typically transcribed by the RNA polymerase II and, even when some miRNAs are individually produced from separate transcription units, they can also be produced as clusters of different miRNAs out from larger transcript-encoding miRNAs (Denli et al., 2004). Directly after transcription, pri-miRNAs are obtained, which will be later processed into pre-miRNAs. These are stem-loop structures that are exported from the cell nuclei to the cytoplasm where the terminal loop is removed by the enzyme Dicer to create a mature miRNA duplex (Bartel, 2004; Carthew and Sontheimer, 2009). Similarly to siRNA, miRNAs are effectors of the RISC complex and mediate the posttranscriptional regulation of a myriad of genes (Carthew and Sontheimer, 2009).

One of the most distinctive features of miRNAs is their ability to interact with hundreds of different mRNA sequences. This is believed to be due to the fact that miRNAs can target mRNA sequences to which they are not perfectly complementary. Furthermore, the degree of miRNA-mRNA complementarity is a crucial determining factor of their regulatory mechanism (Bartel, 2004; Carthew and Sontheimer, 2009). Perfect complementarity often leads to the degradation of the mRNA by the RISC complex while partial complementarity can sequester the mRNA without achieving cleavage of the mRNA strand. In the latter scenario, the recycling of the miRNA to the RISC complex can be delayed, and the miRNA-mRNA interaction accelerates the decay of the miRNA strand, as was demonstrated by a kinetic analysis of the fate of miRNAs after target regulation by Baccarini et al. (Carthew and Sontheimer, 2009; Baccarini et al., 2011).

As miRNAs are endogenous to the cell, miRNA-based therapies can rely either on miRNA replacement or inhibition. Typically, miRNA replacement is done through the use of miRNA mimics, while miRNA inhibition is achieved with the use of antagomirs or miRNA inhibitors (Gori et al., 2015).

In tendon tissue engineering, a plethora of miRNAs have been and continue to be investigated for their potential regulation over relevant tenogenic pathways. The miR-29 family is one of the best studied in the context of tendon healing (Millar et al., 2015; Liu et al., 2021). miR-29a, a member of this family, is known to regulate the production of collagen type III in tendon fibroblasts. This inspired Watts et al. who used intralesional injections of miRNA-29a in an equine tendon model to achieve improved tendon healing by reducing the expression of *COLIII* while increasing the

expression of *COLI*. Likewise, miR-29b is reported to regulate collagen production by interacting with the *SMAD3/TGF-β1* pathway. According to a study by Chen et al., the overexpression of miR-29b in the Achilles tendons of rats improved tendon healing and reduced scar formation after surgery (Chen et al., 2014). Once again, the regulation of the *SMAD3/TGF-β1* pathway by ncRNAs is a target for potential therapeutic approaches to achieve tendon healing. Furthermore, miRNA-based regulation of collagen production in tendon cells has been reported via alternative pathways. miR-124-3p was found to inhibit *EGR1*, which is known to activate the expression of the tendon markers *MKX*, *SCX*, and *COLI* (Guerquin et al., 2013). The inhibition of EGR1 by the overexpression of miR-124-3p in hTDSCs (tendon-derived stem cells) prevented tendon differentiation whilst the inhibition of miR-124-3p promoted the opposite effect (Wang et al., 2016). Thus, suggesting that miR-124-3p is a promising therapeutic target for tendon injury and healing.

In a recent study by our group, fibrosis-related miRNA profiling in a rodent patellar injury model allowed for the identification of dysregulated miRNAs at different time points after injury (Peniche Silva et al., 2023). A total of 13 miRNAs known or predicted to interact with important tenogenic pathways were identified to be dysregulated upon tendon-to-bone enthesis injury. Among them, the previously mentioned miR-124-3p was found upregulated while *EGR1* was downregulated. Additionally, miR-16-5p and miR-133-3p were strongly upregulated in the fibrotic portion of the tendon side of the enthesis 10 days after injury. Interestingly, both miRNAs are known for their anti-fibrotic potential and are reported to inhibit myofibroblasts activation by regulating *SMAD3* and *COLI* respectively (Wei et al., 2019; Yao et al., 2020). Hence, their upregulation at the tendon side of the enthesis after injury suggested an antifibrotic role for these miRNAs in tendon healing. This highlights the relevance of miR16-5p and miR-133-3p as therapeutic candidates to aid tendon healing and regeneration.

In another study involving miRNA profiling, Plachel et al. profiled miRNAs in samples from sera and biopsy samples from the supraspinatus and subscapularis tendons from patients suffering from degenerative rotator cuff tears (RCT), chronic rotator cuff tendinopathy, and healthy patients. They reported at least six circulating miRNAs (i.e., miR-18, miR-19a, miR19b, miR-25, mR-93, and miR192) that were downregulated both in sera and biopsy samples in patients from degenerative RCT when normalized against healthy controls. Furthermore, another six miRNAs were dysregulated in both chronic tendinopathy and degenerative RCT: miR-30-5p, miR-140-3p, miR-210-3p, miR-222-3p, miR-324-3p, miR-425-5p (Plachel et al., 2020). Such data contribute to the identification and establishment of miRNA signatures not only as therapeutic tools but also as diagnostic and prognostic tools for degenerative and chronic rotator cuff tendinopathies.

Inflammation is well known to play a major role in tendon healing and scar formation (Arvind and Huang, 2021; Chartier et al., 2021). Moreover, miR-205 has been found implicated in the secretion of inflammatory factors and the amplification of the NF-kβ-induced inflammatory response in cancer cells (Yeh et al., 2016). However, it has been reported that the inhibition of miR-205 in rat tenocytes from the Achilles tendon leads to an increase in the expression of the anti-inflammatory effector MECP2 (methylated binding protein 2). Furthermore, the inhibition of miR-205 improved tenocyte proliferation and migration and increased the expression of *COLI*, *COLIII*, *SCX*, and *TNC*. Hence, suggesting a tenogenic effect for the inhibition of miR-205.

The RNAi mechanisms of siRNA and miRNA replacement therapy are in many ways similar. They both are based on the administration of synthetic siRNAs or miRNAs to achieve gene silencing. However, miRNAs have the potential to interact with a multitude of different pathways while siRNAs are specifically designed to target one gene of interest. This highlights an important difference that sets these classes of molecules apart, in particular when considering aspects of their sequence design and therapeutic approach. Additionally, miRNA-based therapy comprehends the inhibition of miRNAs by means of miRNA inhibitors, an approach that has no equivalent in the work with siRNAs. Nevertheless, these types of small RNA molecules face similar challenges that hinder their applications in the clinic such as poor *in vivo* stability, the need for efficient transfection vectors, and off-target effects (Lam et al., 2015).

2.3 LncRNAs

LncRNAs are non-coding transcripts longer than 200 nucleotides, although such length cut-off appears to be somewhat arbitrary (Ponting et al., 2009; Cao, 2014). When first discovered, lncRNAs were thought to be non-functional. However, there is now plenty of evidence for the roles of lncRNAs as genomic regulators as well as regulators of transcription and translation, interacting either directly with DNA, and mRNA or acting as miRNA sponges, thus, affecting cell identity, fate, and function (Cao, 2014; DiStefano and Gerhard, 2022; Nemeth et al., 2023).

RNA-sequencing has allowed for lncRNA profiling, hence, facilitating the identification of differentially expressed lncRNAs in specific settings. In a conjoint analysis of lncRNA and mRNA expression in the context of a RCT, Ge et al. identified 419 lncRNAs and 1,542 mRNAs that were differentially expressed in patients with RCT in comparison with the expression in normal tendon. Furthermore, competitive endogenous RNA network analysis based on those results revealed interactions between 139 lncRNA, 126 mRNA and 35 miRNAs, most of which were related to the citrate cycle, p53 signaling, and the renin-angiotensin system. Additionally, they found differentially expressed genes involved in VEGF signaling, which is in line with the changes in vascularity typically observed in RCT. Thus, providing insights into the potential lncRNA-mRNA-mediated mechanism underlying tendon pathology (Ge et al., 2020).

Among the lncRNAs described to be dysregulated in tendinopathy, lncRNA X-inactive specific transcript (lncRNA XIST) has been found highly expressed in relationship with tendon injury (Peffers et al., 2015). Nevertheless, contrasting functions have been described for XIST. In ligament fibroblasts, XIST promotes osteogenic differentiation via the lncRNA XIST/miR-302a-3p/USP8 axis (Yuan et al., 2021). While cancer research acknowledges XIST as a cancer-promoting gene due to its association with tumor occurrence and development via targeting of the tumor-suppressing miR-34a-5p and miR-137 (Wang et al., 2017; Sun et al., 2018). However, in the context of tendon injury in mice models, the overexpression of XIST in populations at high risk of tendon injury was linked to the decreased expression of miR-26-

5p and the increased expression of cyclooxygenase 2 (COX2), with the consequent increase in fibroblast proliferation, collagen production, and the occurrence of tendon adhesion. Indicating a role for lncRNA XIST targeted miR-26-5p in the healing of tendon injury (Chen et al., 2022).

Other reports highlight the tenogenic role of lncRNAs. Such is the case of the lncRNA H19, which has been described to significantly accelerate the TGF-β1-induced tenogenic differentiation *in vitro* and accelerate tendon healing in mouse tendon defect models *in vivo* (Lu et al., 2017). H19 promotes tenogenesis by directly targeting miR-29b-3p. As mentioned before, miR-29b-3p has the potential to suppress the expression of *TGF-β1* and collagen type I (Chen et al., 2014; Lu et al., 2017). Thus, the TGF-β1/H19/miR-29b-3p regulatory loop could be the target of new strategies for treating tendon injuries. Similarly, lncRNA MALAT1 has been shown to promote tendon healing in rat models of tendinopathy by regulating the miR-378a-3p/MAPK1 axis. MiR-378a is a biomarker for tendon injury and its over-expression is associated with decreased *COL1A1, SCX, MKX, MMP3* and other tendon markers. Thus, MALAT1-mediated regulation of miR-378a-3p could be another potential target of molecular therapies to aid tendon healing.

3 Future perspectives

Non-coding RNAs are increasingly present in the development of novel tissue engineering approaches to treat tendon injuries. As molecular tools to modulate gene expression and protein production, they hold the promise to lead the field of regenerative medicine toward a more personalized kind of medicine. Tailoring the ncRNA-based strategies to individual patient profiles may improve the efficacy of the treatments. Moreover, even when the possibility for off-target effects is still a concern when working with ncRNAs, they offer superior specificity to the traditional gene manipulation methods (Kohn et al., 2023). Additionally, they can be integrated into various biomaterials and scaffolds to achieve enhanced regeneration capacity.

In our consideration, there is enough evidence of the potential benefits associated with the use of ncRNAs in regenerative medicine applications to justify the increasing interest in researching this class of molecules, their mechanisms of action, and potential applications in tendon tissue engineering. Nevertheless, siRNAs, miRNAs, and lncRNAs exhibit individual strengths and limitations that should be carefully considered when investigating their potential applications (Figure 1). The RNAi mechanisms of siRNA and miRNA are similar. However, siRNA can be specifically designed to target one mRNA sequence. Alternatively, miRNAs can interact with many distinct biological pathways and many pathways can regulate one specific miRNA. This is both a curse and a blessing, and extensive research is still required to fully understand the implications of dysregulating miRNAs in a tissue-specific manner. Moreover, antagomirs or miRNA inhibitors provide a valuable tool to research the effects of the suppression or knockdown of miRNAs. Similarly, lncRNAs can directly interact with miRNAs, acting as miRNA inhibitors thus

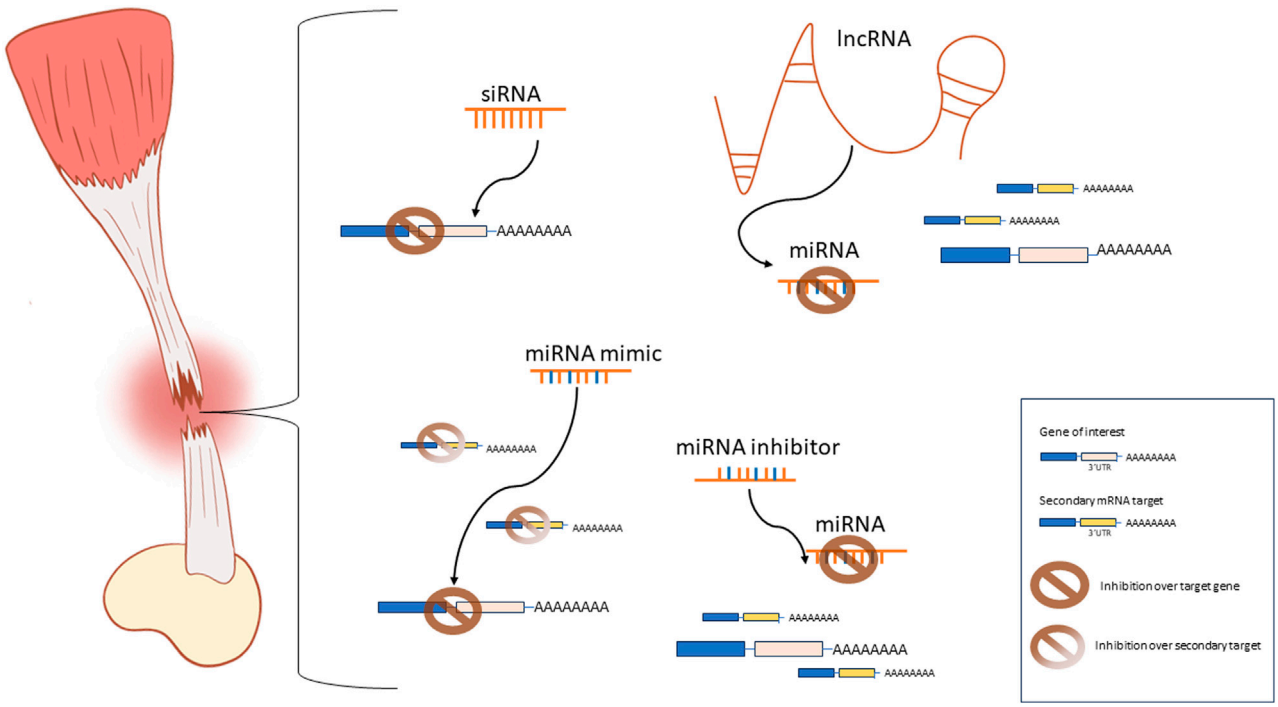


FIGURE 1
Schematic representation of the most commonly used ncRNA-based approaches for tendon tissue engineering applications. siRNAs are designed to specifically interact with one mRNA sequence to achieve inhibition of gene expression. miRNA mimics inhibit one specific mRNA target while interactions with secondary targets and alternative pathways are possible. miRNA inhibitors prevent the miRNA-mediated repression of the mRNA target by inhibiting the activity of the endogenous miRNAs. lncRNAs are used as miRNA sponges to inhibit the miRNA-mediated repression of a target gene.

restoring the function of the miRNA-targeted mRNA. However, lncRNAs exhibit a wide range of targets beyond miRNAs. They can interact with DNA, proteins, or mRNA. Additionally, they can be found in either the nucleus, affecting chromatin structure, or in the cytoplasm, modulating transcriptional and post-transcriptional processes (Jin et al., 2021; Winkle et al., 2021). Compared to siRNAs and miRNAs, lncRNA are functionally very complex and their function is reported to be context dependent. Hence, the understanding of the precise mechanisms and specific function of individual lncRNAs in each tissue is an ongoing challenge. For the moment, the focus on lncRNA in tendon tissue engineering applications seems to be more or less limited to their regulation over miRNAs. Future studies may unveil new applications addressed to aid tendon healing and regeneration.

4 Conclusion

Tendons play a fundamental role in movement and locomotion. Thus, injuries at the tendon can greatly impair the quality of life of patients and represent a significant societal and economic burden. Moreover, patients suffering from tendon injury undergo a long and often painful path to recovery. Advances in the field of tendon tissue engineering are expected to lead to better tissue healing with less scar formation and superior recapitulation of the native tendon morphology and function. ncRNAs offer a set of powerful tools to fine-tune at the molecular levels processes of cell differentiation, proliferation, matrix deposition, and tissue remodeling that could greatly aid tissue regeneration and healing. However, siRNAs, miRNAs, and lncRNA have only recently emerged as molecular candidates for tendon tissue engineering applications. Thus, extensive research is still required to fully harness their potential for better healing of tendons.

Author contributions

CP: Conceptualization, Writing–original draft, Writing–review and editing. EB: Conceptualization, Writing–original draft, Writing–review and editing. MV: Conceptualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Province of Limburg's Limburg Invests in its Knowledge Economy (LINK) program.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission.

Publisher's note

References

Ali Zaidi, S. S., Fatima, F., Zhou, D., Deng, W., and Liu, S. (2023). Engineering siRNA therapeutics: challenges and strategies. *J. Nanobiotechnology* 21 (1), 381. doi:10.1186/s12951-023-02147-z

Arvind, V., and Huang, A. H. (2021). Reparative and maladaptive inflammation in tendon healing. *Front. Bioeng. Biotechnol.* 9, 719047. doi:10.3389/fbioe.2021.719047

Baccarini, A., Chauhan, H., Gardner, T., Jayaprakash, A., Sachidanandam, R., and Brown, B. (2011). Kinetic analysis reveals the fate of a MicroRNA following target regulation in mammalian cells. *Curr. Biol.* 21 (5), 369–376. doi:10.1016/j.cub.2011.01.067

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116 (2), 281–297. doi:10.1016/s0092-8674(04)00045-5

Beg, M. S., Brenner, A. J., Sachdev, J., Borad, M., Kang, Y. K., Stoudemire, J., et al. (2017). Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Investig. new drugs* 35, 180–188. doi:10.1007/s10637-016-0407-y

Buckley, M. R., Evans, E. B., Matuszewski, P. E., Chen, Y. L., Satchel, L. N., Elliott, D. M., et al. (2013). Distributions of types I, II and III collagen by region in the human supraspinatus tendon. *Connect. Tissue Res.* 54 (6), 374–379. doi:10.3109/03008207.2013.847096

Cai, C., Zhang, X., Li, Y., Liu, X., Wang, S., Lu, M., et al. (2022). Self-healing hydrogel embodied with macrophage-regulation and responsive-gene-silencing properties for synergistic prevention of peritendinous adhesion. *Adv. Mater.* 34 (5), 2106564. doi:10.1002/adma.202106564

Cao, J. (2014). The functional role of long non-coding RNAs and epigenetics. *Biol. Proced. Online* 16 (1), 42. doi:10.1186/1480-9222-16-11

Carthew, R. W., and Sontheimer, E. J. (2009). Origins and Mechanisms of miRNAs and siRNAs. *Cell* 136 (4), 642–655. doi:10.1016/j.cell.2009.01.035

Cerqueira, D. M., Tayeb, M., and Ho, J. (2022). MicroRNAs in kidney development and disease. *JCI Insight* 7 (9), e158277. doi:10.1172/jci.insight.158277

Chartier, C., ElHawary, H., Baradaran, A., Vorstenbosch, J., Xu, L., and Efanov, J. I. (2021). "Tendon: principles of healing and repair," in *Seminars in plastic surgery* (Thieme Medical Publishers, Inc).

Chen, L., Liu, J., Tao, X., Wang, G., Wang, Q., and Liu, X. (2015b). The role of Pin1 protein in aging of human tendon stem/progenitor cells. *Biochem. biophysical Res. Commun.* 464 (2), 487–492. doi:10.1016/j.bbrc.2015.06.163

Chen, Q., Hou, D., Suo, Y., and Zhu, Z. (2022). LncRNA XIST prevents tendon adhesion and promotes tendon repair through the miR-26a-5p/COX2 pathway. *Mol. Biotechnol.* 64 (4), 424–433. doi:10.1007/s12033-021-00419-3

Chen, Q., Lu, H., and Yang, H. (2014). Chitosan inhibits fibroblasts growth in Achilles tendon via TGF-β1/Smad3 pathway by miR-29b. *Int. J. Clin. Exp. Pathology* 7 (12), 8462–8470.

Chen, S., Jiang, S., Zheng, W., Tu, B., Liu, S., Ruan, H., et al. (2017). RelA/p65 inhibition prevents tendon adhesion by modulating inflammation, cell proliferation, and apoptosis. *Cell death Dis.* 8 (3), e2710. doi:10.1038/cddis.2017.135

Chen, Y., Gu, H., Wang, X., Liu, T., Zhang, D. S. z., Wang, Y., et al. (2015a). Highly effective antiangiogenesis via magnetic mesoporous silica-based siRNA vehicle targeting the VEGF gene for orthotopic ovarian cancer therapy. *Int. J. nanomedicine* 10, 2579–2594. doi:10.2147/ijn.s78774

Colpaert, R. M. W., and Calore, M. (2019). MicroRNAs in cardiac diseases. *Cells* 8 (7), 737. doi:10.3390/cells8070737

Denli, A. M., Tops, B. B. J., Plasterk, R. H. A., Ketting, R. F., and Hannon, G. J. (2004). Processing of primary microRNAs by the Microprocessor complex. *Nature* 432 (7014), 231–235. doi:10.1038/nature03049

Dietrich, F. L, Duré, G. P., Klein, C. F., Bampi, V. V., Padoin, A. D., Silva, V., et al. (2015). Platelet-rich fibrin promotes an accelerated healing of Achilles tendon when compared to platelet-rich plasma in rat. *World J. Plast. Surg.*, 2228–7914. (Print)).

DiStefano, J. K., and Gerhard, G. S. (2022). Long noncoding RNAs and human liver disease. *Annu. Rev. Pathol.* 17, 1–21. doi:10.1146/annurev-pathol-042320-115255

Felekkis, K., and Deltas, C. (2006). RNA Intereference: a powerful laboratory tool and its therapeutic implications. *Hippokratia* 10 (3), 112–115.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *nature* 391 (6669), 806–811. doi:10.1038/35888

Font Tellado, S., Bonani, W., Balmayor, E. R., Foehr, P., Motta, A., Migliaresi, C., et al. (2017). <sup/>Fabrication and characterization of biphasic silk fibroin scaffolds for tendon/ligament-to-bone tissue engineering. *Tissue Eng. Part A* 23, 859–872. 1937-335X (Electronic)). doi:10.1089/ten.tea.2016.0460

Gargano, G., Oliviero, A., Oliva, F., and Maffulli, N. (2021). Small interfering RNAs in tendon homeostasis. *Br. Med. Bull.* 138 (1), 58–67. doi:10.1093/bmb/ldaa040

Ge, Z., Tang, H., Lyu, J., Zhou, B., Yang, M., Tang, K., et al. (2020). Conjoint analysis of lncRNA and mRNA expression in rotator cuff tendinopathy. *Ann. Transl. Med.* 8 (6), 335. doi:10.21037/atm.2020.02.149

Geisbert, T. W., Hensley, L., Kagan, E., Yu, E., Geisbert, J., Daddario-DiCaprio, K., et al. (2006). Postexposure protection of Guinea pigs against a lethal ebola virus challenge is conferred by RNA interference. *J. Infect. Dis.* 193 (12), 1650–1657. doi:10.1086/504267

Gori, M., Trombetta, M., Santini, D., and Rainer, A. (2015). Tissue engineering and microRNAs: future perspectives in regenerative medicine. *Expert Opin. Biol. Ther.* 15 (11), 1601–1622. doi:10.1517/14712598.2015.1071349

Guerquin, M.-J., Charvet, B., Nourissat, G., Havis, E., Ronsin, O., Bonnin, M. A., et al. (2013). Transcription factor EGR1 directs tendon differentiation and promotes tendon repair. *J. Clin. investigation* 123 (8), 3564–3576. doi:10.1172/jci67521

Huang, L., Chen, L., Chen, H., Wang, M., Jin, L., Zhou, S., et al. (2023). Biomimetic scaffolds for tendon tissue regeneration. *Biomimetics* 8, 246. doi:10.3390/biomimetics8020246

Iacomino, G. (2023). miRNAs: the road from bench to bedside. *Genes (Basel)* 14 (2), 314. doi:10.3390/genes14020314

Ipsaro, J. J., and Joshua-Tor, L. (2015). From guide to target: molecular insights into eukaryotic RNA-interference machinery. *Nat. Struct. Mol. Biol.* 22 (1), 20–28. doi:10.1038/nsmb.2931

Jin, H., Du, W., Huang, W., Yan, J., Tang, Q., Chen, Y., et al. (2021). lncRNA and breast cancer: progress from identifying mechanisms to challenges and opportunities of clinical treatment. *Mol. Ther. Nucleic Acids* 25, 613–637. doi:10.1016/j.omtn.2021.08.005

Kapranov, P., Willingham, A. T., and Gingeras, T. R. (2007). Genome-wide transcription and the implications for genomic organization. *Nat. Rev. Genet.* 8 (6), 413–423. doi:10.1038/nrg2083

Kempfert, M., Willbold, E., Loewner, S., Blume, C., Pitts, J., Menzel, H., et al. (2022). Polycaprolactone-based 3D-printed scaffolds as potential implant materials for tendon-defect repair. *LID* 160, 160–4983. (Print). doi:10.3390/jfb13040160

Kohn, D. B., Chen, Y. Y., and Spencer, M. J. (2023). Successes and challenges in clinical gene therapy. *Gene Ther.* 30 (10), 738–746. doi:10.1038/s41434-023-00390-5

Lam, J. K., Chow, M. Y. T., Zhang, Y., and Leung, S. W. S. (2015). siRNA versus miRNA as therapeutics for gene silencing. *Mol. Ther. Nucleic Acids* 4 (9), e252. doi:10.1038/mtna.2015.23

LeCher, J. C., Nowak, S. J., and McMurry, J. L. (2017). Breaking in and busting out: cell-penetrating peptides and the endosomal escape problem. *Biomol. concepts* 8 (3-4), 131–141. doi:10.1515/bmc-2017-0023

Li, Y., Zhou, M., Zheng, W., Yang, J., and Jiang, N. (2022). Scaffold-based tissue engineering strategies for soft-hard interface regeneration. *Regen. Biomater.* 10, rbac091. doi:10.1093/rb/rbac091

Liao, X., Falcon, N. D., Mohammed, A. A., Paterson, Y. Z., Mayes, A. G., Guest, D. J., et al. (2020). Synthesis and formulation of four-arm PolyDMAEA-siRNA polyplex for transient downregulation of collagen type III gene expression in TGF-β1 stimulated tenocyte culture. *ACS Omega* 5 (3), 1496–1505. doi:10.1021/acsomega.9b03216

Lim, W. L., Liau, L. L., Ng, M. H., Chowdhury, S. R., and Law, J. X. (2019). Current progress in tendon and ligament tissue engineering. *Tissue Eng. Regen. Med.* 16 (6), 549–571. doi:10.1007/s13770-019-00196-w

Liu, Q., Zhu, Y., Zhu, W., Zhang, G., Yang, Y. P., and Zhao, C. (2021). The role of MicroRNAs in tendon injury, repair, and related tissue engineering. *Biomaterials* 277, 121083. doi:10.1016/j.biomaterials.2021.121083

Lu, P., Zhang, G. R., Song, X. H., Zou, X. H., Wang, L. L., and Ouyang, H. W. (2011). Col V siRNA engineered tenocytes for tendon tissue engineering. *PLoS One* 6 (6), e21154. doi:10.1371/journal.pone.0021154

Lu, Y. F., Liu, Y., Fu, W. M., Xu, J., Wang, B., Sun, Y. X., et al. (2017). Long noncoding RNA H19 accelerates tenogenic differentiation and promotes tendon healing through targeting miR-29b-3p and activating TGF-β1 signaling. *Faseb J.* 31 (3), 954–964. doi:10.1096/fj.201600722r

Millar, N. L., Gilchrist, D. S., Akbar, M., Reilly, J. H., Kerr, S. C., Campbell, A. L., et al. (2015). MicroRNA29a regulates IL-33-mediated tissue remodelling in tendon disease. *Nat. Commun.* 6, 6774. doi:10.1038/ncomms7774

Nemeth, K., Bayraktar, R., Ferracin, M., and Calin, G. A. (2023). Non-coding RNAs in disease: from mechanisms to therapeutics. *Nat. Rev. Genet.* 25, 211–232. doi:10.1038/s41576-023-00662-1

Nguyen, M. T., and Hsu, W. K. (2020). Performance-based outcomes following patellar tendon repair in professional athletes. *Physician Sportsmed.* 48 (1), 110–115. doi:10.1080/00913847.2019.1642809

Oh, Y.-K., and Park, T. G. (2009). siRNA delivery systems for cancer treatment. *Adv. drug Deliv. Rev.* 61 (10), 850–862. doi:10.1016/j.addr.2009.04.018

Pal, A., Ahmad, A., Khan, S., Sakabe, I., Zhang, C., Kasid, U., et al. (2005). Systemic delivery of RafsiRNA using cationic cardiolipin liposomes silences Raf-1 expression and inhibits tumor growth in xenograft model of human prostate cancer. *Int. J. Oncol.* 26 (4), 1087–1091. doi:10.3892/ijo.26.4.1087

Pardo, A., Bakht, S. M., Gomez-Florit, M., Rial, R., Monteiro, R. F., Teixeira, S. P. B., et al. (2022). Magnetically-assisted 3D bioprinting of anisotropic tissue-mimetic constructs. *Adv. Funct. Mater.* 32 (50), 2208940. doi:10.1002/adfm.202208940

Peffers, M. J., Fang, Y., Cheung, K., Wei, T., Clegg, P., and Birch, H. (2015). Transcriptome analysis of ageing in uninjured human Achilles tendon. *Arthritis Res. Ther.* 17 (1), 33–18. doi:10.1186/s13075-015-0544-2

Peniche Silva, C. J., De La Vega, R. E., Panos, J., Joris, V., Evans, C. H., Balmayor, E. R., et al. (2023). MiRNAs as potential regulators of enthesis healing: findings in a rodent injury model. *Int. J. Mol. Sci.* 24, 8556. doi:10.3390/ijms24108556

Plachel, F., Heuberer, P., Gehwolf, R., Frank, J., Tempfer, H., Lehner, C., et al. (2020). MicroRNA profiling reveals distinct signatures in degenerative rotator cuff pathologies. *J. Orthop. Res.* 38 (1), 202–211. doi:10.1002/jor.24473

Ponting, C. P., Oliver, P. L., and Reik, W. (2009). Evolution and functions of long noncoding RNAs. *Cell* 136 (4), 629–641. doi:10.1016/j.cell.2009.02.006

Richardson, S. H., Starborg, T., Lu, Y., Humphries, S. M., Meadows, R. S., and Kadler, K. E. (2007). Tendon development requires regulation of cell condensation and cell shape via cadherin-11-mediated cell-cell junctions. *Mol. Cell. Biol.* 27 (17), 6218–6228. doi:10.1128/mcb.00261-07

Ruiz-Alonso, S., Lafuente-Merchan, M., Ciriza, J., Saenz-del-Burgo, L., and Pedraz, J. L. (2021). Tendon tissue engineering: cells, growth factors, scaffolds and production techniques. *J. Control. Release* 333, 448–486. doi:10.1016/j.jconrel.2021.03.040

Sakurai, Y., Hatakeyama, H., Akita, H., and Harashima, H. (2014). Improvement of doxorubicin efficacy using liposomal anti-polo-like kinase 1 siRNA in human renal cell carcinomas. *Mol. Pharm.* 11 (8), 2713–2719. doi:10.1021/mp500245z

Sarıkaya, B., and Gümüşderelioğlu, M. (2021). Aligned silk fibroin/poly-3-hydroxybutyrate nanofibrous scaffolds seeded with adipose-derived stem cells for tendon tissue engineering. *Int. J. Biol. Macromol.* 193, 276–286. doi:10.1016/j.ijbiomac.2021.10.104

Sensini, A., Gualandi, C., Focarete, M. L., Belcari, J., Zucchelli, A., Boyle, L., et al. (2019). Multiscale hierarchical bioresorbable scaffolds for the regeneration of tendons and ligaments. *Biofabrication* 11 (3), 035026. doi:10.1088/1758-5090/ab20ad

Shen, L., Qinglin, K., Rui, Z., Yanhao, L., and Rong, B. (2022). "Tendon adhesion and novel solutions," in *Tendons* Editor R. Nahum (Rijeka: IntechOpen). 3. doi:10.5772/intechopen.108019

Sun, Z., Zhang, B., and Cui, T. (2018). Long non-coding RNA XIST exerts oncogenic functions in pancreatic cancer via miR-34a-5p. *Oncol. Rep.* 39 (4), 1591–1600. doi:10.3892/or.2018.6245

Tabernero, J., Shapiro, G. I., LoRusso, P. M., Cervantes, A., Schwartz, G. K., Weiss, G. J., et al. (2013). First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. *Cancer Discov.* 3 (4), 406–417. doi:10.1158/2159-8290.cd-12-0429

Tiwari, P., Kumar, A., Das, R. N., Malhotra, V., and VijayRaghavan, K. (2015). A tendon cell specific RNAi screen reveals novel candidates essential for muscle tendon interaction. *PLoS One* 10 (10), e0140976. doi:10.1371/journal.pone.0140976

Uszczynska-Ratajczak, B., Lagarde, J., Frankish, A., Guigó, R., and Johnson, R. (2018). Towards a complete map of the human long non-coding RNA transcriptome. *Nat. Rev. Genet.* 19 (9), 535–548. doi:10.1038/s41576-018-0017-y

Uyanik, O., Pekkoc-Uyanik, K. C., Findik, S., Avci, A., and Altuntas, Z. (2022). Prevention of peritendinous adhesions with electrospun poly (lactic acid-co-glycolic acid) (PLGA) bioabsorbable nanofiber: an experimental study. *Colloids Surfaces B Biointerfaces* 209, 112181. doi:10.1016/j.colsurfb.2021.112181

Wang, B., Guo, J., Feng, L., Suen, C. w., Fu, W. m., Zhang, J. f., et al. (2016). MiR124 suppresses collagen formation of human tendon derived stem cells through targeting egr1. *Exp. Cell Res.* 347 (2), 360–366. doi:10.1016/j.yexcr.2016.08.018

Wang, H., Zhang, S., Lv, J., and Cheng, Y. (2021). Design of polymers for siRNA delivery: recent progress and challenges. *View* 2 (3), 20200026. doi:10.1002/viw.20200026

Wang, Z., Yuan, J., Li, L., Yang, Y., Xu, X., and Wang, Y. (2017). Long non-coding RNA XIST exerts oncogenic functions in human glioma by targeting miR-137. *Am. J. Transl. Res.* 9 (4), 1845–1855.

Wei, P., Xie, Y., Abel, P. W., Huang, Y., Ma, Q., Li, L., et al. (2019). Transforming growth factor (TGF)-β1-induced miR-133a inhibits myofibroblast differentiation and pulmonary fibrosis. *Cell Death Dis.* 10 (9), 670. doi:10.1038/s41419-019-1873-x

Winkle, M., El-Daly, S. M., Fabbri, M., and Calin, G. A. (2021). Noncoding RNA therapeutics — challenges and potential solutions. *Nat. Rev. Drug Discov.* 20 (8), 629–651. doi:10.1038/s41573-021-00219-z

Xue, Y., Kim, H., Lee, J., Liu, Y., Hoffman, T., Chen, Y., et al. (2022). Co-electrospun silk fibroin and gelatin methacryloyl sheet seeded with mesenchymal stem cells for tendon regeneration. *Small* 18 (21), 2107714. doi:10.1002/smll.202107714

Yan, X. (2017). "3 - chitosan for tendon engineering and regeneration," in *Chitosan based biomaterials volume 2* Editors J. A. Jennings and J. D. Bumgardner (Tissue Engineering and Therapeutics: Woodhead Publishing), 73–87. doi:10.1016/B978-0-08-100228-5.00003-1

Yao, Q., Xing, Y., Wang, Z., Liang, J., Lin, Q., Huang, M., et al. (2020). MiR-16-5p suppresses myofibroblast activation in systemic sclerosis by inhibiting NOTCH signaling. *Aging (Albany NY)* 13 (2), 2640–2654. doi:10.18632/aging.202308

Yao, Z., Wang, W., Ning, J., Zhang, X., Zheng, W., Qian, Y., et al. (2019). Hydroxycamptothecin inhibits peritendinous adhesion via the endoplasmic reticulum stress-dependent apoptosis. *Front. Pharmacol.* 10, 967. doi:10.3389/fphar.2019.00967

Yeh, D. W., Chen, Y. S., Lai, C. Y., Liu, Y. L., Lu, C. H., Lo, J. F., et al. (2016). Downregulation of COMMD1 by miR-205 promotes a positive feedback loop for amplifying inflammatory- and stemness-associated properties of cancer cells. *Cell Death Differ.* 23 (5), 841–852. doi:10.1038/cdd.2015.147

Yuan, X., Shi, L., Guo, Y., Sun, J., Miao, J., Shi, J., et al. (2021). METTL3 regulates ossification of the posterior longitudinal ligament via the lncRNA XIST/miR-302a-3p/USP8 Axis. *Front. Cell Dev. Biol.* 9, 629895. doi:10.3389/fcell.2021.629895

Zamora, M. R., Budev, M., Rolfe, M., Gottlieb, J., Humar, A., DeVincenzo, J., et al. (2011). RNA interference therapy in lung transplant patients infected with respiratory syncytial virus. *Am. J. Respir. Crit. care Med.* 183 (4), 531–538. doi:10.1164/rccm.201003-0422oc

frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Review
PUBLISHED 08 August 2024
DOI 10.3389/fbioe.2024.1357696

OPEN ACCESS

EDITED BY
Dai Fei Elmer Ker,
The Chinese University of Hong Kong, China

REVIEWED BY
Takashi Taguchi,
Western University of Health Sciences, United States
Hakan Darici,
University of Istinye, Türkiye

*CORRESPONDENCE
SongOu Zhang,
zso010@163.com

†These authors share first authorship

RECEIVED 18 December 2023
ACCEPTED 29 July 2024
PUBLISHED 08 August 2024

CITATION
He W, Jiang C, Zhou P, Hu X, Gu X and Zhang S (2024) Role of tendon-derived stem cells in tendon and ligament repair: focus on tissue engineer.
Front. Bioeng. Biotechnol. 12:1357696.
doi: 10.3389/fbioe.2024.1357696

Role of tendon-derived stem cells in tendon and ligament repair: focus on tissue engineer

Wei He[1†], Chao Jiang[1†], Ping Zhou[1], Xujun Hu[1], XiaoPeng Gu[2,3] and SongOu Zhang[1,2,3*]

[1]Shaoxing People's Hospital, Shaoxing, Zhejiang, China, [2]Department of Clinical Medicine, Health Science Center, Ningbo University, Ningbo, Zhejiang, China, [3]Department of Orthopedics, Zhoushan Guhechuan Hospital, Zhoushan, Zhejiang, China

This review offered a comprehensive analysis of tendon and ligament injuries, emphasizing the crucial role of tendon-derived stem cells (TDSCs) in tissue engineering as a potential solution for these challenging medical conditions. Tendon and ligament injuries, prevalent among athletes, the elderly, and laborers, often result in long-term disability and reduced quality of life due to the poor intrinsic healing capacity of these avascular structures. The formation of biomechanically inferior scar tissue and a high rate of reinjury underscore the need for innovative approaches to enhance and guide the regenerative process. This review delved into the complexities of tendon and ligament structure and function, types of injuries and their impacts, and the limitations of the natural repair process. It particularly focused on the role of TDSCs within the context of tissue engineering. TDSCs, with their ability to differentiate into tenocytes, are explored in various applications, including biocompatible scaffolds for cell tracking, co-culture systems to optimize tendon-bone healing, and graft healing techniques. The review also addressed the challenges of immunoreactivity post-transplantation, the importance of pre-treating TDSCs, and the potential of hydrogels and decellularized matrices in supporting tendon regeneration. It concluded by highlighting the essential roles of mechanical and molecular stimuli in TDSC differentiation and the current challenges in the field, paving the way for future research directions.

KEYWORDS

tendon-derived stem cell, tendon injury, tissue engineer, nanotechnology, seed cell

1 Introduction

Tendon and ligament injuries are common among athletes, the elderly, and individuals engaged in physical labor, comprising a significant portion of musculoskeletal disorders (Leong et al., 2020). These injuries not only cause pain and dysfunction but can also lead to long-term disability and a substantial decrease in quality of life. The complex structure and biomechanical properties of tendons and ligaments make them particularly challenging to heal, with current treatments often failing to fully restore the original strength and functionality (Cottrell et al., 2016). This highlights the clinical importance of developing more effective repair strategies.

The intrinsic healing capacity of tendons and ligaments is notably poor due to their avascular nature, which restricts the influx of cells and nutrients to the injury site (Andarawis-Puri et al., 2015). Consequently, the repair process is slow and often leads to the formation of scar tissue, lacking the original tissue's biomechanical properties. As a

result, there is a high rate of reinjury and an extended recovery period. These challenges necessitate innovative approaches that can enhance and direct the regenerative process, ensuring the restoration of the tendon or ligament to its native state (Nichols et al., 2019).

Tissue engineering has emerged as a promising field offering potential therapeutic strategies for the repair and regeneration of damaged tendons and ligaments (Lim et al., 2019). By incorporating principles from cell biology, material science, and engineering, tissue engineering aims to develop biological substitutes that can restore, maintain, or enhance tissue function (Ruiz-Alonso et al., 2021). This multidisciplinary approach often involves the utilization of stem cells (Tevlin et al., 2016), biocompatible scaffolds (Stace et al., 2018), and bioactive molecules (Atienza-Roca et al., 2018) to create an environment conducive to healing. With the capability to differentiate into tenocytes, the cells responsible for maintaining tendon structure and function, TDSCs are at the forefront of research as a cellular source for tissue-engineered constructs (Zhang et al., 2023). This literature review will explore the role of TDSCs within the context of tissue engineering and investigate how this synergy might pave the way for effective tendon and ligament repair.

2 Basic science of tendon and TDSCs

2.1 Structure and function of tendons and ligaments

Tendons and ligaments are vital components of the musculoskeletal system, serving as crucial connectors within the body. Tendons attach muscle to bone, enabling movement by transmitting forces generated by muscle contractions to the skeleton. Ligaments connect bones to each other, offering joint stability and guiding motion. Both structures consist of dense fibrous connective tissue, primarily composed of collagen fibers, which provide tensile strength and elasticity (Thorpe and Screen, 2016). The main structural component of tendons is type I collagen (Col I), a protein arranged in a highly ordered manner that forms the tendon's primary load-bearing structure. Collagen molecules themselves have an elongated triple helix structure, and these molecules are further packed tightly together to form microfibers. Microfibers aggregate into thicker fiber bundles, and these fiber bundles are then aggregated to form large fiber bundles visible in tendons. These thicker fiber bundles can be called tendon clusters. Tendon clusters are composed of tendon inner chambers and tendon outer chambers. The inner compartment is composed of tenocytes and multiscale assembled collagen clusters, and the outer compartment is synovium-like tissue that connects blood vessels and the nervous system. Each level of structure increases the overall strength and elasticity of the tendon (Franchi et al., 2007; Thorpe and Screen, 2016). As show in Figures 1A, 2. The main cell types in tendons are tenocytes and tendon-specific stem cells which we evaluate in this review as TDSCs. These cells are distributed between collagen fibers and are responsible for synthesizing and breaking down collagen, thus participating in the maintenance, repair and regeneration of tendons. Tenocytes are flat and aligned parallel to the direction of collagen fibers, which helps them transmit mechanical and chemical signals efficiently throughout the tendon. In addition to collagen fibers and tenocytes, tendons also contain a certain amount of matrix and interstitium, which are filled between cells and fibers (Chen et al., 2022). The matrix is primarily composed of proteoglycans and glycoproteins, which are highly absorbent and help maintain tendon lubrication and serve as a medium for nutrient transport (Subramanian and Schilling, 2015). The presence of interstitium is critical to the overall health and function of the tendon, providing a supportive environment that helps maintain the tendon's structural stability and transmit force. The microstructure of tendons shows a hierarchical tissue structure, from single collagen molecules to microfibers, to fiber bundles, and finally to the entire tendon. This hierarchical structure enables tendons to have extremely high mechanical properties, which can not only withstand high-intensity tensile forces, but also have a certain degree of elasticity, ensuring effective force transmission between muscles and bones (Zabrzyński et al., 2018). This distinctive composition and alignment enable tendons and ligaments to endure substantial mechanical stress during daily activities and athletic pursuits (Zitnay and Weiss, 2018).

2.2 Definition and characteristics of TDSCs

From a developmental perspective, tendon tissue originates from the mesoderm in the embryo. Some mesoderm cells separate and concentrate in the ectoderm. These cells differentiate into bones, cartilage, tendons, and other connective tissues. Cells characterized by Sox9 positive expression in tendon tissue are considered to be the source of TDSCs. One of the defining characteristics of TDSCs lies in their capacity for self-renewal, which denotes their ability to replicate while retaining an undifferentiated state—an essential aspect for continual tissue maintenance. These cells exhibit multipotency, suggesting their potential to differentiate into various cell types, primarily tenocytes, but also potentially including adipocytes, chondrocytes, and osteocytes under specific conditions (Bi et al., 2007; Rui et al., 2010; Liu Q. et al., 2018). The morphological characteristics of TDSC mainly show spindle or fibroblast morphology (Figure 1B). The initial growth rate is slow, and it generally reaches 40%–50% confluence about 10 days after separation (Zhang Z. et al., 2021). Identification of TDSCs involves specific surface markers, such as Scleraxis (Scx), a critical transcription factor in tendon development, and Mohawk (Mkx), another tendon-specific transcription factor. Additional markers include tenomodulin (Tnmd), a type II transmembrane glycoprotein, and CD44, a cell surface glycoprotein involved in cell-cell and cell-matrix interactions. These markers are used to isolate and identify TDSCs from tendon tissues. Moreover, markers such as CD146, CD105, CD90, Oct4, SSEA-4, and Nucleostemin are also employed for identifying TDSCs (Yang et al., 2016; Lee et al., 2018; Jo et al., 2019; Li et al., 2021; Wei and Lu, 2021). At the cellular marker level, the molecular markers of TDSCs closely resemble those of bone marrow mesenchymal stem cells (BM-MSCs). However, TDSCs specifically express tendon markers such as Scx, Mkx, Tnmd, and Col I (Zhang et al., 2018). Similar to BM-MSCs, TDSCs possess differentiation potential toward osteoblasts,

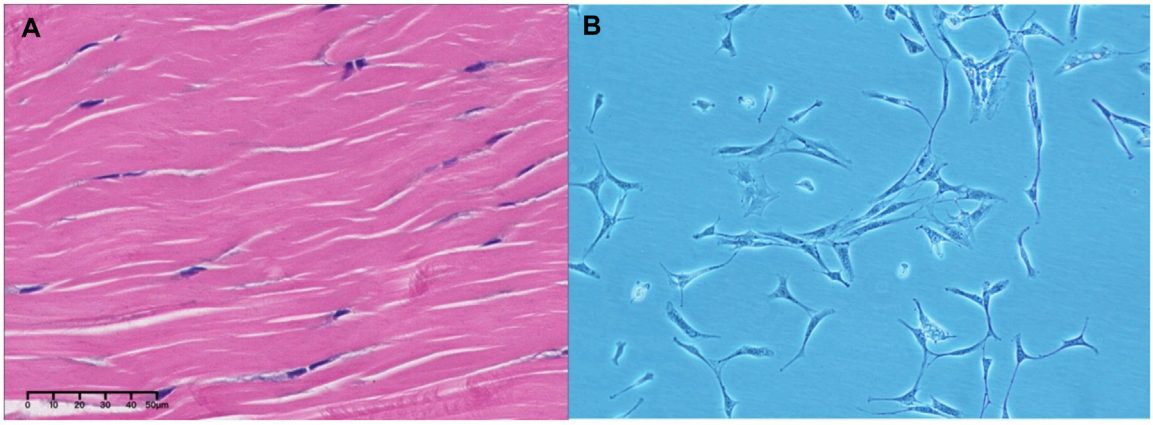


FIGURE 1
Morphology and structure of tendons and TDSCs. **(A)** Morphological picture of tendon tissue; **(B)** Morphological picture of TDSCs. **(A,B)** are pictures obtained by the author in previous research.

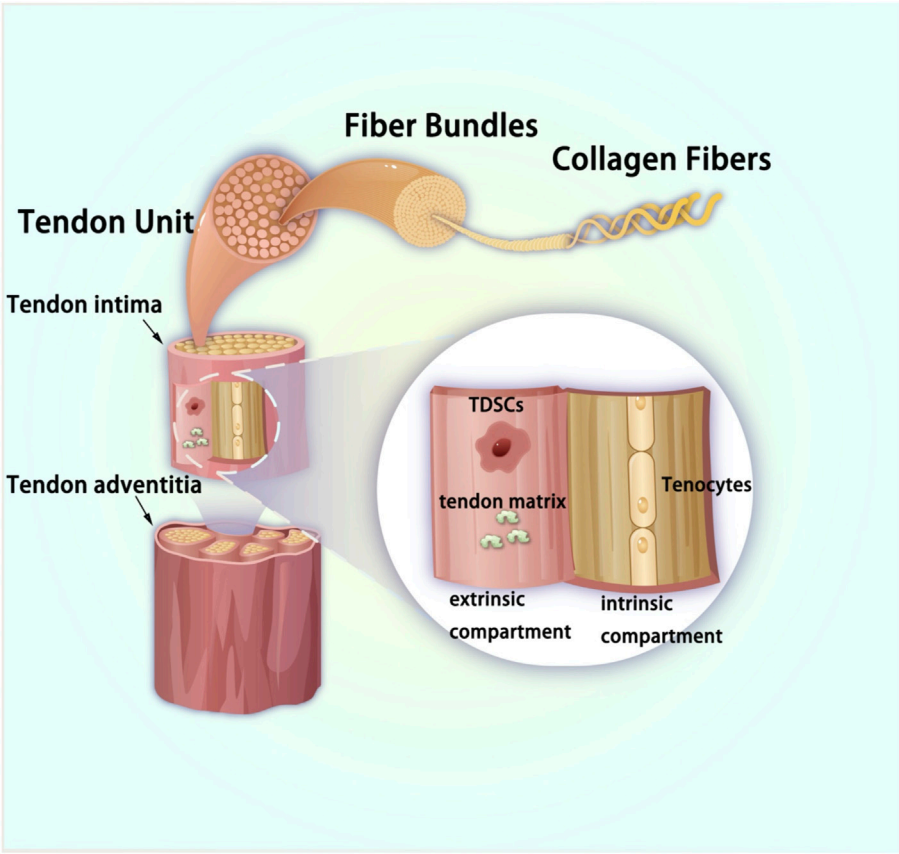


FIGURE 2
Pattern picture of tendon tissue.

chondrocytes, tendons, and adipocytes. Notably, TDSCs exhibit enhanced chondrogenic and tenogenic differentiation capabilities (Jiang et al., 2023). Following tendon injury, the altered local microenvironment may induce aberrant differentiation of TDSCs, leading to impaired tendon repair (Zhang and Wang, 2014). TDSCs play a pivotal role in maintaining tendon homeostasis by contributing to the continuous turnover of the extracellular matrix within tendons, thereby ensuring structural integrity and functionality. Furthermore, they exhibit responsiveness to mechanical stimuli, a crucial aspect given the primary function of

tendons in force transmission (Wang HN. et al., 2020). Changes in mechanical loading can influence TDSC behavior, impacting both their proliferation and differentiation.

Upon tendon injury, TDSCs become activated, migrating to the site of damage. Subsequently, they proliferate and differentiate to replace lost or damaged cells, playing a fundamental role in the initial stages of tendon repair. However, their response may occasionally lead to aberrant healing, characterized by scar tissue formation, highlighting the necessity for precise modulation of TDSC activity to optimize tendon repair (Lui and Chan, 2011; Huang et al., 2021).

TDSCs exhibit substantial promise in the realms of regenerative medicine and tissue engineering. These cells can be harvested, cultured, and potentially subjected to genetic modification to augment their regenerative potential. They are explored in various therapeutic approaches, including stem cell injections (Yin et al., 2018; Chen et al., 2021), tissue-engineered constructs (Zhang and Cheng, 2013), and gene therapy (Lai et al., 2022), to improve tendon repair and function. TDSCs play an integral role in tendon biology, contributing significantly to both the maintenance and repair of tendon tissues. Their capacity for self-renewal and differentiation, combined with their responsiveness to mechanical stimuli, positions them as pivotal targets in strategies designed to improve tendon regeneration and repair (Harvey et al., 2019). Understanding the detailed characteristics and behaviors of TDSCs is imperative for advancing therapies related to tendons and ameliorating outcomes in tendon injuries and disorders.

2.3 Methods for isolation and culture of TDSCs

The isolation of TDSCs entails enzymatic digestion of tendon tissue to release the cells, followed by employing cell sorting techniques like flow cytometry to isolate stem cell markers. The digestive juice currently used to digest tendon tissue is mainly type I collagenase (Randelli et al., 2016a) or type II collagenase (Brown et al., 2014). Upon isolation, TDSCs are cultured in specialized media that sustains their growth and stemness. These conditions often incorporate a three-dimensional scaffold mirroring the native tendon environment, thereby fostering natural behavior and differentiation potential in the cells. However, reported culture conditions exhibit variance across studies. While the most commonly used medium is low-glucose. Dulbecco's modified Eagle medium (DMEM) medium (Chen et al., 2012; Qiu et al., 2019; Ni et al., 2021), others include high-glucose DMEM medium (Brown et al., 2014; Rajpar and Barrett, 2020), α-MEM medium (Feng et al., 2020), or DMEM/F12 medium (Song et al., 2017). Moreover, experiments commonly supplement with 10%–20% fetal bovine serum (Randelli et al., 2016b; Yin et al., 2019; Lai et al., 2022; Ni et al., 2023). In addition to culture media and serum, researchers often add certain small molecule substances to the culture system to promote cell growth. The most common ones are ascorbic acid (Popov et al., 2015a) and L-glutamine (Ni et al., 2012). A few researchers also add Growth factor fibroblast growth factor-2 (FGF2) (Di Meglio et al., 2020) or connective tissue growth factor (CTGF) (Lui et al., 2016). However, there is currently no absolutely unified standard for the training system, which may be related to the habits of researchers and the specific purpose of the experiment.

Progress in culture techniques, exemplified by dynamic bioreactors applying mechanical stimulation, has notably amplified both the proliferation and tenogenic differentiation of TDSCs, yielding a more physiologically relevant model for investigating tendon biology and regenerative therapies. Table 1 illustrates 10 representative cell isolation and culture conditions. Recent research has indicated that 3D culture systems favor the growth and differentiation of TDSCs, establishing them as a pivotal entry point for tissue engineering (Hsieh et al., 2018; Yin et al., 2020).

3 Application of TDSCs in tendon tissue engineering

TDSCs represent a distinct subset of stem cells located within tendons, characterized by their unique ability to differentiate into tenocytes, the fundamental cells responsible for maintaining tendon structure and function. The domain of tendon tissue engineering endeavors to harness the regenerative potential of these cells to mend or substitute damaged tendon tissue, posing a considerable challenge owing to the restricted healing capabilities of tendons. Tables 2–4 provides an overview of ongoing research concerning TDSCs in tendon tissue engineering.

3.1 Tissue engineering seed cell optimization

Pre-treatment of TDSCs involves subjecting these cells to specific growth factors or environmental cues before transplantation. This process aims to augment the cells' inherent healing capabilities, rendering them more effective once introduced into the injury site. Furthermore, this approach can be customized to promote specific cellular behaviors, such as increased proliferation or directed differentiation, which are indispensable for facilitating effective tendon repair (Wang et al., 2023).

In the research on TDSCs treatment of tendon-related diseases, the study of molecular mechanisms has always been a hot topic. A clear molecular mechanism can provide a reference for pretreatment of seed cells for tissue engineering. Cheng et al. (2014) intervened the expression of TNF alpha-stimulated gene/protein 6 (TSG-6) in TDSC and found in the experiment that TDSCs can promote the repair of rotator cuff injury. Therefore, TSG-6 plays an important role in TDSCs repairing rotator cuff injuries. In a human TDSC study, TDSCs from fetal Achilles tendons were isolated and cultured to overexpress FGF-2. The researchers found that after overexpression of FGF-2, the expression of tendon-related factors Scx and type III collagen increased. Transplanting FGF-2 overexpressing TDSC into the Achilles tendon notch in rats can promote Achilles tendon healing (Guo et al., 2020). In the process of tendinopathy, inflammation and programmed death are pathological processes that inhibit tendon self-repair. Tripartite Motif Containing 54 (TRIM54) inhibits inflammation and

TABLE 1 Ten representative cell isolation and culture conditions.

Cell source	Digestive enzymes	Digestion time	Medium	FBS (%)	Reference
Human	3 mg/mL type I collagenase and 4 mg dispase II	1.5 h	α-MEM	20	Randelli et al. (2016a)
Human	4 mg/mL type I collagenase	2 h	LG-DMEM	10	Qiu et al. (2019)
Rat	3 mg/mL type I collagenase	2 h	HG-DMEM	10	Han et al. (2019)
Rat	3 mg/mL type I collagenase	2 h	LG-DMEM	20	Li et al. (2019)
Rat	3 mg/mL type I collagenase	2.5 h	HG-DMEM	10	Wang et al. (2020b)
Mice	1% type II collagenase	0.75 h	HG-DMEM	10	Brown et al. (2014)
Mice	1.5 mg/mL II collagenase and 2 mg dispase II	0.5 h	α-MEM	10	Feng et al. (2020)
Rabbit	3 mg/mL type I collagenase	Not given	LG-DMEM	10	Ni et al. (2021)
Rabbit	0.25% collagenase	Overnight	LG-DMEM	10	Shen et al. (2012)
Horse	Collagenase (details not given)	Overnight	HG-DMEM	10	Rajpar and Barrett (2020)

FBS, represent fetal bovine serum; LG, represent low glucose; HG, represent high glucose.

apoptosis in rat TDSCs by stabilizing YOD1 deubiquitinase (YOD1) (Chen et al., 2024).

Promoting tendon directional differentiation of TDSCs is an important research topic in this field. The study found that STIP1 homology and U-box containing protein 1 (STUB1) (Han W. et al., 2017), transforming growth factor-beta 1 (TGF-β1) (Han P. et al., 2017), and focal adhesion kinase- Extracellular signal-regulated kinase 1/2 (FAK-ERK1/2) signaling pathways (Liu C. et al., 2018) are factors that promote the differentiation of TDSC into tendon, while tumor necrosis factor α (TNF-α) (Han P. et al., 2017), interleukin 10 (IL -10) (Deng et al., 2018) and the janus kinase/signal transducer and activator of transcription 3 (JAK/Stat3) signaling pathways (Deng et al., 2018) are inhibitors of this process. In tendon diseases, in addition to the repair of tendon tissue, the repair of the tendon-bone connection is a more important issue. Because in most tendon diseases, such as rotator cuff injuries, cruciate ligament reconstruction, etc., it is necessary to restore the connection and normal physiological gradient between the tendon and bone. TDSCs are often used to study the role of tendon-bone junction repair. The normal tendon-bone insertion gradient is a bone-mineralized fibrocartilage-nonmineralized fibrocartilage-tendon structure. Researchers study the gradient of tendon-bone insertion point repair by TDSCs from different angles and mechanisms. Studies have found that bone morphogenetic protein 2 (BMP-2) and TGF-β1 can promote osteogenic and tenogenic differentiation of mouse TDSC *in vivo* and *in vitro* (Wei et al., 2023). As an upstream signal of TGF-β1, transforming growth interacting factor 1 (TGIF1) plays an inhibitory role in the chondrogenic differentiation process of TDSCs. Manipulating the expression of TGIF1 can promote the repair of tendon-bone junctions (Chen et al., 2015). The aging of stem cells is manifested by the decrease in differentiation and proliferation capabilities, apoptosis or fibrosis, resulting in a decrease in the tendon's ability to self-repair. Targeting the aging mechanism of TDSCs can provide important targets for the treatment of tendon-related diseases. A study on human-derived TDSCs found that the expression of CBP/p300-interacting transactivator with Glu/Asp-rich C-terminal domain, 2 (CITED2) decreased in aging tendon, and TGFβ2 regulated the aging of TDSC through CITED2 (Hu et al., 2017). Inflammation can induce premature senescence of TDSCs, and the NF-κB signaling pathway plays an important regulatory role in this process (Xu K. et al., 2021; Wang et al., 2022). During tendon repair, excessive osteogenic differentiation can cause complications of heterotopic ossification. In rat TDSCs, ERK1/2 is involved in the osteogenic differentiation of TDSC (Li et al., 2016).

Co-culture systems, involving the simultaneous cultivation of diverse cell types like BM-MSCs and TDSCs, play a pivotal role in enhancing tendon-bone healing in tissue engineering. The study by Liu et al. (2019) demonstrated that optimal co-culture ratios, particularly a 1:1 ratio of BM-MSCs and TDSCs, significantly boost cell proliferation, differentiation, and tenogenic activities. These interactions are further influenced by factors such as Tenascin C, capable of impacting cellular behaviors like osteogenesis and chondrogenesis. Additionally, molecular pathways involving Rho-associated kinase (ROCK), insulin-like growth factor 1 receptor (IGF-1R), and methyl ethyl ketone (MEK) are implicated in these processes, highlighting a complex regulatory network. Another study suggests that a balanced co-culture of BM-MSCs and TDSCs not only enhances proliferation and collagen production but also elevates tenogenic marker gene expression and collagen matrix production, particularly in equal proportion co-cultures (Wu et al., 2016). This approach has been proven to effectively promote tendon healing in a rat model, underscoring its potential as an enhanced cell source for tendon tissue engineering (Figure 3).

Moreover, interactions between human adipose-derived stem cells and tendon cells have shown temporal regulation of tenogenesis, emphasizing the significance of extracellular matrix (ECM) synthesis and remodeling in co-cultured systems. These interactions lead to controlled cell proliferation and elongation, along with an improved ratio of collagen type I to III, crucial for generating high-quality tendon tissue. Co-culture systems in tendon tissue engineering are indispensable for optimizing tendon-bone healing (Costa-Almeida et al., 2018). The synergy between different cell types in co-cultures, coupled with the regulation of specific molecular pathways and ECM remodeling, plays a vital role in enhancing tendon repair and regeneration.

TABLE 2 Application of TDSCs in tissue engineering seed cell optimization.

Cell source	Stimulus method	Assessment methods	Main evaluation index	Main finding	Reference
Rat	TSG-6 knockdown	Biomechanical Testing	Failure load	The ultimate stress was greater in the TDSCs group (4.91 ± 1.41 N/mm^2) as compared with the Control group (2.99 ± 1.04 N/mm^2) ($p < 0.05$). The TSG-6 silenced group (3.36 ± 0.96N/mm^2) showed no benefit over the control group	Cheng et al. (2014)
Rat	FGF-2 overexpression	Biomechanical Testing	Failure load	The stiffness of the Achilles tendon in Control group, Vector group, and FGF-2 group at 4 weeks postoperative were 3.87 ± 0.63 N/m, 6.72 ± 1.72 N/m, and 16.21 ± 1.97 N/m	Guo et al. (2020)
Rat	Trim54 overexpression	Biomechanical Testing and Morphological analysis	failure load and histological scores	Overexpressed TRIM54 in these injured rats and observed a rescue effect in morphology and Biomechanical Testing. However, the author did not give specific values	Chen et al. (2024)
Rat	Chip overexpression	cytological experiments	proliferation and differentiation ability	Overexpression of Chip can significantly increase the proliferation and tenogenic differentiation ability of TDSCs	Han et al. (2017a)
Rat	TNF-α, TGF-β	cytological experiments	differentiation ability	The combined use of 5 ng/mL TGF-β1 and 0.0025 ng/mL TNF-α can significantly increase the differentiation ability of TDSCs	Han et al. (2017b)
Rat	different matrix stiffness	cytological experiments	proliferation and differentiation ability	The higher the matrix stiffness, the stronger the proliferation and differentiation ability of TDSCs in culture	Liu et al. (2018b)
Rat	Different concentrations of IL-10	cytological experiments	migration and differentiation ability	10 ng/mL concentration of IL-10 significantly inhibited the migration and differentiation ability of TDSCs	Deng et al. (2018)
Rat	Cell sheet with BMP and TGF-β gene intervention	Radiology, biomechanics	failure load and bone formation	These results indicate that the Ad-BMP-2/TGF-β1-transfected TDSC sheet promotes biomechanical strength and reduces inflammatory infiltration	Wei et al. (2023)
Rat	TGIF1 knockdown	Morphological analysis	Cartilage-related staining	Knockdown of Tgif can promote chondrogenic differentiation of TDSCs	Chen et al. (2015)
Human	CITED2 knockdown	Cytology and Molecular Biology Experiments	β-gal staining	Downregulation of CITED2 contributes to TGFβ-mediated senescence in TDSCs	Hu et al. (2017)
Rat	AuNC-siRNA	Cytological and biomechanical experiments	β-gal staining and failure load	Gold nanoparticles can improve inflammation-induced aging by blocking the IKKβ/NF-κB pathway. It can promote an increase in failure load of 10% in aged tendons	Wang et al. (2022)
Rat	Spironolactone	Cytological and morphological experiments	β-gal staining and heterotopic ossification	Spironolactone (1 μM and 10 μM) can improve TDSC aging and tendon heterotopic ossification. Its mechanism may be through the NF-κB/MAPK Pathway	Xu et al. (2021a)

(Continued on following page)

TABLE 2 (*Continued*) Application of TDSCs in tissue engineering seed cell optimization.

Cell source	Stimulus method	Assessment methods	Main evaluation index	Main finding	Reference
Rat	Low oxygen environment	Cytology and Molecular Biology Experiments	alizarin red staining and Osteogenic differentiation related indicators	Hypoxic environment (3% O_2) can inhibit the osteogenic differentiation of TDSCs	Li et al. (2016)
Rat	Co-culture with BM-MSC	Cytology and Molecular Biology Experiments	Cell proliferation ability and Osteogenic differentiation related indicators	1:1 Co-culture can increase the proliferation capacity of TDSCs compared with single culture	Li et al. (2016)
Rat	Co-culture with BM-MSC	Cell and animal experiments	Cell proliferation and tendon differentiation ability	In the group with a cell ratio of 1:1, TDSCs had stronger proliferation and tenogenic differentiation abilities	Wu et al. (2016)
Human	Co-culture with ADSC	Cytology and Molecular Biology Experiments	tendon differentiation ability	On the seventh day of co-culture of tendon stem cells and adipose-derived mesenchymal stem cells, type I collagen deposition increased significantly	Costa-Almeida et al. (2018)
Mice	TGF-β, FGF-4 treatment	Cytology and Molecular Biology Experiments	tendon differentiation ability	Similar responses as TPCs to specific treatments suggest MSCs have tenogenic potential	Brown et al. (2015)
Mice	Tnmd Knockout	Cytology and Molecular Biology Experiments	β-gal staining and tendon differentiation ability	Tnmd knockout causes TDSC to be susceptible to aging but does not affect their differentiation ability	Alberton et al. (2015)

3.2 Advances in graft healing techniques

The application of TDSC sheets has significantly advanced graft healing techniques in tendon repair, particularly in anterior cruciate ligament (ACL) reconstruction. These sheets, often combined with growth factors, serve as a direct source of reparative cells and bioactive molecules at the injury site, thereby fostering improved graft integration and healing outcomes. In a rat model, TDSC sheets were employed to encase ACL grafts. Treated with connective tissue growth factor and ascorbic acid, the sheets augmented graft healing by elevating tunnel bone mineral density and bone volume, enhancing osteointegration, and preserving greater intra-articular graft integrity. These findings were supported by the presence of GFP-positive cells, signifying the successful integration of the TDSC sheets into the healing process (Lui et al., 2014). Zhang Y. et al. (2021) implemented a stepwise culture strategy for TDSCs, resulting in a significant upregulation of tendon-related genes and proteins. By integrating this culture system with 3D printing technology, researchers embedded chemically empowered TSPCs within a biocompatible hydrogel to engineer tendon grafts, displaying an enhanced capacity to promote functional tendon repair and regeneration both *in vivo* and *in situ*. Using genetically modified TDSCs to improve cell sheet properties. Wei et al. (2023) found that BMP-2 can promote osteogenic differentiation of TDSC, while TGF-β1 can promote tenogenic differentiation of TDSC. Simultaneous transfection of BMP-2 and TGF-β1 into TDSCs can significantly improve the maturation of tendon-bone junctions. If the Cell sheet is made with allogeneic TDSC, it may cause rejection (Dang et al., 2021). Therefore, decellularization and leaving the extracellular matrix will greatly reduce the possibility of rejection (Inci et al., 2020). Yao et al. (2023) investigated the use of decellularized TDSC (dTDSC) sheets, which retained the bioactive factors of the natural extracellular matrix and fostered graft healing after ACLR. These sheets exhibited heightened bone formation, improved graft osteointegration, and enhanced midsubstance graft integrity. This method also influenced macrophage polarization and MMP/TIMP expression, contributing to better healing outcomes. These advancements underscore the potential of TDSC sheets in enhancing graft healing in tendon repair, particularly in ACL reconstruction. Through leveraging the unique properties of TDSCs and innovative techniques such as 3D printing and decellularization, these approaches offer promising strategies to improve graft integration, facilitate tissue regeneration, and ultimately enhance the outcomes of tendon repair surgeries.

3.3 Mechanical stimuli in TDSC differentiation

Mechanical and molecular stimuli play pivotal roles in influencing the behavior and differentiation of TDSCs. Mechanical stimuli, such as stretch or compression, mimic the natural mechanical environment of tendons, while molecular stimuli encompass signaling molecules that can guide cell behavior. Understanding the impact of these stimuli on TSPC differentiation is crucial for devising effective strategies for tendon repair. Several research endeavors elucidate these mechanisms. In their study, Liu et al. (2017) delved into the influence of mechanical stretching on TDSC differentiation, revealing the crucial role of the cystic fibrosis transmembrane conductance regulator (CFTR) in tenogenic differentiation. Their findings indicated irregularities, diminished mechanical properties, and reduced matrix formation in tendon tissues of CFTR-dysfunctional mice. Furthermore, RNA sequencing analysis

TABLE 3 Summary of TDSC research in the field of mechanical stress.

Cell source	Mechanical stimuli type	Stimulation frequency	Elongation	Stimulation time	Research focus	Main finding	Reference
Rat	UCMT	1 Hz	4%	0.5 h	Mechanism of cystic fibrosis transmembrane conductance regulator regulating tendon stem cell differentiation	Cystic fibrosis transmembrane conductance regulator plays an important role in tenogenic differentiation and tendon regeneration by inhibiting the b-catinin/pERK1/2 signaling pathway	Liu et al. (2017)
Rat	UCMT	0.5 Hz	4% or 8%	4 h	Does mechanical stimulation affect TDSC osteogenic differentiation	Repetitive tensile loading increased the expression of BMP-2 and addition of BMP-2 enhanced osteogenic differentiation of TDSCs	Rui et al. (2011)
Rat	UCMT	0.5 Hz	2%	4 h	Mechanism of heterotopic ossification after overuse of tendon tissue	UMT induced osteogenic differentiation of rTDSCs via the Wnt5a-RhoA pathway	Shi et al. (2012)
Rabbit	UCMT	0.5 Hz	4% or 8%	12 h	Exploring the mechanobiological responses of TDSCs	Low mechanical stretching (4%) may be beneficial to tendons by enabling differentiation of TSCs into tenocytes to maintain tendon homeostasis. However, large mechanical loading (8%) may be detrimental, as it directs differentiation of TSCs into non-tenocytes in tendons	Zhang and Wang (2010)
Rat	UCMT	0.3 Hz, 0.5 Hz, and 1.0 Hz	2%, 4%, and 8%	3 h/day for 7 days	Exploring the effects of different stretch intensities on the proliferation and differentiation of TDSCs	Stretching had a significant effect on type I collagen, tenascin-C, tenomodulin, and scleraxis of TDSC, especially at 0.5 Hz frequency with 4% amplitude	Xu et al. (2015)
Rat	UCMT	1 Hz	8%	48, 60, or 72 h	Exploring the causes of heterotopic ossification after tendon overuse	Osteogenic differentiation of TDSCs via the Wnt5a/Wnt5b/JNK signaling pathway	Liu et al. (2015)
Rat	UCMT	0.5 Hz	8%	12 h	Exploring the effect of PRP on TDSCs under strong tension	PRCR promotes tenocyte differentiation while inhibiting adipocyte, chondrocyte, and osteocyte lineages, which are believed to hinder tendon healing	Chen et al. (2012)
Rat	UCMT	0.2 Hz	10%	48 h	To investigate the effect of mechanical stress on the co-culture system of BMSCs and TDSCs	Mechanical stimulation enhances the regenerative potential of BMSCs and TCs in tendon-bone healing by promoting the proliferation and differentiation of local precursor cells	Song et al. (2017)

(Continued on following page)

TABLE 3 (*Continued*) Summary of TDSC research in the field of mechanical stress.

Cell source	Mechanical stimuli type	Stimulation frequency	Elongation	Stimulation time	Research focus	Main finding	Reference
Rat	UCMT	0.5 Hz	4 or 8%	2 h	To explore the effects and mechanisms of excessive mechanical stimulation on TDSCs *in vitro*	Mechanical loading activates mTOR signaling in TDSCs	Nie et al. (2021)
Mice	treadmill running	3 h/day, 4 h/day, and 5 h/day	/	5 days/week	Exploring the responses of TDSCs and mature tenocytes in tendons to mechanical stress	mTOR maintains tendon homeostasis by promoting TDSC differentiation into tenocytes. In contrast, improper tension causes tendinopathy by inducing non-tenocyte differentiation in TSCs	Zhang et al. (2020)
Human	UCMT	1 Hz	1%, 5% or 8%	1 h/day for 3 days	Comparison of the effects of different mechanical stimulation protocols on TDSCs	8% mechanical loading had a positive effect on matrix proteins, integrins and matrix metalloproteinases, and activation of integrin downstream kinases p38 and ERK1/2 in TDSCs	Popov et al. (2015b)
Mice	3D UCMT	0.25 Hz	6%	8 h	Establishing a protocol to simulate the stress effects of TDSCs during tendon development	This protocol,6% strain, 0.25 Hz, 8 h followed by 16 h rest for 6 days, could mimic cell differentiation in the tendon	Chen et al. (2020)
Mice	UCMT and 3D UCMT	0.25 Hz	6%	8 h	Comparison of the effects of uniaxial and biaxial mechanical stretching on TDSCs	3D cell niches are essential for tendon tissue development	Wang et al. (2018)

UCMT, represent uniaxial cyclic mechanical tension.

unveiled abnormal activation of the Wnt/β-catenin signaling pathway in TDSCs from these mice. Notably, inhibiting the pERK1/2 signaling pathway fostered tenogenic differentiation and tendon regeneration, suggesting that CFTR regulates tendon differentiation through the β-catenin/pERK1/2 pathway.

Zhang and Wang (2010) investigated the impact of mechanical stretching on TDSC behavior, observing that low mechanical stretching facilitated TDSC differentiation into tenocytes, potentially maintaining tendon homeostasis. However, excessive stretching led to differentiation into non-tenocyte lineages, contributing to tendinopathy features such as lipid accumulation and tissue calcification. Additionally, Chen et al. (2012) explored the effect of autologous platelet-rich clot releasate (PRCR) on TDSCs subjected to mechanical stretching, finding that PRCR increased TDSC numbers and the production of collagen types I and III and TGF-β1, favoring tenocyte differentiation while suppressing adipocyte, chondrocyte, and osteocyte lineages.

Researchers have also discovered that mechanical stress impacts the tendon-bone healing process. Continuous passive motion (CPM) therapy has been shown to enhance healing by promoting fibrocartilage formation, increasing load-bearing capacity, and up-regulating key genes at the tendon-bone interface. Moreover, mechanical stretch has been found to improve cell proliferation and matrix synthesis in a co-culture of BM-MSCs and TCs, underscoring the regenerative potential of local precursor cells in tendon-bone healing (Song et al., 2017). The interplay between mechanical and molecular stimuli in TDSC differentiation and tendon healing offers valuable insights for future tendon injury management strategies.

For TDSCs, the matrix morphology and surface morphology in the cell culture environment have an important impact on the differentiation of TDSCs. Culturing normal TDSCs on a stiff matrix can promote TDSCs maturation and tendon differentiation (Kim et al., 2018). Preserving the stemness of TDSCs *in vitro* culture is a very important issue (Zhang and Wang, 2013; Chen et al., 2016; Yu et al., 2017). Compared with ordinary plastic culture plates, culturing TDSC on the surface of decellularized tendons can preserve the stemness of TDSC (Zhang et al., 2011). The use of a specially designed parallel microgrooved polydimethylsiloxane (PDMS) membrane to culture TDSCs can guide the elongation of TDSCs. Under such culture conditions, the ability of TDSCs to differentiate into adipogenesis, chondrogenesis, and osteogenesis is significantly inhibited. The ability of tendon differentiation is enhanced (Shi et al., 2017). Therefore, the application of hard, regular, and parallel culture matrix morphology in vitro culture systems may be meaningful for subsequent stem cell treatments.

TABLE 4 Application of TDSC in scaffold-based tendon regeneration.

Cell source	Scaffold type	Favorable factors in scaffold	Disease models	Main finding	Reference
Rat	cell sheet	TDSC and ECM	ACL rupture	The TDSC sheet improved early graft healing after ACL reconstruction in the rat model	Lui et al. (2014)
Rat	Decellularized Cell Sheet	ECM	ACL rupture	dTDSC sheets alleviate the quality control and safety concerns of cell transplantation and can be used as a cell-free alternative for the promotion of graft healing in ACLR.	Yao et al. (2023)
Rat	different Culture matrix	nanotopographic cues and substrate stiffness	tendinopathy	The differentiation of TDSCs is affected by the mechanical stiffness and nanotopography of the culture substrate, which has implications for tendon regeneration and healing	Kim et al. (2018)
Rabbit	engineered tendon matrix	TDSC and ECM	Tendon regeneration	ETM can effectively expand TDSCs *in vitro* and improve tendon repair *in vivo*	Zhang et al. (2011)
Rat	Decellularized tendon matrix	TDSC and ECM	Achilles tendon rupture	Tendon-derived decellularized matrices combined with tendon stem/progenitor cells provide a promising strategy for functional tendon tissue regeneration	Song et al. (2018)
Macaca mulatta	Decellularized tendon matrix	ECM	Tendon regeneration	T-gel retains the nanofibrous structure and bioactive factors of native tendon ECM, making it a potential hydrogel for tendon regeneration	Ning et al. (2021)
Human	Decellularized matrix (tendon, bone and dermis)	ECM	Tendon regeneration	Implantation of this cell-scaffold construct led to a more mature structure (histology score: 4.08 ± 0.61 vs 8.51 ± 1.66), larger collagen fibrils (52.2 ± 1.6 nm vs 47.5 ± 2.8 nm) and stronger mechanical properties [stiffness: 21.68 ± 7.1 Nm m (-1) vs13.2 ± 5.9 Nm m (-1)] of repaired tendons compared to the control group	Yin et al. (2013)
Rat	Decellularized Small Intestinal Submucosa	ECM	Achilles tendon rupture	Biologically prepared SIS scaffolds synergistically promote tendon regeneration with TDSCs, while achieving anti-adhesion through M2 macrophage polarization	Mao et al. (2022)
Rat	Young Decellularized tendon matrix	ECM	Tendon regeneration	The impaired capacity of aged TDSCs can be rejuvenated by exposure to young DECM	Jiang et al. (2018)
Rat	Synthetic Materials	Bioactive agent (KGN and MBGs)	rotator cuff injury	Bioactive agent-loaded hydrogels add value to biomaterials used in chronic tendon-bone junction injuries	Huang et al. (2022)
Human	Synthetic Materials	3D nanofiber	Achilles tendon rupture	the RADA-based hydrogels exert a rejuvenating effect by recapitulating *in vitro* specific features of the natural microenvironment of human TSPCs, which strongly indicates their potential to direct cell behaviour and overcome the challenge of cell aging and degeneration in tendon repair	Yin et al. (2020)
Human	Synthetic Materials	3D microenvironment	Tendon regeneration	Thermosensitive BC hydrogel holds great potential as an injectable cell delivery carrier of TSPCs for tendon tissue engineering	Yin et al. (2018)
Human	Synthetic Materials	aligned focal contact points	Tendon regeneration	These results showed a novel strategy for directing stem cell behavior without the use of exogenous growth factors or pre-aligned COL I fibers, and propose that anisotropic nanocomposite hydrogels hold great potential for tendon tissue engineering applications	Xu et al. (2021b)

(Continued on following page)

TABLE 4 (*Continued*) Application of TDSC in scaffold-based tendon regeneration.

Cell source	Scaffold type	Favorable factors in scaffold	Disease models	Main finding	Reference
Human	Synthetic Materials	3D RADA peptide	Patellar Tendon rupture	The combination of TSPC and nanofiber hydrogel provide an optimistic alternative method to accelerate functional tendon repair with reduced heterotopic ossification	Zhang et al. (2023)
Rat	Synthetic Materials	Chitosan/β-Glycerophosphate/ Collagen	Achilles tendon rupture	The improved healing was indicated by the improvement in histological and immunohistochemistry outcomes and the increase in the biomechanical properties of the regenerated tissue at both 4 and 6 weeks post-injury	Yang et al. (2017)
Rat	Synthetic Materials	chitosan	Tendon regeneration	TSPC-seeded chitosan scaffolds offer a feasible approach for tendon repair	Chen et al. (2018)
Rat	Synthetic Materials	3D nanofiber	Tendon regeneration	Electrospun bundled nanofiber yarns (NFYs) replicate native tendon tissue structure, highlighting their potential as biomimetic scaffolds for tendon regeneration	Yang et al. (2022)
Rabbit	Synthetic Materials	aligned tissue morphology	Achilles tendon rupture	Engineered scaffolds facilitate TDSC proliferation and migration, promote tenogenesis, and enhance mechanical properties, indicating their value in tendon tissue engineering	Ning et al. (2022)
Rat	Synthetic Materials	3D aligned microenvironment	Achilles tendon rupture	3D-aligned TSPCs in a biomimetic topology are promising for functional tendon regeneration	Li et al. (2023)
Rat	Synthetic Materials	Silver nanoparticles	Tendon regeneration	Silver nanoparticles (75–150 μg mL^{-1}) have a certain degree of toxicity to tendon stem cells. NAC can reduce the toxicity	Cheung et al. (2016)
Human	Synthetic Materials	3D aligned electrospun nanofiber threads	Tendon regeneration	Key scaffold features mimicking native tissue are crucial for developing engineered tendon substitutes	Laranjeira et al. (2017)
Rat	Synthetic Materials	Silk fibroin	Tendon regeneration	The 10 μm SF film group had the highest percentage of oriented cells and the most significant changes in cell morphology, as well as the highest expression of COL1A1, TNC, TNMD, and SCX.	Lu et al. (2020a)
Rat	Synthetic Materials	Silk fibroin	Tendon regeneration	SF films with a bionic microstructure may serve as a scaffold, provide biophysical cues to alter the cellular adherence arrangement and cell morphology, and enhance the tenogenic gene and protein expression in TSPCs. FAK activation plays a key role during this biological response process	Lu et al. (2020b)
Rabbit	silk-collagen	silk scaffold	rotator cuff injury	Allogeneic TSPC-seeded knitted silk-collagen sponge scaffolds show clinical potential for tendon tissue engineering	Shen et al. (2012)
Human	Synthetic Materials	aligned nanofibers	Tendon regeneration	An aligned electrospun nanofiber structure creates an instructive microenvironment for hTSPC differentiation, useful for engineered tendon development	Yin et al. (2010)
Rat	Synthetic Materials	hBM-MSC secretome with keratin electrospun scaffolds	rotator cuff injury	Human mesenchymal stem cell-conditioned medium (hMSCs-CM) increases hTCs viability and density *in vitro*, and the cells integrated into keratin scaffolds show significant benefits	Sevivas et al. (2018)

(Continued on following page)

TABLE 4 (*Continued*) Application of TDSC in scaffold-based tendon regeneration.

Cell source	Scaffold type	Favorable factors in scaffold	Disease models	Main finding	Reference
Mice	Synthetic Materials	Bioinspired bimodal micro-nanofibrous	Achilles tendon rupture	Micro-nanofibrous scaffolds improve the structural and mechanical properties of regenerated Achilles tendons, presenting significant potential for improving tendon tissue engineering outcomes	Yin et al. (2022)
Rat	Synthetic Materials	bioactive electrospun nanofiber membranes	Tendon regeneration	Bioactive electrospun nanofiber membranes are suitable as biomimetic scaffolds in tendon-bone tissue engineering, enhancing tendon-bone healing	Lin et al. (2019)
Rat	Synthetic Materials	3D printed scaffolds	rotator cuff injury	An *in situ* tissue engineering approach could improve rotator cuff repair outcomes	Tarafder et al. (2019)

3.4 Scaffold-based tendon regeneration

Scaffold-based regeneration involves the use of engineered structures specifically designed to facilitate cell attachment, growth, and differentiation. Collagen fiber scaffold used in research on tendon-related diseases (Fan et al., 2008; Sun et al., 2014; Tabesh et al., 2022). Ouyang's team used homemade collagen fiber scaffolds (Chen et al., 2008) as carriers for TDSCs. *In vivo* experiments observed that the combination of collagen fiber scaffolds and TDSCs resulted in significant collagen production and reduced immune rejection (Shen et al., 2012). Compared with randomly arranged collagen fiber scaffolds, parallel-arranged collagen fiber scaffolds can achieve better mechanical properties as carriers of TDSCs (Zheng et al., 2017). Decellularized matrices represent scaffolds derived from natural tissues, wherein cellular components are removed to minimize immunogenicity while retaining the structural and functional cues of the extracellular matrix. Within tendon tissue engineering, these matrices serve as a foundational support guiding TDSC differentiation and promoting tissue regeneration, thus playing a pivotal role in the development of tissue-engineered tendon constructs. Investigative studies have delved into their influence on TDSCs and tendon repair. Collagen matrices extracted from various tissues (tendon, bone, and dermis) facilitated TSPC adhesion and proliferation. Notably, tendon-derived matrix encouraged a tendinous phenotype and suppressed osteogenesis in hTDSCs (Yin et al., 2013). Moreover, engineered tendon matrix (ETM) from decellularized tendon tissues stimulated TSPC proliferation and preserved stemness *in vitro*, subsequently enhancing the formation of tendon-like tissue *in vivo*, underscoring the potential of ETM in tendon healing (Zhang et al., 2011). When combined with TSPCs, decellularized extracellular matrix from porcine tendons demonstrated superior results in promoting tendon regeneration compared to mesenchymal stromal cells (Song et al., 2018). Additionally, decellularized tendon hydrogel from *Macaca mulatta* supported mTDSC proliferation, migration, and tenogenic differentiation, with the native ECM components of T-gel augmenting these behaviors (Ning et al., 2021). The application of decellularized matrices presents promising prospects in tendon tissue engineering, effectively steering TSPC behavior and fostering tendon repair.

Hydrogels utilized in tendon repair constitute water-swollen, cross-linked polymeric networks simulating the extracellular matrix, thus establishing an environment conducive to cell growth and tissue development. Numerous studies have investigated the potential of hydrogel systems combined with TSPCs to enhance tendon healing and regeneration. In a particular study, a sequential culture strategy using small molecules was developed to potentiate TSPCs for enhanced therapeutic applications. Through high-throughput screening, specific small molecules were identified, leading to heightened TSPC proliferation, initiation of tenogenesis, and subsequent maturation. When these chemically empowered TSPCs were embedded within a biocompatible hydrogel using 3D printing technology, they demonstrated improved functional tendon repair and regeneration *in vivo* (Zhang Y. et al., 2021). Another investigation assessed the use of chitosan/β-glycerophosphate/collagen (C/GP/Co) hydrogel in combination with TDSCs for Achilles tendon healing in a rat model. The research illustrated that local application of TDSCs with C/GP/Co hydrogel significantly improved tendon healing compared to control groups, as evidenced by histological, immunohistochemical, and biomechanical assessments (Yang et al., 2017).

Furthermore, Ying et al. (2020) explored the rejuvenation of aged/degenerative human TDSCs using a self-assembling nanofiber matrix composed of RADA peptide hydrogel. This nanocomposite hydrogel supported the survival, proliferation, and rejuvenation of TDSCs, potentially overcoming challenges associated with cell aging and degeneration in tendon repair. Thermosensitive hydrogels, including a butane diisocyanate (BDI)-collagen hydrogel and methylcellulose/polyvinyl alcohol/polyvinylpyrrolidone-based hydrogel, were examined as injectable cell delivery carriers for TDSCs. These hydrogels exhibited biocompatibility, supported TDSC behavior, and induced differentiation toward tenogenic and osteogenic lineages, making them valuable candidates for tendon tissue engineering (Yin et al., 2018). A magnetically-responsive nanocomposite hydrogel composed of collagen type I and aligned iron oxide nanoparticles showed potential for promoting the alignment and tenogenesis of TDSCs, addressing the issue of uniform cell arrangement in tendon tissue engineering (Xu Y. et al., 2021). Mao et al. (2022) utilized TDSCs combined with small intestinal submucosa (SIS) scaffolds to improve Achilles tendon repair. *In vitro*, SIS facilitated TDSC adhesion and tenogenic differentiation. *In vivo*, TDSCs-SIS scaffolds augmented tendon regeneration and reduced adhesion formation through M2 macrophage polarization. An injectable thermosensitive

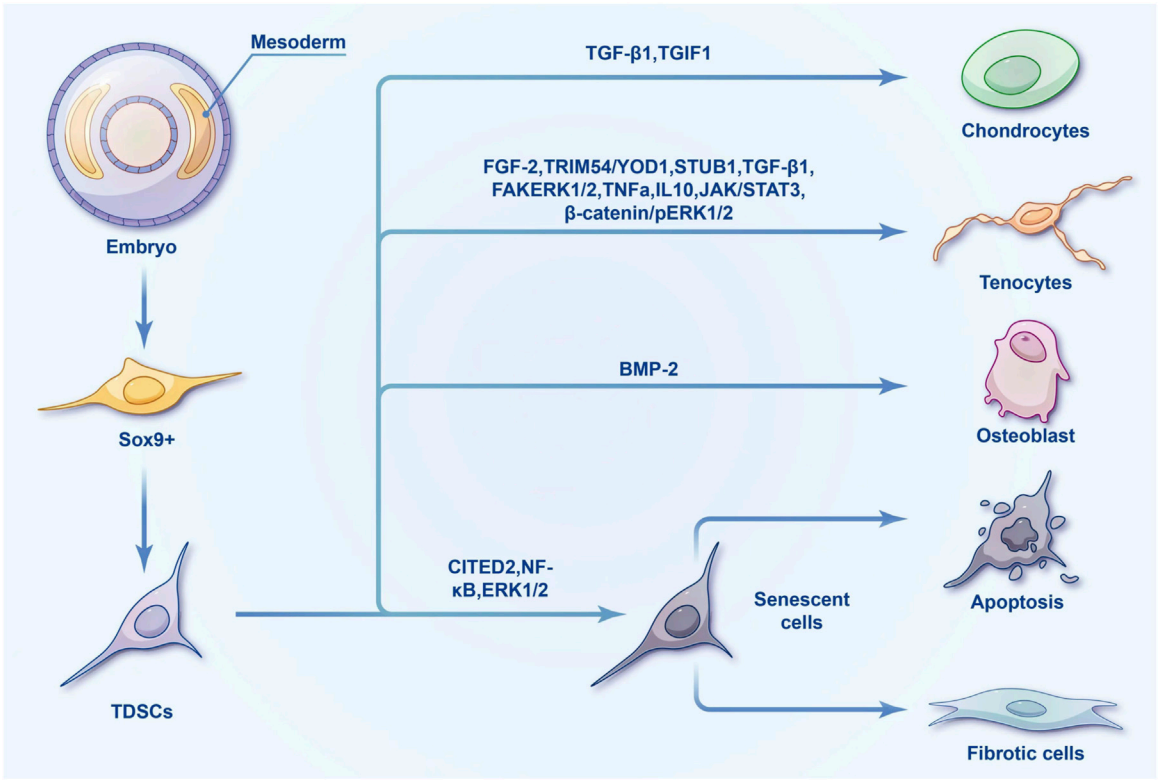


FIGURE 3
Differentiation tree of TDSCs.

hydrogel containing kartogenin-loaded bioactive glass nanoparticles displayed promise in addressing rotator cuff injuries. It induced chondrogenesis and osteogenesis in TDSCs, promoting fibrocartilage and bone layer regeneration in a rabbit model of chronic cuff tears (Huang et al., 2022). Zhang et al. (2023) employed a RADA peptide hydrogel with human TSPCs for patellar tendon repair. Results demonstrated improved function recovery, enhanced matrix organization, reduced complications, and decreased heterotopic ossification, suggesting a promising approach for clinical tendon repair. The utilization of hydrogel-based approaches in conjunction with TSPCs presents compelling prospects for enhancing tendon repair and regeneration. Collectively, these studies underscored the potential of hydrogel systems to furnish a conducive microenvironment for TSPCs, foster tenogenesis, and ameliorate functional outcomes in tendon tissue engineering and clinical applications.

Allogeneic TSPCs, exhibiting typical stem cell characteristics, were employed in a study focusing on shoulder repair. These cells were seeded onto knitted silk-collagen sponge scaffolds, demonstrating clonogenicity, high proliferation, multidifferentiation potential, non-immunogenicity, and immunosuppressive properties. In a rabbit model, these TSPCs facilitated tendon regeneration by differentiating into tenocytes, diminishing immunological rejection, and augmenting collagen deposition and biomechanical properties (Chen et al., 2012). Silk-Based Materials in Tendon Engineering: Silk fibroin (SF) films, both with bionic microstructures and smooth surfaces, have exhibited promise in tendon tissue engineering. The 10 μm bionic SF film showcased mechanical properties comparable to native tendons. When TSPCs were seeded on these films, they underwent significant changes in cell morphology, prompted the upregulation of tenogenic genes (COL1A1, TNC, TNMD, SCX), and facilitated TSPC adherence and differentiation (Lu et al., 2020a). Furthermore, SF films with bionic microstructures activated focal adhesion kinase (FAK), playing a pivotal role in enhancing TSPC differentiation. In brief, silk-based materials, particularly bionic SF films, present potential for tendon repair by guiding TSPC morphology and promoting tenogenic differentiation through FAK activation (Lu et al., 2020b). These materials, especially SF films with bionic microstructures, demonstrate substantial promise in tendon tissue engineering. They not only guide TSPC morphology but also enhance tenogenic differentiation through FAK activation, making them customizable biomaterials for tendon repair applications.

The domain of TDSCs and tissue engineering is evolving towards a more comprehensive approach integrating advanced biomaterials, cellular therapies, and an understanding of the biomechanical environment. This multifaceted strategy holds significant promise for surmounting traditional challenges in tendon repair and regeneration, offering new pathways for effective treatments of tendon injuries and disorders.

4 Clinical study for tendon with stem cells

Although there are currently no clinical studies specifically on tendon stem cells, several clinical studies on stem cells in tendon-

related diseases have been initiated. Some of these studies have already reached conclusions. The stem cells used in these clinical studies are primarily derived from bone marrow and adipose tissue. Stem cell therapy has shown good safety in the treatment of tendon diseases. In the treatment of partial tears in the supraspinatus tendon, although stem cell injections did not show significant effects compared to placebo, all participants only reported transient pain at the injection site with no persistent adverse events (Chun et al., 2022). Additionally, the use of adipose-derived stem cells in treating lateral epicondylosis demonstrated no significant adverse effects, confirming their safety (Lee et al., 2015). Preoperative bone channelling combined with stem cell treatment in rotator cuff repair did not result in any adverse events or significant differences in healing rates compared to sham procedures (Lapner et al., 2021). Stem cell therapy has shown potential efficacy in the repair of tendon ruptures. Adipose-derived stem cells significantly improved structural outcomes in rotator cuff repair, although there were no clinical differences during a 28-month follow-up period; MRI data indicated a significantly lower retear rate in the treatment group compared to the control group (Kim et al., 2017). Moreover, bone marrow-derived stem cells used in the acute repair of achilles tendons showed no reruptures and allowed earlier resumption of walking and sporting activities (Stein et al., 2015). In another study, bone marrow-derived MSCs used in rotator cuff repair enhanced healing rates and prevented reruptures over a 10-year period, showing substantial improvements in tendon integrity (Hernigou et al., 2014). In the treatment of patellar tendinopathy, bone marrow-derived stem cells showed significant improvements in tendon structure at 6 months compared to leukocyte-poor platelet-rich plasma (Rodas et al., 2021). In chronic tendinopathy, adipose-derived stem cells provided faster recovery in pain relief and functional improvement compared to SVF treatment (Lee et al., 2015). For non-insertional Achilles tendinopathy, both PRP and SVF treatments significantly improved clinical scores, but SVF-treated patients achieved faster improvements (Gomes et al., 2012). Stem cell therapy has demonstrated good safety and potential efficacy in tendon-related diseases. Although in some cases stem cell treatments did not show significant improvements over conventional treatments, they have shown considerable advantages in improving tendon structural integrity. We believe that in current clinical research, the means of using stem cell therapy is simply injection. No optimization methods such as improving the microenvironment or using induction factors have been considered. These are the factors that are studied more in basic medical research. Future research should continue to explore optimal strategies and long-term effects in clinical applications to fully leverage the regenerative potential of stem cells.

5 Conclusion and future outlook

In conclusion, the field of tendon tissue engineering, particularly involving TDSCs, is rapidly progressing, providing novel insights and strategies for addressing tendon and ligament injuries. The utilization of TDSCs in various therapeutic approaches, such as scaffold-based regeneration, has demonstrated promising results in enhancing tendon repair and function. Nonetheless, challenges remain in optimizing cell-scaffold interactions, sustaining long-term cell viability and function, and translating research findings into clinical applications. There are currently no clinical trials on TDSCs. Therefore clinical research is also very important in this field. Future research should concentrate on integrated approaches that combine advanced biomaterials, precise cellular manipulation, and a deeper understanding of the biomechanical environment of tendons. This multifaceted strategy possesses the potential to overcome the traditional challenges in tendon repair and regeneration, furnishing effective treatments for tendon injuries and disorders. As the discipline advances, it holds great promise for improving the outcomes of tendon repair, potentially revolutionizing the management of tendon and ligament injuries in clinical practice.

Author contributions

WH: Writing–original draft, Writing–review and editing. CJ: Conceptualization, Writing–review and editing. PZ: Writing–review and editing. XH: Writing–review and editing. XG: Writing–review and editing. SZ: Conceptualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by Shaoxing Science and Technology Plan Project (2023A14009) and the Shaoxing Health Science and Technology Plan (2023SK013).

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.

Publisher's note

References

Alberton, P., Dex, S., Popov, C., Shukunami, C., Schieker, M., and Docheva, D. (2015). Loss of tenomodulin results in reduced self-renewal and augmented senescence of tendon stem/progenitor cells. *Stem Cells Dev.* 24 (5), 597–609. doi:10.1089/scd.2014.0314

Andarawis-Puri, N., Flatow, E. L., and Soslowsky, L. J. (2015). Tendon basic science: development, repair, regeneration, and healing. *J. Orthop. Res.* 33 (6), 780–784. doi:10.1002/jor.22869

Atienza-Roca, P., Cui, X., Hooper, G. J., Woodfield, T. B. F., and Lim, K. S. (2018). Growth factor delivery systems for tissue engineering and regenerative medicine. *Adv. Exp. Med. Biol.* 1078, 245–269. doi:10.1007/978-981-13-0950-2_13

Bi, Y., Ehirchiou, D., Kilts, T. M., Inkson, C. A., Embree, M. C., Sonoyama, W., et al. (2007). Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat. Med.* 13 (10), 1219–1227. doi:10.1038/nm1630

Brown, J. P., Finley, V. G., and Kuo, C. K. (2014). Embryonic mechanical and soluble cues regulate tendon progenitor cell gene expression as a function of developmental stage and anatomical origin. *J. Biomech.* 47 (1), 214–222. doi:10.1016/j.jbiomech.2013.09.018

Brown, J. P., Galassi, T. V., Stoppato, M., Schiele, N. R., and Kuo, C. K. (2015). Comparative analysis of mesenchymal stem cell and embryonic tendon progenitor cell response to embryonic tendon biochemical and mechanical factors. *Stem Cell Res. Ther.* 6 (1), 89. doi:10.1186/s13287-015-0043-z

Chen, B., Liang, Y., Zhang, J., Bai, L., Xu, M., Han, Q., et al. (2021). Synergistic enhancement of tendon-to-bone healing via anti-inflammatory and pro-differentiation effects caused by sustained release of Mg(2+)/curcumin from injectable self-healing hydrogels. *Theranostics* 11 (12), 5911–5925. doi:10.7150/thno.56266

Chen, E., Yang, L., Ye, C., Zhang, W., Ran, J., Xue, D., et al. (2018). An asymmetric chitosan scaffold for tendon tissue engineering: *in vitro* and *in vivo* evaluation with rat tendon stem/progenitor cells. *Acta Biomater.* 73, 377–387. doi:10.1016/j.actbio.2018.04.027

Chen, H., Chen, X., Yang, L., Sheng, S., Yang, J., Lu, Y., et al. (2024). TRIM54 alleviates inflammation and apoptosis by stabilizing YOD1 in rat tendon-derived stem cells. *J. Biol. Chem.* 300 (1), 105510. doi:10.1016/j.jbc.2023.105510

Chen, H., Ge, H. A., Wu, G. B., Cheng, B., Lu, Y., and Jiang, C. (2016). Autophagy prevents oxidative stress-induced loss of self-renewal capacity and stemness in human tendon stem cells by reducing ROS accumulation. *Cell Physiol. Biochem.* 39 (6), 2227–2238. doi:10.1159/000447916

Chen, L., Dong, S. W., Tao, X., Liu, J. P., Tang, K. L., and Xu, J. Z. (2012). Autologous platelet-rich clot releasate stimulates proliferation and inhibits differentiation of adult rat tendon stem cells towards nontenocyte lineages. *J. Int. Med. Res.* 40 (4), 1399–1409. doi:10.1177/147323001204000418

Chen, L., Jiang, C., Tiwari, S. R., Shrestha, A., Xu, P., Liang, W., et al. (2015). TGIF1 gene silencing in tendon-derived stem cells improves the tendon-to-bone insertion site regeneration. *Cell Physiol. Biochem.* 37 (6), 2101–2114. doi:10.1159/000438568

Chen, X., Qi, Y. Y., Wang, L. L., Yin, Z., Yin, G. L., Zou, X. H., et al. (2008). Ligament regeneration using a knitted silk scaffold combined with collagen matrix. *Biomaterials* 29 (27), 3683–3692. doi:10.1016/j.biomaterials.2008.05.017

Chen, Z., Chen, P., Ruan, R., Chen, L., Yuan, J., Wood, D., et al. (2020). Applying a three-dimensional uniaxial mechanical stimulation bioreactor system to induce tenogenic differentiation of tendon-derived stem cells. *J. Vis. Exp.* 162. doi:10.3791/61278

Chen, Z., Chen, P., Zheng, M., Gao, J., Liu, D., Wang, A., et al. (2022). Challenges and perspectives of tendon-derived cell therapy for tendinopathy: from bench to bedside. *Stem Cell Res. Ther.* 13 (1), 444. doi:10.1186/s13287-022-03113-6

Cheng, B., Ge, H., Zhou, J., and Zhang, Q. (2014). TSG-6 mediates the effect of tendon derived stem cells for rotator cuff healing. *Eur. Rev. Med. Pharmacol. Sci.* 18 (2), 247–251.

Cheung, T. S., Lau, P. M., Lu, H., Ho, H. P., Lui, P. P. Y., and Kong, S. K. (2016). Cytotoxic and sublethal effects of silver nanoparticles on tendon-derived stem cells - implications for tendon engineering. *Toxicol. Res. (Camb).* 5 (1), 318–330. doi:10.1039/c5tx00349k

Chun, S. W., Kim, W., Lee, S. Y., Lim, C. Y., Kim, K., Kim, J. G., et al. (2022). A randomized controlled trial of stem cell injection for tendon tear. *Sci. Rep.* 12 (1), 818. doi:10.1038/s41598-021-04656-z

Costa-Almeida, R., Calejo, I., Reis, R. L., and Gomes, M. E. (2018). Crosstalk between adipose stem cells and tendon cells reveals a temporal regulation of tenogenesis by matrix deposition and remodeling. *J. Cell Physiol.* 233 (7), 5383–5395. doi:10.1002/jcp.26363

Cottrell, J. A., Turner, J. C., Arinzeh, T. L., and O'Connor, J. P. (2016). The biology of bone and ligament healing. *Foot Ankle Clin.* 21 (4), 739–761. doi:10.1016/j.fcl.2016.07.017

Dang, L. H., Hung, S. H., Tseng, Y., Quang, L. X., Le, N. T. N., Fang, C. L., et al. (2021). Partial decellularized scaffold combined with autologous nasal epithelial cell sheet for tracheal tissue engineering. *Int. J. Mol. Sci.* 22 (19), 10322. doi:10.3390/ijms221910322

Deng, G., Li, K., Chen, S., Chen, P., Zheng, H., Yu, B., et al. (2018). Interleukin-10 promotes proliferation and migration, and inhibits tendon differentiation via the JAK/Stat3 pathway in tendon-derived stem cells *in vitro*. *Mol. Med. Rep.* 18 (6), 5044–5052. doi:10.3892/mmr.2018.9547

Di Meglio, F., Sacco, A., Belviso, I., Romano, V., Sirico, F., Loiacono, C., et al. (2020). Influence of supplements and drugs used for the treatment of musculoskeletal disorders on adult human tendon-derived stem cells. *Muscles, Ligaments and Tendons J. (MLTJ).* 10 (3), 376. doi:10.32098/mltj.03.2020.04

Fan, H., Liu, H., Wang, Y., Toh, S. L., and Goh, J. C. (2008). Development of a silk cable-reinforced gelatin/silk fibroin hybrid scaffold for ligament tissue engineering. *Cell Transpl.* 17 (12), 1389–1401. doi:10.3727/096368908787648047

Feng, H., Xing, W., Han, Y., Sun, J., Kong, M., Gao, B., et al. (2020). Tendon-derived cathepsin K-expressing progenitor cells activate Hedgehog signaling to drive heterotopic ossification. *J. Clin. Invest.* 130 (12), 6354–6365. doi:10.1172/jci132518

Franchi, M., Trirè, A., Quaranta, M., Orsini, E., and Ottani, V. (2007). Collagen structure of tendon relates to function. *ScientificWorldJournal* 7, 404–420. doi:10.1100/tsw.2007.92

Gomes, J. L. E., da Silva, R. C., Silla, L. M., Abreu, M. R., and Pellanda, R. (2012). Conventional rotator cuff repair complemented by the aid of mononuclear autologous stem cells. *Knee Surg. Sports Traumatol. Arthrosc.* 20 (2), 373–377. doi:10.1007/s00167-011-1607-9

Guo, D., Li, H., Liu, Y., Yu, X., Zhang, X., Chu, W., et al. (2020). Fibroblast growth factor-2 promotes the function of tendon-derived stem cells in Achilles tendon restoration in an Achilles tendon injury rat model. *Biochem. Biophys. Res. Commun.* 521 (1), 91–97. doi:10.1016/j.bbrc.2019.10.082

Han, P., Cui, Q., Lu, W., Yang, S., Shi, M., Li, Z., et al. (2019). Hepatocyte growth factor plays a dual role in tendon-derived stem cell proliferation, migration, and differentiation. *J. Cell Physiol.* 234 (10), 17382–17391. doi:10.1002/jcp.28360

Han, P., Cui, Q., Yang, S., Wang, H., Gao, P., and Li, Z. (2017b). Tumor necrosis factor-α and transforming growth factor-β1 facilitate differentiation and proliferation of tendon-derived stem cells *in vitro*. *Biotechnol. Lett.* 39 (5), 711–719. doi:10.1007/s10529-017-2296-3

Han, W., Chen, L., Liu, J., and Guo, A. (2017a). Enhanced tenogenic differentiation and tendon-like tissue formation by CHIP overexpression in tendon-derived stem cells. *Acta Biochim. Biophys. Sin. (Shanghai)* 49 (4), 311–317. doi:10.1093/abbs/gmx005

Harvey, T., Flamenco, S., and Fan, C. M. (2019). A Tppp3(+)Pdgfra(+) tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis. *Nat. Cell Biol.* 21 (12), 1490–1503. doi:10.1038/s41556-019-0417-z

Hernigou, P., Flouzat Lachaniette, C. H., Delambre, J., Zilber, S., Duffiet, P., Chevallier, N., et al. (2014). Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: a case-controlled study. *Int. Orthop.* 38 (9), 1811–1818. doi:10.1007/s00264-014-2391-1

Hsieh, C. F., Yan, Z., Schumann, R. G., Milz, S., Pfeifer, C. G., Schieker, M., et al. (2018). *In vitro* comparison of 2D-cell culture and 3D-cell sheets of scleraxis-programmed bone marrow derived mesenchymal stem cells to primary tendon stem/progenitor cells for tendon repair. *Int. J. Mol. Sci.* 19 (8), 2272. doi:10.3390/ijms19082272

Hu, C., Zhang, Y., Tang, K., Luo, Y., Liu, Y., and Chen, W. (2017). Downregulation of CITED2 contributes to TGFβ-mediated senescence of tendon-derived stem cells. *Cell Tissue Res.* 368 (1), 93–104. doi:10.1007/s00441-016-2552-1

Huang, K., Du, J., Xu, J., Wu, C., Chen, C., Chen, S., et al. (2022). Tendon-bone junction healing by injectable bioactive thermo-sensitive hydrogel based on inspiration of tendon-derived stem cells. *Mater. Today Chem.* 23, 100720. doi:10.1016/j.mtchem.2021.100720

Huang, Z., Yin, Z., Xu, J., Fei, Y., Heng, B. C., Jiang, X., et al. (2021). Tendon stem/progenitor cell subpopulations and their implications in tendon biology. *Front. Cell Dev. Biol.* 9, 631272. doi:10.3389/fcell.2021.631272

Inci, I., Norouz Dizaji, A., Ozel, C., Morali, U., Dogan Guzel, F., and Avci, H. (2020). Decellularized inner body membranes for tissue engineering: a review. *J. Biomater. Sci. Polym. Ed.* 31 (10), 1287–1368. doi:10.1080/09205063.2020.1751523

Jiang, D., Xu, B., and Gao, P. (2018). Effects of young extracellular matrix on the biological characteristics of aged tendon stem cells. *Adv. Clin. Exp. Med.* 27 (12), 1625–1630. doi:10.17219/acem/75503

Jiang, L., Lu, J., Chen, Y., Lyu, K., Long, L., Wang, X., et al. (2023). Mesenchymal stem cells: an efficient cell therapy for tendon repair (Review). *Int. J. Mol. Med.* 52 (2), 70. doi:10.3892/ijmm.2023.5273

Jo, C. H., Lim, H. J., and Yoon, K. S. (2019). Characterization of tendon-specific markers in various human tissues, tenocytes and mesenchymal stem cells. *Tissue Eng. Regen. Med.* 16 (2), 151–159. doi:10.1007/s13770-019-00182-2

Kim, S. J., Tatman, P. D., Song, D. H., Gee, A. O., Kim, D. H., and Kim, S. J. (2018). Nanotopographic cues and stiffness control of tendon-derived stem cells from diverse conditions. *Int. J. Nanomedicine* 13, 7217–7227. doi:10.2147/ijn.s181743

Kim, Y. S., Sung, C. H., Chung, S. H., Kwak, S. J., and Koh, Y. G. (2017). Does an injection of adipose-derived mesenchymal stem cells loaded in fibrin glue influence rotator cuff repair outcomes? A clinical and magnetic resonance imaging study. *Am. J. Sports Med.* 45 (9), 2010–2018. doi:10.1177/0363546517702863

Lai, F., Wang, J., Tang, H., Huang, P., Liu, J., He, G., et al. (2022). VEGF promotes tendon regeneration of aged rats by inhibiting adipogenic differentiation of tendon stem/progenitor cells and promoting vascularization. *Faseb J.* 36 (8), e22433. doi:10.1096/fj.202200213r

Lapner, P., Pollock, J. W., Laneuville, O., Uhthoff, H. K., Zhang, T., Sheikh, A., et al. (2021). Preoperative bone marrow stimulation does not improve functional outcomes in arthroscopic cuff repair: a prospective randomized controlled trial. *Bone Jt. J.* 103-b (1), 123–130. doi:10.1302/0301-620x.103b1.bjj-2020-0011.r2

Laranjeira, M., Domingues, R. M. A., Costa-Almeida, R., Reis, R. L., and Gomes, M. E. (2017). 3D mimicry of native-tissue-fiber architecture guides tendon-derived cells and adipose stem cells into artificial tendon constructs. *Small* 13 (31). doi:10.1002/smll.201700689

Lee, K. J., Clegg, P. D., Comerford, E. J., and Canty-Laird, E. G. (2018). A comparison of the stem cell characteristics of murine tenocytes and tendon-derived stem cells. *BMC Musculoskelet. Disord.* 19 (1), 116. doi:10.1186/s12891-018-2038-2

Lee, S. Y., Kim, W., Lim, C., and Chung, S. G. (2015). Treatment of lateral epicondylosis by using allogeneic adipose-derived mesenchymal stem cells: a pilot study. *Stem Cells* 33 (10), 2995–3005. doi:10.1002/stem.2110

Leong, N. L., Kator, J. L., Clemens, T. L., James, A., Enamoto-Iwamoto, M., and Jiang, J. (2020). Tendon and ligament healing and current approaches to tendon and ligament regeneration. *J. Orthop. Res.* 38 (1), 7–12. doi:10.1002/jor.24475

Li, K., Deng, G., Deng, Y., Chen, S., Wu, H., Cheng, C., et al. (2019). High cholesterol inhibits tendon-related gene expressions in tendon-derived stem cells through reactive oxygen species-activated nuclear factor-κB signaling. *J. Cell Physiol.* 234 (10), 18017–18028. doi:10.1002/jcp.28433

Li, P., Xu, Y., Gan, Y., Song, L., Zhang, C., Wang, L., et al. (2016). Role of the ERK1/2 signaling pathway in osteogenesis of rat tendon-derived stem cells in normoxic and hypoxic cultures. *Int. J. Med. Sci.* 13 (8), 629–637. doi:10.7150/ijms.16045

Li, S., Sun, Y., Chen, Y., Lu, J., Jiang, G., Yu, K., et al. (2023). Sandwich biomimetic scaffold based tendon stem/progenitor cell alignment in a 3D microenvironment for functional tendon regeneration. *ACS Appl. Mater Interfaces* 15 (3), 4652–4667. doi:10.1021/acsami.2c16584

Li, Y., Wu, T., and Liu, S. (2021). Identification and distinction of tenocytes and tendon-derived stem cells. *Front. Cell Dev. Biol.* 9, 629515. doi:10.3389/fcell.2021.629515

Lim, W. L., Liau, L. L., Ng, M. H., Chowdhury, S. R., and Law, J. X. (2019). Current progress in tendon and ligament tissue engineering. *Tissue Eng. Regen. Med.* 16 (6), 549–571. doi:10.1007/s13770-019-00196-w

Lin, Y., Zhang, L., Liu, N. Q., Yao, Q., Van Handel, B., Xu, Y., et al. (2019). *In vitro* behavior of tendon stem/progenitor cells on bioactive electrospun nanofiber membranes for tendon-bone tissue engineering applications. *Int. J. Nanomedicine* 14, 5831–5848. doi:10.2147/ijn.s210509

Liu, C., Luo, J. W., Liang, T., Lin, L. X., Luo, Z. P., Zhuang, Y. Q., et al. (2018b). Matrix stiffness regulates the differentiation of tendon-derived stem cells through FAK-ERK1/2 activation. *Exp. Cell Res.* 373 (1-2), 62–70. doi:10.1016/j.yexcr.2018.08.023

Liu, Q., Zhu, Y., Amadio, P. C., Moran, S. L., Gingery, A., and Zhao, C. (2018a). Isolation and characterization of multipotent Turkey tendon-derived stem cells. *Stem Cells Int.* 2018, 3697971. doi:10.1155/2018/3697971

Liu, X., Chen, W., Zhou, Y., Tang, K., and Zhang, J. (2015). Mechanical tension promotes the osteogenic differentiation of rat tendon-derived stem cells through the wnt5a/wnt5b/JNK signaling pathway. *Cell Physiol. Biochem.* 36 (2), 517–530. doi:10.1159/000430117

Liu, Y., Xu, J., Xu, L., Wu, T., Sun, Y., Lee, Y. W., et al. (2017). Cystic fibrosis transmembrane conductance regulator mediates tenogenic differentiation of tendon-derived stem cells and tendon repair: accelerating tendon injury healing by intervening in its downstream signaling. *Faseb J.* 31 (9), 3800–3815. doi:10.1096/fj.201601181r

Liu, Y., Yuan, C., Zhou, M., and Tang, K. (2019). Co-Cultured bone-marrow derived and tendon stem cells: novel seed cells for bone regeneration. *Open Life Sci.* 14, 568–575. doi:10.1515/biol-2019-0063

Lu, K., Chen, X., Tang, H., Zhou, M., He, G., Liu, J., et al. (2020a). Bionic silk fibroin film induces morphological changes and differentiation of tendon stem/progenitor cells. *Appl. Bionics Biomech.* 2020, 8865841. doi:10.1155/2020/8865841

Lu, K., Chen, X., Tang, H., Zhou, M., He, G., Lu, Z., et al. (2020b). Bionic silk fibroin film promotes tenogenic differentiation of tendon stem/progenitor cells by activating focal adhesion kinase. *Stem Cells Int.* 2020, 1–10. doi:10.1155/2020/8857380

Lui, P. P., and Chan, K. M. (2011). Tendon-derived stem cells (TDSCs): from basic science to potential roles in tendon pathology and tissue engineering applications. *Stem Cell Rev. Rep.* 7 (4), 883–897. doi:10.1007/s12015-011-9276-0

Lui, P. P., Wong, O. T., and Lee, Y. W. (2014). Application of tendon-derived stem cell sheet for the promotion of graft healing in anterior cruciate ligament reconstruction. *Am. J. Sports Med.* 42 (3), 681–689. doi:10.1177/0363546513517539

Lui, P. P., Wong, O. T., and Lee, Y. W. (2016). Transplantation of tendon-derived stem cells pre-treated with connective tissue growth factor and ascorbic acid *in vitro* promoted better tendon repair in a patellar tendon window injury rat model. *Cytotherapy* 18 (1), 99–112. doi:10.1016/j.jcyt.2015.10.005

Mao, X., Yao, L., Li, M., Zhang, X., Weng, B., Zhu, W., et al. (2022). Enhancement of tendon repair using tendon-derived stem cells in small intestinal submucosa via M2 macrophage polarization. *Cells* 11 (17), 2770. doi:10.3390/cells11172770

Ni, M., Lui, P. P., Rui, Y. F., Lee, Y. W., Lee, Y. W., Tan, Q., et al. (2012). Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defect model. *J. Orthop. Res.* 30 (4), 613–619. doi:10.1002/jor.21559

Ni, M., Sun, W., Li, Y., Ding, L., Lin, W., Peng, H., et al. (2021). Sox11 modified tendon-derived stem cells promote the repair of osteonecrosis of femoral head. *Cell Transpl.* 30, 096368972110538. doi:10.1177/09636897211053870

Ni, Q., Zhu, J., Li, Z., Li, B., Wang, H., and Chen, L. (2023). Simvastatin promotes rat Achilles tendon-bone interface healing by promoting osteogenesis and chondrogenic differentiation of stem cells. *Cell Tissue Res.* 391 (2), 339–355. doi:10.1007/s00441-022-03714-w

Nichols, A. E. C., Best, K. T., and Loiselle, A. E. (2019). The cellular basis of fibrotic tendon healing: challenges and opportunities. *Transl. Res.* 209, 156–168. doi:10.1016/j.trsl.2019.02.002

Nie, D., Zhou, Y., Wang, W., Zhang, J., and Wang, J. H. (2021). Mechanical overloading induced-activation of mTOR signaling in tendon stem/progenitor cells contributes to tendinopathy development. *Front. Cell Dev. Biol.* 9, 687856. doi:10.3389/fcell.2021.687856

Ning, C., Gao, C., Li, P., Fu, L., Chen, W., Liao, Z., et al. (2022). Dual-phase aligned composite scaffolds loaded with tendon-derived stem cells for achilles tendon repair. *Adv. Ther.* 5. doi:10.1002/adtp.202200081

Ning, L. J., Zhang, Y. J., Zhang, Y. J., Zhu, M., Ding, W., Jiang, Y. L., et al. (2021). Enhancement of migration and tenogenic differentiation of Macaca mulatta tendon-derived stem cells by decellularized tendon hydrogel. *Front. Cell Dev. Biol.* 9, 651583. doi:10.3389/fcell.2021.651583

Popov, C., Burggraf, M., Kreja, L., Ignatius, A., Schieker, M., and Docheva, D. (2015b). Mechanical stimulation of human tendon stem/progenitor cells results in upregulation of matrix proteins, integrins and MMPs, and activation of p38 and ERK1/2 kinases. *BMC Mol. Biol.* 16, 6. doi:10.1186/s12867-015-0036-6

Popov, C., Kohler, J., and Docheva, D. (2015a). Activation of EphA4 and EphB2 reverse signaling restores the age-associated reduction of self-renewal, migration, and actin turnover in human tendon stem/progenitor cells. *Front. Aging Neurosci.* 7, 246. doi:10.3389/fnagi.2015.00246

Qiu, S., Sun, Y., Xu, J., Wen, G., Yu, Y., Wu, T., et al. (2019). Ferulic acid improves self-renewal and differentiation of human tendon-derived stem cells by upregulating early growth response 1 through hypoxia. *Genesis* 57 (9), e23291. doi:10.1002/dvg.23291

Rajpar, I., and Barrett, J. G. (2020). Multi-differentiation potential is necessary for optimal tenogenesis of tendon stem cells. *Stem Cell Res. Ther.* 11 (1), 152. doi:10.1186/s13287-020-01640-8

Randelli, P., Menon, A., Ragone, V., Creo, P., Alfieri Montrasio, U., Perucca, O. C., et al. (2016a). Effects of the pulsed electromagnetic field PST® on human tendon stem cells: a controlled laboratory study. *BMC Complement. Altern. Med.* 16, 293. doi:10.1186/s12906-016-1261-3

Randelli, P., Menon, A., Ragone, V., Creo, P., Bergante, S., Randelli, F., et al. (2016b). Limoges product treatment increases the proliferation rate of human tendon stem cells without affecting their stemness and differentiation capability. *Stem Cells Int.* 2016, 1–11. doi:10.1155/2016/4373410

Rodas, G., Soler-Rich, R., Rius-Tarruella, J., Alomar, X., Balius, R., Orozco, L., et al. (2021). Effect of autologous expanded bone marrow mesenchymal stem cells or leukocyte-poor platelet-rich plasma in chronic patellar tendinopathy (with gap >3 mm): preliminary outcomes after 6 Months of a double-blind, randomized, prospective study. *Am. J. Sports Med.* 49 (6), 1492–1504. doi:10.1177/0363546521998725

Rui, Y. F., Lui, P. P., Li, G., Fu, S. C., Lee, Y. W., and Chan, K. M. (2010). Isolation and characterization of multipotent rat tendon-derived stem cells. *Tissue Eng. Part A* 16 (5), 1549–1558. doi:10.1089/ten.tea.2009.0529

Rui, Y. F., Lui, P. P., Ni, M., Chan, L. S., Lee, Y. W., and Chan, K. M. (2011). Mechanical loading increased BMP-2 expression which promoted osteogenic differentiation of tendon-derived stem cells. *J. Orthop. Res.* 29 (3), 390–396. doi:10.1002/jor.21218

Ruiz-Alonso, S., Lafuente-Merchan, M., Ciriza, J., Saenz-Del-Burgo, L., and Pedraz, J. L. (2021). Tendon tissue engineering: cells, growth factors, scaffolds and production techniques. *J. Control Release* 333, 448–486. doi:10.1016/j.jconrel.2021.03.040

Sevivas, N., Teixeira, F. G., Portugal, R., Direito-Santos, B., Espregueira-Mendes, J., Oliveira, F. J., et al. (2018). Mesenchymal stem cell secretome improves tendon cell viability *in vitro* and tendon-bone healing *in vivo* when a tissue engineering strategy is used in a rat model of chronic massive rotator cuff tear. *Am. J. Sports Med.* 46 (2), 449–459. doi:10.1177/0363546517735850

Shen, W., Chen, J., Yin, Z., Chen, X., Liu, H., Heng, B. C., et al. (2012). Allogenous tendon stem/progenitor cells in silk scaffold for functional shoulder repair. *Cell Transpl.* 21 (5), 943–958. doi:10.3727/096368911x627453

Shi, Y., Fu, Y., Tong, W., Geng, Y., Lui, P. P., Tang, T., et al. (2012). Uniaxial mechanical tension promoted osteogenic differentiation of rat tendon-derived stem cells (rTDSCs) via the Wnt5a-RhoA pathway. *J. Cell Biochem.* 113 (10), 3133–3142. doi:10.1002/jcb.24190

Shi, Y., Zhou, K., Zhang, W., Zhang, Z., Zhou, G., Cao, Y., et al. (2017). Microgrooved topographical surface directs tenogenic lineage specific differentiation of mouse tendon derived stem cells. *Biomed. Mater* 12 (1), 015013. doi:10.1088/1748-605x/12/1/015013

Song, F., Jiang, D., Wang, T., Wang, Y., Chen, F., Xu, G., et al. (2017). Mechanical loading improves tendon-bone healing in a rabbit anterior cruciate ligament reconstruction model by promoting proliferation and matrix formation of mesenchymal stem cells and tendon cells. *Cell Physiol. Biochem.* 41 (3), 875–889. doi:10.1159/000460005

Song, H., Yin, Z., Wu, T., Li, Y., Luo, X., Xu, M., et al. (2018). Enhanced effect of tendon stem/progenitor cells combined with tendon-derived decellularized extracellular matrix on tendon regeneration. *Cell Transpl.* 27 (11), 1634–1643. doi:10.1177/0963689718805383

Stace, E. T., Nagra, N. S., Tiberwel, S., Khan, W., and Carr, A. J. (2018). The use of electrospun scaffolds in musculoskeletal tissue engineering: a focus on tendon and the rotator cuff. *Curr. Stem Cell Res. Ther.* 13 (8), 619–631. doi:10.2174/1574888x13666180129105707

Stein, B. E., Stroh, D. A., and Schon, L. C. (2015). Outcomes of acute Achilles tendon rupture repair with bone marrow aspirate concentrate augmentation. *Int. Orthop.* 39 (5), 901–905. doi:10.1007/s00264-015-2725-7

Subramanian, A., and Schilling, T. F. (2015). Tendon development and musculoskeletal assembly: emerging roles for the extracellular matrix. *Development* 142 (24), 4191–4204. doi:10.1242/dev.114777

Sun, L., Li, H., Qu, L., Zhu, R., Fan, X., Xue, Y., et al. (2014). Immobilized lentivirus vector on chondroitin sulfate-hyaluronate acid-silk fibroin hybrid scaffold for tissue-engineered ligament-bone junction. *Biomed. Res. Int.* 2014, 1–10. doi:10.1155/2014/816979

Tabesh, H., Elahi, Z., Amoabediny, Z., and Rafiei, F. (2022). Elimination of induced hypoxic regions in depth of 3D porous silk scaffolds by the introduction of channel configuration. *Biomed. Res. Int.* 2022, 1–12. doi:10.1155/2022/9767687

Tarafder, S., Brito, J. A., Minhas, S., Effiong, L., Thomopoulos, S., and Lee, C. H. (2019). *In situ* tissue engineering of the tendon-to-bone interface by endogenous stem/progenitor cells. *Biofabrication* 12 (1), 015008. doi:10.1088/1758-5090/ab48ca

Tevlin, R., Walmsley, G. G., Marecic, O., Hu, M. S., Wan, D. C., and Longaker, M. T. (2016). Stem and progenitor cells: advancing bone tissue engineering. *Drug Deliv. Transl. Res.* 6 (2), 159–173. doi:10.1007/s13346-015-0235-1

Thorpe, C. T., and Screen, H. R. (2016). Tendon structure and composition. *Adv. Exp. Med. Biol.* 920, 3–10. doi:10.1007/978-3-319-33943-6_1

Wang, C., Zhou, Z., Song, W., Cai, Z., Ding, Z., Chen, D., et al. (2022). Inhibition of IKKβ/NF-κB signaling facilitates tendinopathy healing by rejuvenating inflamm-aging induced tendon-derived stem/progenitor cell senescence. *Mol. Ther. Nucleic Acids* 27, 562–576. doi:10.1016/j.omtn.2021.12.026

Wang, H. N., Huang, Y. C., and Ni, G. X. (2020a). Mechanotransduction of stem cells for tendon repair. *World J. Stem Cells* 12 (9), 952–965. doi:10.4252/wjsc.v12.i9.952

Wang, T., Thien, C., Wang, C., Ni, M., Gao, J., Wang, A., et al. (2018). 3D uniaxial mechanical stimulation induces tenogenic differentiation of tendon-derived stem cells through a PI3K/AKT signaling pathway. *Faseb J.* 32 (9), 4804–4814. doi:10.1096/fj.201701384r

Wang, Y., He, G., Tang, H., Shi, Y., Zhu, M., Kang, X., et al. (2020b). Aspirin promotes tenogenic differentiation of tendon stem cells and facilitates tendinopathy healing through regulating the GDF7/Smad1/5 signaling pathway. *J. Cell Physiol.* 235 (5), 4778–4789. doi:10.1002/jcp.29355

Wang, Y., Li, M., Lin, Y., Yin, F., Shan, H., and Wu, T. (2023). Dimethyl oxalylglycine activates tendon-derived stem cells to promote regeneration of achilles tendon rupture in rats via HIF-1α. *Adv. Ther.* 6 (3), 2200164. doi:10.1002/adtp.202200164

Wei, B., Li, Z., Lin, Y., Hu, X., Xu, L., Wang, S., et al. (2023). BMP-2/TGF-β1 gene insertion into ligament-derived stem cells sheet promotes tendon-bone healing in a mouse. *Biotechnol. J.* 18 (5), e2200470. doi:10.1002/biot.202200470

Wei, B., and Lu, J. (2021). Characterization of tendon-derived stem cells and rescue tendon injury. *Stem Cell Rev. Rep.* 17 (5), 1534–1551. doi:10.1007/s12015-021-10143-9

Wu, T., Liu, Y., Wang, B., Sun, Y., Xu, J., Yuk-Wai, L. W., et al. (2016). The use of cocultured mesenchymal stem cells with tendon-derived stem cells as a better cell source for tendon repair. *Tissue Eng. Part A* 22 (19-20), 1229–1240. doi:10.1089/ten.tea.2016.0248

Xu, K., Lin, C., Ma, D., Chen, M., Zhou, X., He, Y., et al. (2021a). Spironolactone ameliorates senescence and calcification by modulating autophagy in rat tendon-derived stem cells via the NF-κB/MAPK pathway. *Oxid. Med. Cell Longev.* 2021, 1–15. doi:10.1155/2021/5519587

Xu, Y., Wang, Q., Li, Y., Gan, Y., Li, P., Li, S., et al. (2015). Cyclic tensile strain induces tenogenic differentiation of tendon-derived stem cells in bioreactor culture. *Biomed. Res. Int.* 2015, 1–13. doi:10.1155/2015/790804

Xu, Y., Yin, H., Chu, J., Eglin, D., Serra, T., and Docheva, D. (2021b). An anisotropic nanocomposite hydrogel guides aligned orientation and enhances tenogenesis of human tendon stem/progenitor cells. *Biomater. Sci.* 9 (4), 1237–1245. doi:10.1039/d0bm01127d

Yang, J., Zhao, Q., Wang, K., Liu, H., Ma, C., Huang, H., et al. (2016). Isolation and biological characterization of tendon-derived stem cells from fetal bovine. *Vitro Cell Dev. Biol. Anim.* 52 (8), 846–856. doi:10.1007/s11626-016-0043-z

Yang, Q., Li, J., Su, W., Yu, L., Li, T., Wang, Y., et al. (2022). Electrospun aligned poly(ε-caprolactone) nanofiber yarns guiding 3D organization of tendon stem/progenitor cells in tenogenic differentiation and tendon repair. *Front. Bioeng. Biotechnol.* 10, 960694. doi:10.3389/fbioe.2022.960694

Yang, Z., Cao, H., Gao, S., Yang, M., Lyu, J., and Tang, K. (2017). Effect of tendon stem cells in chitosan/β-glycerophosphate/collagen hydrogel on achilles tendon healing in a rat model. *Med. Sci. Monit.* 23, 4633–4643. doi:10.12659/msm.906747

Yao, S., Liang, Z., Lee, Y. W., Yung, P. S. H., and Lui, P. P. Y. (2023). Bioactive decellularized tendon-derived stem cell sheet for promoting graft healing after anterior cruciate ligament reconstruction. *Am. J. Sports Med.* 51 (1), 66–80. doi:10.1177/03635465221135770

Yin, H., Caceres, M. D., Yan, Z., Schieker, M., Nerlich, M., and Docheva, D. (2019). Tenomodulin regulates matrix remodeling of mouse tendon stem/progenitor cells in an *ex vivo* collagen I gel model. *Biochem. Biophys. Res. Commun.* 512 (4), 691–697. doi:10.1016/j.bbrc.2019.03.063

Yin, H., Strunz, F., Yan, Z., Lu, J., Brochhausen, C., Kiderlen, S., et al. (2020). Three-dimensional self-assembling nanofiber matrix rejuvenates aged/degenerative human tendon stem/progenitor cells. *Biomaterials* 236, 119802. doi:10.1016/j.biomaterials.2020.119802

Yin, H., Yan, Z., Bauer, R. J., Peng, J., Schieker, M., Nerlich, M., et al. (2018). Functionalized thermosensitive hydrogel combined with tendon stem/progenitor cells as injectable cell delivery carrier for tendon tissue engineering. *Biomed. Mater* 13 (3), 034107. doi:10.1088/1748-605x/aaadd1

Yin, Z., Chen, X., Chen, J. L., Shen, W. L., Hieu Nguyen, T. M., Gao, L., et al. (2010). The regulation of tendon stem cell differentiation by the alignment of nanofibers. *Biomaterials* 31 (8), 2163–2175. doi:10.1016/j.biomaterials.2009.11.083

Yin, Z., Chen, X., Zhu, T., Hu, J. J., Song, H. X., Shen, W. L., et al. (2013). The effect of decellularized matrices on human tendon stem/progenitor cell differentiation and tendon repair. *Acta Biomater.* 9 (12), 9317–9329. doi:10.1016/j.actbio.2013.07.022

Yin, Z., Sun, L., Shi, L., Nie, H., Dai, J., and Zhang, C. (2022). Bioinspired bimodal micro-nanofibrous scaffolds promote the tenogenic differentiation of tendon stem/progenitor cells for achilles tendon regeneration. *Biomater. Sci.* 10 (3), 753–769. doi:10.1039/d1bm01287h

Yu, Y., Lin, L., Zhou, Y., Lu, X., Shao, X., Lin, C., et al. (2017). Effect of hypoxia on self-renewal capacity and differentiation in human tendon-derived stem cells. *Med. Sci. Monit.* 23, 1334–1339. doi:10.12659/msm.903892

Zabrzyński, J., Łapaj, Ł., Paczesny, Ł., Zabrzyńska, A., and Grzanka, D. (2018). Tendon - function-related structure, simple healing process and mysterious ageing. *Folia Morphol. Warsz.* 77 (3), 416–427. doi:10.5603/fm.a2018.0006

Zhang, H., Dai, Y., Long, H., Cao, R., Shi, L., Zhao, J., et al. (2023). Tendon stem/progenitor cell-laden nanofiber hydrogel enhanced functional repair of patellar tendon. *Tissue Eng. Part A* 29 (5-6), 150–160. doi:10.1089/ten.tea.2022.0183

Zhang, J., Li, B., and Wang, J. H. (2011). The role of engineered tendon matrix in the stemness of tendon stem cells *in vitro* and the promotion of tendon-like tissue formation *in vivo*. *Biomaterials* 32 (29), 6972–6981. doi:10.1016/j.biomaterials.2011.05.088

Zhang, J., Nie, D., Williamson, K., McDowell, A., Hogan, M. V., and Wang, J. H. (2020). Moderate and intensive mechanical loading differentially modulate the phenotype of tendon stem/progenitor cells *in vivo*. *PLoS One* 15 (12), e0242640. doi:10.1371/journal.pone.0242640

Zhang, J., and Wang, J. H. (2010). Mechanobiological response of tendon stem cells: implications of tendon homeostasis and pathogenesis of tendinopathy. *J. Orthop. Res.* 28 (5), 639–643. doi:10.1002/jor.21046

Zhang, J., and Wang, J. H. (2013). Human tendon stem cells better maintain their stemness in hypoxic culture conditions. *PLoS One* 8 (4), e61424. doi:10.1371/journal.pone.0061424

Zhang, J., and Wang, J. H. (2014). Prostaglandin E2 (PGE2) exerts biphasic effects on human tendon stem cells. *PLoS One* 9 (2), e87706. doi:10.1371/journal.pone.0087706

Zhang, Q., and Cheng, B. (2013). Tendon-derived stem cells as a new cell source for tendon tissue engineering. *Front. Biosci. (Landmark Ed.* 18 (2), 756–764. doi:10.2741/4138

Zhang, Y., Lei, T., Tang, C., Chen, Y., Liao, Y., Ju, W., et al. (2021b). 3D printing of chemical-empowered tendon stem/progenitor cells for functional tissue repair. *Biomaterials* 271, 120722. doi:10.1016/j.biomaterials.2021.120722

Zhang, Y. J., Chen, X., Li, G., Chan, K. M., Heng, B. C., Yin, Z., et al. (2018). Concise review: stem cell fate guided by bioactive molecules for tendon regeneration. *Stem Cells Transl. Med.* 7 (5), 404–414. doi:10.1002/sctm.17-0206

Zhang, Z., Li, Y., Zhang, T., Shi, M., Song, X., Yang, S., et al. (2021a). Hepatocyte growth factor-induced tendon stem cell conditioned medium promotes healing of injured achilles tendon. *Front. Cell Dev. Biol.* 9, 654084. doi:10.3389/fcell.2021.654084

Zheng, Z., Ran, J., Chen, W., Hu, Y., Zhu, T., Chen, X., et al. (2017). Alignment of collagen fiber in knitted silk scaffold for functional massive rotator cuff repair. *Acta Biomater.* 51, 317–329. doi:10.1016/j.actbio.2017.01.041

Zitnay, J. L., and Weiss, J. A. (2018). Load transfer, damage, and failure in ligaments and tendons. *J. Orthop. Res.* 36 (12), 3093–3104. doi:10.1002/jor.24134

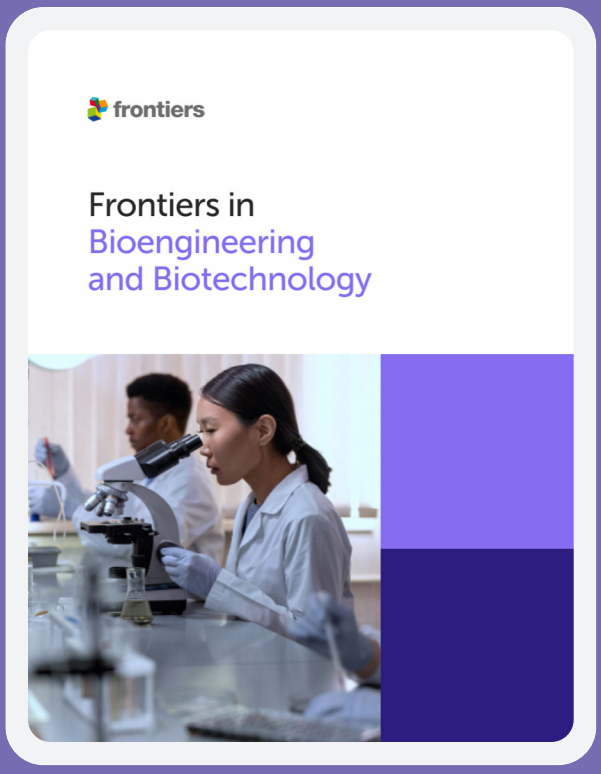
Frontiers in
Bioengineering and Biotechnology

Accelerates the development of therapies, devices, and technologies to improve our lives

A multidisciplinary journal that accelerates the development of biological therapies, devices, processes and technologies to improve our lives by bridging the gap between discoveries and their application.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact