CULTURED MEAT - ARE WE GETTING IT RIGHT?

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CULTURED MEAT - ARE WE GETTING IT RIGHT?

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EDITORIAL
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Editorial: Cultured Meat—Are We Getting it Right?

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Keywords: cultured meat, cellular agriculture, food system, nutrition, sustainability

Editorial on the Research Topic

Cultured Meat—Are We Getting it Right?

Since the launch of this Research Topic in the second half of 2019, the world has changed. The Covid-19 pandemic puts on hold many societal activities, upending normalcy for the majority of human life. Interestingly, not only has the pandemic impacted the progress of this Research Topic but it underscores the importance, opportunities, and relevance of cultured meats in a post-pandemic era.

The pandemic brings to light the extraordinary frailty in our global healthcare and food systems. We are just learning of the inter-relationship between diet and disease etiology and progression, and that certain dietary patterns can be associated with either an increase or decrease in disease risk and progression. Sugar intake, and gut microbial diversity appear to be only a few of the putative Covid-19 related nutritional metrics. More related to the Research Topic, we also witnessed Covid-19 outbreaks among food handlers in meat production and packaging plants, leading to plant closures.

At the outset of this Research Topic, we had written an overview outlining that the "*dietary consumption of meat is a hallmark of most human cultures and civilizations.*" However, maybe it is time to redefine this statement and consider that the "*dietary consumption of meat has been a hallmark of most human cultures and civilizations, but the twenty-first century necessitates more strategic technologies and sustainable lifestyles.*"

Six articles are published in the final Research Topic in the following order: Bryant and Dillard's article on "The Impact of Framing on Acceptance of Cultured Meat" highlights impressively how the perception of any novel food-related concept depends upon the way and context it is being presented. By using three different frames on cultured meat, i.e., "societal benefits," "high tech," and "same meat" they illustrate how the overall reception of these novel materials is context dependent.

The work by Bodiou et al. from the laboratory of Mark Post and Mosa Meat BV explores technology development, discussing the role of "Microcarriers for Upscaling Cultured Meat Production." Investigations into proper substrates and scaffolds for cultured meat are rapidly advancing. It is becoming clear, that any discussion about bioreactor design will have to be informed by the type of scaffolding and substrate that are being used to make biomass proliferate and differentiate. Later in the Research Topic, Bellani et al. return to this important point.

By choosing the title "Cultured Meat—*Are We Getting it Right*?" we aimed to encourage a critical assessment for this developing technology. In their review article "The Myth of Cultured Meat: A Review" Chriki and Hocquette provide exactly that, while keeping a balanced and analytical view on the topic.

"Sensorial and Nutritional Aspects of Cultured Meat in Comparison to Traditional Meat: Much to Be Inferred" by Fraeye et al. is a glimpse into the sensorial and organoleptic properties of future products to come. This paper also compares cultured meat to traditional meat from a tissue engineering and meat technological point of view.

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A relevant question to emerge from this contribution might be: To what degree will cultivated meat have to mimic existing animal-based meat products such as cuts and steaks and ribs?

Bogueva and Marinova explore "Cultured Meat and Australia's Generation Z." Interestingly, this contribution received a lot of attention—possibly because of the very local Australian aspects that are discussed in great detail. Concerns about masculinity and betraying Australia as a country of quality animal meat are raised. However, a significant number of young people (28%) are prepared to try cultured meat. Environmental and health concerns may encourage a broader section of society to embrace it as a novelty.

With their paper "Scale-Up Technologies for the Manufacture of Adherent Cells" Bellani et al. present their work on the overarching key issue in the cultured meat arena: scaling. Scaling at an affordable cost will be the key critical parameter to ensure the technology will prevail. The article gives a well-balanced overview of the different approaches in bioreactor design necessary to develop production plants for the upper kg range. Bioreactor design in the cultured meat field is becoming a formidable challenge for engineering departments across the globe.

The exploration and evolution of a second agricultural domestication (1) cannot be ignored any longer. It took mankind 10, 000 years to domesticate multicellular macroorganisms such as plants and animals. It will now potentially only take a few decades to domesticate the tissue analogues of these organisms at scale starting from the cellular level. The ambition to develop cultivated meat is only one facet in the growing domain of cellular agriculture and the discussion is about to reach a mainstream audience (2). As with many waves in the development of technologies, there are bumps along the way, there are dead-end roads, there are duplicate inventions and yet there is an underlying slow and steady way forward. It is disciplines such as biology and nutrition science that provide us with a deeper understanding of our physiological needs, states, and health requirements. Such understanding will eventually enable consumer acceptance of cellular agriculture. Overall, these advances are the result of the global necessity to renovate our approaches to nutrition and health systems toward providing for both individual and planetary health (3).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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ORIGINAL RESEARCH
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The Impact of Framing on Acceptance of Cultured Meat

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Cultured meat can be produced from growing animal cells *in-vitro* rather than as part of a living animal. This technology has the potential to address several of the major ethical, environmental, and public health concerns associated with conventional meat production. However, research has highlighted some consumer uncertainty regarding the concept. Although several studies have examined the media coverage of this new food technology, research linking different frames to differences in consumer attitudes is lacking. In an experimental study, we expose U.S. adults (n = 480) to one of three different frames on cultured meat: "societal benefits," "high tech," and "same meat." We demonstrate that those who encounter cultured meat through the "high tech" frame have significantly more negative attitudes toward the concept, and are significantly less likely to consume it. Worryingly, this has been a very dominant frame in early media coverage of cultured meat. Whilst this is arguably inevitable, since its technologically advanced nature is what makes it newsworthy, we argue that this high tech framing may be causing consumers to develop more negative attitudes toward cultured meat than they otherwise might. Implications for producers and researchers are discussed.

Keywords: clean meat, cultured meat, cell-based meat, consumer psychology, framing

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INTRODUCTION

Framing

The ways in which humans strive to make sense of the world they inhabit has long been of interest to scholars in a variety of fields. Goffman (1) set the course for much of this research when he conceptualized framing as a "schemata of interpretation," the manner by which humans organize information to make meaning both for themselves and others. Later research, especially in the fields of sociology and psychology, flushed out the way that frames work. Frames were seen as condensing reality, particularly in terms of fore-fronting certain aspects of reality, while back-dropping others (2–4). In the last four decades, an impressive body of literature on framing has developed in fields ranging from economics to cognitive linguistics (5–8).

Researchers in the various communication fields have focused their attention on the intentional use of frames, particularly in public life. Entman's well-known definition, "to frame is to select some aspects of a perceived reality and make them more salient in a communicating text, in such a way as to promote a particular problem definition, causal interpretation, moral evaluation, and/or treatment recommendation for the item described" [(9), p. 52] has undergirded and directed much of the research in this area. Frames have been investigated in terms of their role in media coverage, particularly news media (10, 11), political communication (12, 13) and advertising (14). One important distinction these scholars have sought to maintain is between the framing activities of those presenting information and those receiving it (15).

While interesting work has been done on the types of frames created by those presenting information (16–18), some of the most generative areas of research have been in terms of framing effects. This vein of research investigates how particular frames, often intentionally created, influence specific audiences (19, 20) and often seeks to establish frame effectiveness (21, 22).

Framing effects in terms of products and product features has more recently become a rich line of investigation. Work has been done on the type of frame employed and its effects in terms of willingness to pay, product preferences, and brand loyalty. For example, scholars have suggested that positive frames are generally more effective than negative ones, while allowing for the fact that there are occasions where a negative frame might be advantageous (23–25). Research has also focused on the effectiveness of marketing products in terms of social causes, particularly the environment. For example, Olsen et al. (26) found that while making green claims enhanced consumer favorability toward the brand, fewer claims rather than more were preferred. Cho (27) found that green frames worked best when they highlighted the consumer's own environmental impact. Ku et al. (28) noted that a consumer's motivations impacted how favorably they responded to green framing techniques.

Recent research in framing effectiveness has also demonstrated a growing curiosity around the role of images, whether stand alone or combined with text. Early theoretical research in this area (29, 30) made the case for the power of visuals, particularly in terms of emotional influence. Researchers have sought to examine this relationship in different contexts. For example, Iyer et al. (31) found that images of victims of the 2005 bombings in London elicited feelings of sympathy, while images of terrorists elicited feelings of fear and anger. Andrews et al. (32) found that cigarette packaging which included graphic images positively impacted young smokers determination to quit over an extended period of time.

Other scholars have taken an interest in the effects of multimodal frames, those which include a combination of texts and visuals. Geise and Baden (33) proposed a theoretical framework for understanding multimodal framing effects which draws attention to the amplifying effect of images. In terms of multimodal frames and products, recent work has suggested that textual framing might be more effective for some types of products, while visual framing or a combination of both works better for others (34, 35).

Of particular relevance here is the research on framing of genetically modified (GM) foods. Media coverage on GM foods has been shown to have a significant impact on public perceptions of, and behavior toward, the technology (36–39), and there is plenty of research on the nature of this coverage. Researchers have identified coverage on GM foods to be primarily driven by specific events such as food scares and environmental events (40, 41). Others have shown how mainstream media coverage diverges somewhat from scientific publications (42), and how stakeholders have been characterized to fit simple narratives (43). This demonstrates how media coverage is dependent on breaking stories, and how complexity is condensed for popular consumption.

Coverage has been different in different countries, however. Listerman (44) argued that, whilst US coverage of GM foods focused on the scientific-economic elements of the technology, German coverage was focused on the practical ethics and British coverage was focused on the public discourse. Coverage in the US was generally more positive than in the UK (41), and in China was universally positive or neutral (45). Whilst Botelho and Kurtz (40) argued that coverage within countries was fairly similar, Vicsek (46) noted that Hungarian coverage was particularly polarized. Interestingly, several researchers have commented on how genetic technology was generally framed much more negatively in relation to food than it was in relation to medicine within the same media outlets (38, 47, 48).

While there has been some important framing research concerning innovations in food products (49–51), there has been surprisingly little work on the intentional use of different frames to introduce audiences to new food products, particularly those closely connected to technological innovation. This article explores the effectiveness of different multimodal frames for a new food innovation, meat produced outside of an animal in a laboratory.

Cultured Meat

In the near future, we will be able to produce meat directly from animal cells (52). Termed "cultured meat," this technology will enable us to sustainably produce meat for a growing global population, whilst reducing animal suffering on an enormous scale (53, 54). However, research into public perceptions of cultured meat has indicated that some consumers may have reservations around the concept (55).

Although many consumers recognize the potential ethical and environmental benefits of cultured meat, some have concerns about its alleged unnaturalness, which can lead to concerns about food safety (56–58). Recent studies have demonstrated how these perceptions can be invoked or avoided by different framings.

The Good Food Institute (59, 60) has given substantial attention to the question of what cultured meat should be called, demonstrating that consumers are significantly more likely to find "clean meat" appealing than other names including "cultured meat" and "cell-based meat." This finding has been replicated by Bryant and Barnett (61). Siegrist et al. (57), meanwhile, have demonstrated that less technical descriptions of cultured meat lead to higher consumer acceptance compared to more technical descriptions.

These findings are relevant for the interpretation of much of the existing research on cultured meat. For instance, Verbeke et al. (58) noted many consumers in their focus groups reacted with disgust to the concept and perceiving few personal benefits—yet, these responses were undoubtedly influenced by the video participants were shown, which describes "synthetic meat" being grown in labs. Likewise, Laestadius and Caldwell (62) conducted an analysis of online comments on news stories about cultured meat, but note "...the framing of the issue in each individual article may have influenced perceptions of [cultured meat]" (p. 2466).

Therefore, the framing of cultured meat is likely to have a substantial impact on consumer perceptions, though this has yet

to be studied empirically (55). Whilst Goodwin and Shoulders (63) reported that European and American media coverage of cultured meat commonly discusses its benefits, production process, timescale, history, and skeptics, Dilworth and McGregor (64) identified naturalness as a key focus in Australian print media. Indeed, stories about cultured meat frequently feature "science themed" photos such as meat in a petri dish in a lab [e.g., (65, 66)]. Meanwhile, Hopkins (67) has commented that coverage in western media has focused disproportionately on the reactions of vegetarians.

While a variety of frames pertaining to cultured meat are available, little is known about how they may affect consumer attitudes. A wealth of existing research indicates that frames have an impact on public attitudes, but this has not yet been formally studied in the context of cultured meat. The present study seeks to understand how different frames affect consumer attitudes, beliefs, and behavioral intentions toward cultured meat.

METHODS

We used an experimental survey to test the effect of different framings of cultured meat on consumer attitudes, beliefs, and behavioral intentions. This study received ethical approval from the Portland State University Institutional Review Board.

TABLE 1 | Demographic breakdown of participants.

		Number	Percentage
Gender	Male	276	57.5
	Female	202	42.1
	Other	2	0.4
Age	18–25	92	19.2
	26–35	229	47.7
	36–45	84	17.5
	46–55	38	7.9
	Over 55	37	7.7
Region	Northeast	109	22.7
	South	185	38.5
	Midwest	81	16.9
	West	105	21.9
Diet	Omnivore	422	87.9
	Pescatarian	35	7.3
	Vegetarian	14	2.9
	Vegan	9	1.9

Participants

Participants were U.S. adults recruited through Amazon MTurk, a microtasking platform frequently used in social research. MTurk enables researchers to get high quality affordable data from a sample which is more representative than college samples which have commonly been used in the past (68). However, we did find evidence of some illegitimate or duplicate responses. After removing these responses, the sample size dropped from 527 to 480. Participants were each paid $0.50 for their time.

The demographic breakdown of participants is shown in **Table 1**:

As shown here, the sample is slightly skewed toward younger age groups (in particular 26–35) and toward males. The south of the country is also slightly over-represented, though overall the sample is reasonably representative.

Procedure

First, participants read some information about the study and gave their consent to take part. They were then asked for demographic information, including gender, age group, region, and which foods they eat. These foods were later used to determine diet.

Next, participants indicated whether they had heard of cultured meat before. They then read the following description of cultured meat:

> "Clean meat (also called cultured meat or *in-vitro* meat) is real meat which is grown from animal cells without the need to raise animals. It should not be confused with meat substitutes such as soy, since it is real animal meat it has the same taste, texture, and the same or better nutritional content as conventionally-produced meat."

Next, participants gave one word that they first thought of when they thought about cultured meat. This was an open question, and was later used to identify illegitimate responses. Participants also indicated how familiar they were with cultured meat on a 5-point Likert scale (1 = Not at all familiar, 5 = very familiar).

Participants were then allocated to one of three experimental conditions. These conditions (see **Table 2**) contained an image

TABLE 2 | Text and images presented to participants in each condition.

Societal benefits

Clean meat has many benefits for society like reducing harm to the environment and helping animals.

High-tech

Clean meat is made using highly advanced technology in a state of the art laboratory.

Same meat

Clean meat tastes like conventional meat, is increasingly affordable and can be healthier to eat.

and a short piece of text. They corresponded to three different framings that cultured meat could be presented in.

Next, participants were asked to rate their attitude toward cultured meat on a 5-point Likert scale (1 = Very favorable, 5 = Very unfavorable).

Participants were then asked to rate their agreement with five statements about cultured meat on 5-point Likert scales (1 = Strongly disagree, 5 = Strongly agree). The statements were about cultured meat's healthiness, safety, environmental friendliness, sensory quality, and benefits for society. Next, participants rated four concerns about cultured meat using 5-point Likert scales (1 = Not at all concerned, 5 = Extremely concerned). The concerns were about cost, taste, naturalness, and safety. These are common concerns and benefits identified by Bryant and Barnett (55).

Finally, participants rated their willingness to eat cultured meat using 5-point Likert scales (1 = Definitely yes, 5 = Definitely No). Participants were asked about their willingness to try cultured meat, willingness to buy cultured meat regularly, willingness to eat cultured meat as a replacement for conventionally produced meat, and willingness to eat cultured meat compared to plant-based meat substitutes. These measures were adapted from Wilks and Phillips (69).

During analysis, we removed 47 illegitimate or duplicate responses. We also computed diet based on foods which participants said they ate. Finally, we recalibrated all Likert scales such that higher numbers represented more positive opinions of cultured meat. This involved reverse coding the attitude rating, concern ratings, and behavioral intentions ratings.

Experimental Design

We opted for an experimental design whereby participants would see one of three framings before answering questions about cultured meat. This approach is fairly common in similar research (57, 70) as it allows for direct comparison between groups who have seen different information. While some authors (71) have used repeated measures designs (before/after information), we decided to avoid this approach since participants might be anchored to responses they give before reading additional information. Indeed, Bekker et al. (71) implemented a Solomon four-group design to rule out such effects.

These three framings were chosen because they represent common discourses on cultured meat. Potential societal benefits, the technical scientific nature of the product, and the sensory similarity to conventional meat are all themes which occur in media coverage of the topic (62). Furthermore, they are well-defined and distinct from one another in that they foreground a different aspect of the technology, and could therefore be expected to produce different perceptions to some extent.

It is worth noting that we did not include a control group as such. We could have asked a control group about their perceptions of cultured meat after reading basic facts about the product with no framing. However, such a presentation of information is unlikely to occur in the media. Moreover, one could argue that there is no such thing as "no framing" in this context—any information we could give about cultured meat would, by definition, focus on some aspects more than others, and therefore would frame the product in some way. Therefore, we decided not to include a control group in the conventional sense.

It is also worth noting that some measures (e.g., about taste, healthiness, and benefits to society) asked about things which were explicitly mentioned in some of the experimental manipulations. For example, the "same meat" framing mentions that "Clean meat tastes like conventional meat," and we might therefore expect responses to reflect this. We should bear in mind the content of the messages when interpreting the results; higher agreement with statements about aspects of the technology mentioned in the descriptions is to be expected, and can be taken as confirmation that participants have engaged with and believed the material. Of course, this may not be the case, and beliefs about specific aspects of the technology may not be sensitive to such information if it is not deemed credible.

RESULTS

Overall Findings

Before examining differences between experimental groups, we looked at the findings across all experimental conditions. Our findings are comparable to those observed in previous U.S. studies: we found that 64.6% of participants were probably or definitely willing to try cultured meat, which is very similar to the rates observed in previous research (69, 70). Only 18.4% were probably or definitely not willing to try cultured meat, whilst 16.9% were unsure.

Similarly optimistic rates were found with regards to participants' willingness to buy cultured meat regularly (49.1% were probably or definitely willing to do this; 24.5% were probably or definitely not willing to; 26.4% were undecided) and willingness to eat cultured meat as a replacement for conventional meat (48.5% were probably or definitely willing to do this; 26.6% were probably or definitely not willing to; 24.9% were undecided). Of the 243 participants who currently ate plant-based meat substitutes, 49.8% were somewhat or much more likely to eat cultured meat; 25.5% were somewhat or much less likely, and 24.7% were undecided.

Overall, this indicates a fairly high willingness to eat cultured meat regardless of framing, with almost two thirds of participants being willing to try it, and almost half willing to buy it regularly and eat it instead of conventional meat. This indicates a substantial potential market for cultured meat, and provides evidence that cultured meat could displace a considerable amount of demand for conventional meat.

Demographic Variations in Acceptance

Previous research has discussed demographic variations in acceptance of clean meat, and some studies have found higher acceptance amongst men, younger people, and omnivores [see (55)]. To test for significant differences in acceptance between demographic groups, we conducted a series of three one-way between-group ANOVAs with gender, age, region, and diet as independent variables, and the range of acceptance measures as dependent variables. No significant differences were found between respondents from different regions.

In terms of gender, we detected several significant differences between men and women. In line with previous research, men had more positive views of cultured meat than women, on average. These differences were significant with respect to attitude, perceived safety, perceived taste, perceived benefits for society, willingness to try, willingness to buy regularly, willingness to replace conventional meat, and willingness to eat over plant-based alternatives ($p < 0.05$). However, men were more concerned about the cost compared to women ($p = 0.01$).

Age was also a factor which affected views on cultured meat. Younger people generally had more positive views than older people, with a steady decline in attitudes in older age groups. Curiously, the 56+ age group was an exception here—people in this group tended to have more positive views than those in the 36–45 and 46–55 age groups. Significant differences were found in the different age groups' attitudes, perceived taste, perceived benefits for society, willingness to try, willingness to buy regularly, willingness to replace conventional meat, and willingness to eat compared to plant-based alternatives ($p < 0.05$).

Participants with different diets also had differing views on cultured meat. We observed interesting differences between vegetarians/vegans and those who eat meat/fish. Vegetarians/vegans were significantly less willing to try cultured meat than meat/fish-eaters ($p = 0.014$) and significantly less willing to eat cultured meat compared to plant-based alternatives ($p = 0.01$), but meat/fish-eaters had significantly higher concerns about the taste, naturalness, and safety of the product ($p < 0.05$). This probably reflects a relative lack concern on the part of vegetarians/vegans, who were not intending to eat the product anyway. This partly reflects the findings of Wilks and Phillips (69), who similarly found vegetarians/vegans to be more positive about some aspects of cultured meat, but relatively unwilling to eat it themselves.

Word Associations

Participants gave word associations immediately after learning about cultured meat. Word associations is a technique which has been used in previous research to explore consumer perceptions of novel products (61, 72). A codebook was developed based on common categories which the word associations fit into. Each word was then categorized independently by both researchers. We agreed on the categories of 83.5% of the words; the remaining words were categorized after consultation between the researchers. The categories of words given by consumers are shown in **Table 3**.

TABLE 3 | Word associations given by participants after learning about cultured meat.

Category	No. of words	Percentage	Example words
Artificial	73	15.2	Fake, unnatural, artificial
Science	54	11.3	Scientific, laboratory, chemicals
Positive	50	10.4	Good, awesome, super
Natural	40	8.3	Natural, no hormones, unprocessed
Unusual	35	7.3	Weird, strange, different
Food	27	5.6	Beef, calories, steak
Healthy	26	5.4	Fat-free, healthy, good for health
Clean	25	5.2	Sterilized, washed, soap
Disgust	24	5.0	Disgusting, yuck, gross
Other	18	3.8	Options, jars, grown
Taste	16	3.3	Tasty, bland, delicious
Food technology	14	2.9	GMOs, cultured meat, laboratory meat
Interesting	12	2.5	Interesting, intriguing
Animals	10	2.1	Chicken, fish, pig
Ethical	10	2.1	Ethical, cruelty-free, humane
Fear	10	2.1	Unsafe, danger, creepy
Negative	9	1.9	Abomination, dystopia, never
Safety	7	1.5	Safe, safety, passes regulation
Uncertainty	7	1.5	Confusing, why, unobtainable
Environment	5	1.0	Sustainable, biofriendly, green
Special diet	5	1.0	Vegetarian, Halal, Kosher
Cost	3	0.6	Expensive, pricey, cost
Total	480	100	

Experimental Findings

Before proceeding with analysis, we wanted to verify that key demographic and familiarity variables associated with cultured meat acceptance had been evenly distributed across experimental conditions. To this end, we tested for significant differences between experimental groups using Chi square and ANOVA tests as appropriate.

Chi square tests reveal that there are no significant differences between conditions in the proportions of participants in each gender ($\chi^2 = 4.009$, $p = 0.405$), age group ($\chi^2 = 8.762$, $p = 0.363$), region ($\chi^2 = 6.726$, $p = 0.347$), or diet ($\chi^2 = 10.463$, $p = 0.106$). ANOVA tests reveal no significant differences between conditions in the proportion of participants who had heard of cultured meat [$F_{(2, 477)} = 1.530$, $p = 0.218$] or the familiarity with cultured meat [$F_{(2, 477)} = 0.895$, $p = 0.409$]. Given no significant differences between experimental conditions with respect to these variables, we can rule this out as a source of bias.

Attitudes and Beliefs

We tested for significant differences in attitudes and beliefs between experimental conditions using one-way ANOVA analyses. The results (shown in **Table 4**) indicate several significant differences ($p < 0.05$) between experimental conditions, indicating that the framing had a statistically significant effect on key attitudes and beliefs about cultured meat.

Within rows, mean values which are significantly different using Tukey's HSD ($p < 0.05$) are denoted using different subscript letters. Values which share a subscript letter are not significantly different.

As shown here, the experimentally manipulated framing had a statistically significant effect on attitude, belief that cultured meat is healthy, belief that cultured meat is safe, and belief that cultured meat is good for the environment (although no pairwise comparisons were significantly different for the latter variable). Conversely, although the omnibus ANOVA showed no significant effect on the belief that cultured meat tastes the

TABLE 4 | ANOVAs showing differences between experimental conditions in attitudes and beliefs.

Variable	ANOVA (2, 477)	Societal benefits M (σ)	High tech M (σ)	Same meat M (σ)
Attitude	$F = 5.711$, $p = 0.004$	3.45_{a} (1.13)	3.11_{b} (1.32)	3.55_{a} (1.20)
Belief that cultured meat is healthy	$F = 5.093$, $p = 0.007$	3.43_{ab} (0.98)	3.23_{b} (1.12)	3.60_{a} (1.00)
Belief that cultured meat is safe	$F = 3.247$, $p = 0.040$	3.56_{ab} (1.08)	3.40_{b} (1.12)	3.71_{a} (1.01)
Belief that cultured meat is good for the environment	$F = 3.336$, $p = 0.036$	3.98_{a} (0.99)	3.40_{b} (1.08)	3.97_{a} (0.94)
Belief that cultured meat tastes the same as conventional meat	$F = 3.003$, $p = 0.051$	3.27_{a} (1.07)	3.40_{ab} (1.08)	3.56_{b} (1.06)
Belief that cultured meat has benefits for society	$F = 0.760$, $p = 0.468$	3.70_{a} (1.02)	3.63_{a} (1.08)	3.78_{a} (1.02)
Concern about cost	$F = 0.935$, $p = 0.393$	2.70_{a} (1.19)	2.53_{a} (1.09)	2.57_{a} (1.19)
Concern about taste	$F = 0.534$, $p = 0.587$	2.38_{a} (1.05)	2.26_{a} (1.06)	2.36_{a} (1.22)
Concern about naturalness	$F = 2.055$, $p = 0.129$	2.40_{a} (1.19)	2.14_{a} (1.18)	2.36_{a} (1.24)
Concern about safety	$F = 1.064$, $p = 0.346$	2.15_{a} (1.15)	1.99_{a} (1.15)	2.16_{a} (1.16)

TABLE 5 | ANOVAs showing differences between experimental conditions in behavioral intentions.

Variable	ANOVA (2, 477)	Societal benefits M (σ)	High tech M (σ)	Same meat M (σ)
Willingness to try cultured meat	$F = 9.808$, $p < 0.001$	3.79_{a} (1.10)	3.30_{b} (1.55)	3.85_{a} (1.62)
Willingness to eat cultured meat regularly	$F = 7.313$, $p = 0.001$	3.50_{a} (1.10)	3.03_{b} (1.33)	3.48_{a} (1.21)
Willingness to replace conventional meat	$F = 5.488$, $p = 0.004$	3.37_{a} (1.16)	3.03_{b} (1.36)	3.49_{a} (1.24)
Willingness to eat compared to plant-based meat substitutes	$F = 4.834$, $p = 0.008$	3.42_{ab} (1.20)	3.10_{b} (1.27)	3.51_{a} (1.23)

same as conventional meat, *post-hoc* tests did show a significant pairwise difference. No significant differences were found on the belief that cultured meat has benefits for society, or on any measures of concern about cost, taste, naturalness, or safety.

In each case, the "same meat" framing was shown to be conducive to the most positive attitudes, whereas the "high tech" framing was shown to be conducive to the least positive attitudes.

Behavioral Intentions

Next, we tested for significant differences between framings in behavioral intentions using a one-way ANOVA. A similar pattern of results emerges with respect to behavioral intentions, as shown in **Table 5**.

Again, participants who saw the "high tech" framing were significantly less willing to try cultured meat, buy cultured meat regularly, eat cultured meat as a replacement for conventional meat, and eat cultured meat compared to plant-based meat substitutes compared to those who saw other framings.

Although these differences were significant, the effect sizes were relatively small. It should be noted that perceptions of cultured meat are likely to be changed by further information, and may not be stable over time.

DISCUSSION AND CONCLUSION

In this study, we demonstrated that the framing of cultured meat has a significant effect on many attitudes and beliefs about the product, as well as behavioral intentions toward it. Our results somewhat mirror the findings of Siegrist et al. (57), who found that more technical descriptions of cultured meat lead to lower acceptance compared to less technical descriptions. This is probably because the information in the "high tech" condition (particularly the image) were evocative of an image of science and unnaturalness. Siegrist and Sütterlin (73) demonstrated that perceived naturalness of cultured meat mediated the acceptability of risk.

Implications

These findings offer important insight for those publicizing and promoting cultured meat. While more research is clearly needed in terms of the frames currently used both by companies in the industry and the media, existing work suggests that the most common frame used thus far may be the least effective in garnering consumer acceptance. As noted previously, many of the media reports have featured images like the petri dish and used terminology like "test tube meat" to introduce this concept and the products associated with it to the public. While fledgling ventures might welcome media interest and the benefits associated with earned media, these findings suggest that the frames favored by the media might do more harm than good. At the same time, this must be weighed against the benefits of increased consumer familiarity (55). Since more familiar consumers are more likely to say they would eat cultured meat, it may be the case that any coverage is better than no coverage, regardless of framing.

The findings may also inform future decisions for the messaging of this product, once the products are close to launching and dedicated advertising and marketing campaigns are underway. A quick perusal of comments by company executives, venture capitalists and supporting institutions in this area suggest a laudable commitment to transparency in terms of the production process. The outcomes of the research here argue for a high level of intentionality in how the process is shared with the public. Perhaps the most effective approach would be to have that information readily available for consumers who seek it, but not to have the high tech process as the dominant frame in promotional materials. Instead, producers should consider shifting their frame from discussing the production process to discussing product features and societal benefits. This should be done both in terms of paid and earned media activities.

Whilst producers and traditional media outlets have a certain degree of control over what framings are employed in discussions of cultured meat, social media represents a domain in which such control is substantially limited. Fellenor et al. (74) have demonstrated how social media, compared to traditional media, can lead to substantially different framings, with certain groups selecting and emphasizing different "frame fragments" (p. 1174) as they share information. As the authors comment, the curated nature of social media news feeds can lead to individuals having different aspects of a concept highlighted or backdropped. In this context, this may lead to a variety of personalized frames. Notably, such frames are likely outside the control of cultured meat producers and traditional media sources. The same is true of those developed through other unconventional media such as blogs.

Contributions to the Field

This article contributes to the field in several important ways. First, it advances the conversation on multimodal frames through its consideration of responses to image and text combinations. As these combinations reflect the type of messaging that most consumers are exposed to in contemporary marketing and promotional efforts, it deepens understanding of consumer reactions in contexts with a variety of messaging modes. Second, this article contributes to the growing field of research on very new products (VNP) and specifically the marketing of products associated with advanced technological processes. As more and more of these types of products are introduced into the marketplace, it is important for the field to further develop a focus on consumer responses to them Finally, and perhaps most importantly, this research offers a noteworthy addition to a fledgling but growing area of interest in a wide host of issues surrounding the food technology of cultured meat. It complements work done by Goodwin and Shoulders (63) and Dilworth and McGregor (64) who identified varied media frames of cultured meat in different countries and offers an invitation for additional research in this area. Indeed, stories about cultured meat frequently feature "science themed" photos similar to the one used in the process framing condition here [e.g., (65, 66)]. As this product moves through the concept phase to the production process and finally to market, researchers in a wide host of disciplines can make significant contributions not only to their fields of study, but also to society as they explore this transformative technology.

Limitations

There are several limitations to acknowledge here. Firstly, the data is subject to well-known concerns about the quality of self-reported data. Data which is self-reported rather than observed is likely to be biased in some predictable ways; participants may report their past behaviors inaccurately due to poor memory, or their intended behaviors may not represent what they actually do due to poor forecasting. Moreover, some participants may give socially desirable answers, particularly when the subject is moralized, potentially leading them to over-report their intention to eat cultured meat in this case.

Secondly, we have some concerns about the data quality. Data was collected from Amazon MTurk, which has recently been subject to concerns about bots answering surveys (75). Indeed, we identified 47 responses which seemed not to be genuine (most had given nonsensical answers to text input questions) but it is difficult to know whether more went unnoticed. This is likely to be a problem for any researchers using online survey response platforms, and such problems have recently been well documented with MTurk.

Finally, the external validity of an online study which asks participants about a future product is inevitably limited. Whilst we gave all participants information about cultured meat, it is possible that this information would be interpreted differently in the context of taking an online survey compared to making actual purchase decisions in a restaurant or store. Indeed, seeing just an image and a strapline may be a contrived way to consume information, although arguably this could be similar to a headline and image in media.

Overall, there are some concerns about data quality and the external validity of the survey, however these are minor concerns and we have taken steps to mitigate these where possible.

Future Research

Future research on the topic of framing new technologies could explore how producers attempt to influence media frames, how successful they are in promoting their preferred frames, and the downstream effect on consumer attitudes. Systematically comparing the frames used by producers with those present in media reports using content analysis could highlight which aspects of reality each choose to foreground. This will be particularly relevant to other consumer technologies which may become available imminently, and which can be readily interpreted in different ways, for example self-driving vehicles.

In terms of consumer research in relation to cultured meat specifically, the field would benefit from rigorous content analyses of frames used by both producers and the media over the last 5–7 years. What are the dominant frames presented to consumers both by producers through their own promotional materials like YouTube videos and by journalists in their stories? Have these frames changed over time? Do these frames differ from those which occur on social media? And finally, how are consumer perceptions and intentions influenced by the frames they encounter and have these changed over time?

Future research on cultured meat acceptance, meanwhile, could attempt to track consumer attitudes over time. Such a longitudinal design could allow researchers to attempt to observe the real effect of relevant news on consumer attitudes. Observing shifts in specific beliefs and attitudes could provide a way to observe the changes that take place when consumer attitudes shift over time, and could provide a method for measuring the master frame through which consumers interpret cultured meat. Moreover, it would be able to test the idea that acceptance will increase over time as people become more familiar with the product and products become commercially available.

Finally, further exploration of public opinions of cultured meat on social media and blogs may be warranted. As we have discussed, social media may lead to a variety of personalized frames which are outside the control of producers and traditional media outlets. Such an environment could lead to further insights about important narratives about cultured meat as they develop.

DATA AVAILABILITY

The participants in this study were not asked for permission to share the data publicly. Therefore, the dataset for this study is not available.

ETHICS STATEMENT

The study received ethical approval from the Portland State University Institutional Review Board. Participants indicated their consent to take part as part of the online survey process. The study was of a general population, no vulnerable participants were specifically recruited.

AUTHOR CONTRIBUTIONS

CD and CB: research design, survey instrument, writing manuscript, and editing manuscript. CD: ethics application and data collection. CB: data analysis.

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REVIEW
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The Myth of Cultured Meat: A Review

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To satisfy the increasing demand for food by the growing human population, cultured meat (also called *in vitro*, artificial or lab-grown meat) is presented by its advocates as a good alternative for consumers who want to be more responsible but do not wish to change their diet. This review aims to update the current knowledge on this subject by focusing on recent publications and issues not well described previously. The main conclusion is that no major advances were observed despite many new publications. Indeed, in terms of technical issues, research is still required to optimize cell culture methodology. It is also almost impossible to reproduce the diversity of meats derived from various species, breeds and cuts. Although these are not yet known, we speculated on the potential health benefits and drawbacks of cultured meat. Unlike conventional meat, cultured muscle cells may be safer, without any adjacent digestive organs. On the other hand, with this high level of cell multiplication, some dysregulation is likely as happens in cancer cells. Likewise, the control of its nutritional composition is still unclear, especially for micronutrients and iron. Regarding environmental issues, the potential advantages of cultured meat for greenhouse gas emissions are a matter of controversy, although less land will be used compared to livestock, ruminants in particular. However, more criteria need to be taken into account for a comparison with current meat production. Cultured meat will have to compete with other meat substitutes, especially plant-based alternatives. Consumer acceptance will be strongly influenced by many factors and consumers seem to dislike unnatural food. Ethically, cultured meat aims to use considerably fewer animals than conventional livestock farming. However, some animals will still have to be reared to harvest cells for the production of *in vitro* meat. Finally, we discussed in this review the nebulous status of cultured meat from a religious point of view. Indeed, religious authorities are still debating the question of whether *in vitro* meat is *Kosher* or *Halal* (e.g., compliant with Jewish or Islamic dietary laws).

Keywords: cultured meat, *in vitro* meat, muscle cells, livestock farming, consumer perception, vegetarian, ethics

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INTRODUCTION: CONTEXT OF ANIMAL FARMING TODAY

The global population, 7.3 billion today, is expected to surpass 9 billion by 2050. The Food and Agriculture Organization (FAO) has forecast that in 2050, 70% more food will be needed to fulfill the demand of the growing population, which is a great challenge due to resource and arable land limitations. Even if meat consumption is decreasing in developed countries, its global consumption is increasing because consumers are generally unwilling to reduce their meat consumption, in particular in developing countries such as in China, India, and Russia (1). These populations becoming more middle-class, they are looking for more luxury products, such as meat or other animal products (e.g., cheese, dairy products).

Livestock systems will contribute to addressing the issue of global food and nutrition security in the world (2). Animal farming must produce larger quantities of high quality and affordable meat, milk, and eggs, through production systems that are environmentally sound, socially responsible, and economically viable (3). Despite the wide range of economic, environmental, cultural and social services at local, regional, and global levels provided by livestock farming (4), a significant proportion of livestock is raised nowadays within the factory farming model. Despite a lower contribution to greenhouse gases (GHG) and water usage than extensive agriculture, factor farming is mainly focused on efficiency (i.e., the quantity of milk or meat produced) rather than on other services and impacts such as interaction with the environment, climate change, less use of antibiotics, animal welfare, or sustainability (5–8).

As a consequence, more efficient ways of protein production are being developed to sustain the growing global population while complying with today's challenges, such as environmental and animal welfare issues (9). Among the solutions, cultured meat is presented by its advocates as a sustainable alternative for consumers who want to be more responsible but do not wish to change the composition of their diet (10–13). The history of cultured meat was detailed by Hamdan et al. (14), and a bibliometric analysis of publications about this subject was carried out by Fernandes et al. (15). Indeed, since the first publication about cultured meat in 2008, the number of publications increased considerably (89% of the total) after 2013. In August of that same year, the first hamburger produced with cultured meat was prepared and tasted on a television program (16).

THE PRODUCTION OF CULTURED MEAT

Pros and Cons of the Culture Process

The objective of this process is to recreate the complex structure of livestock muscles with a few cells. A biopsy is taken from a live animal. This piece of muscle will be cut to liberate the stem cells, which have the ability to proliferate but can also transform themselves into different types of cells, such as muscle cells and fat cells (16).

The cells will start to divide after they are cultured in an appropriate culture medium, which will provide nutrients, hormones and growth factors. The best medium is known to contain fetal bovine serum (FBS), a serum made from the blood of a dead calf, which is going to be rate-limiting, and not acceptable for vegetarians nor vegans. More than one trillion cells can be grown, and these cells naturally merge to form myotubes which are no longer than 0.3 mm; the myotubes are then placed in a ring growing into a small piece of muscle tissue as described in different reviews (17, 18). This piece of muscle can multiply up to more than a trillion strands (13). These fibers are attached to a sponge-like scaffold that floods the fibers with nutrients and mechanically stretches them, "exercising" the muscle cells to increase their size and protein content (17, 18). Based on this process, fewer animals will be necessary to produce huge amounts of meat due to cell proliferation, thereby avoiding killing as too many animals but potentially lots of calves if FBS is still used.

Throughout this process, the cells are kept in a monitored environment that replicates the temperature inside the body of a cow, for example, to speed up the development of the lab-grown meat (17, 18).

One initial problem with this type of culture is the serum used, as *in vitro* meat aims to be slaughter-free. So it is contradictory to use a medium made from the blood of dead calves. In addition, this serum is expensive and affects to a large extent the production cost of the meat. One of the main goals of the laboratory start-ups (about 25–30) as of this writing, scattered over the globe and working on cultured meat is to find a cheaper medium derived from plant ingredients and as efficient as FBS. Apparently (from personal communications), this problem has been solved, at least in research prototypes to produce cultured meat. Once this problem has been solved on an industrial scale (and it is likely to be solved), *in vitro* meat could become competitive in terms of production costs and animal ethics compared to regular meat from livestock. In addition to FBS, antibiotics and fungicides have been commonly used to avoid contamination of cell cultures. All the start-ups claim that this problem has also been solved.

However, as farm animals, like all mammals including humans, naturally produce hormones and growth factors to sustain their own growth, cell culture needs hormones, growth factors, etc., in the culture medium to sustain cell proliferation and differentiation. The research questions are now: how can these compounds be produced on an industrial scale, and how can be ensured that none of them will have negative effects on human health in the short and long term? This is an important issue since hormone growth promoters are prohibited in farming systems for conventional meat production in the European Union (unlike in some other parts of the world).

Finally, we are still far away from real muscle, which is made up of organized fibers, blood vessels, nerves, connective tissue and fat cells (19–21). This is why the different start-ups working in this area have developed different strategies: some of them work with stem cells or muscle cells to reproduce unorganized muscle fibers, which is the simplest approach, while others are trying to reproduce thin slices of muscles (i.e., muscle fibers and other cell types quite well imbricated together). Nevertheless, the production of a thick piece of meat like a real steak is still a dream, due to the necessity of perfusing oxygen inside the meat to mimic the diffusion of oxygen as it occurs in real tissue.

In addition, it is difficult to imagine that laboratory meat producers will be in a position in the near future to offer consumers a wide range of meats reflecting the diversity of animal muscles or cuts. Indeed, the sensory quality (i.e., flavor) of meat differs across species (pork, poultry, ovines, bovines, etc), and within a species, between breeds, genders, animal types (i.e., young bulls, steers, heifers, and cows in the case of bovines), farming conditions (depending for instance on breeding location), and mainly between muscles with a different anatomic location (22). So, many complex processes still need to be controlled to make *in vitro* meat more attractive to consumers as it is more or less the case for any other new food product.

Health and Safety

Advocates of *in vitro* meat claim that it is safer than conventional meat, based on the fact that lab-grown meat is produced in an environment fully controlled by researchers or producers, without any other organism, whereas conventional meat is part of an animal in contact with the external world, although each tissue (including muscles) is protected by the skin and/or by mucosa. Indeed, without any digestive organs nearby (despite the fact that conventional meat is generally protected from this), and therefore without any potential contamination at slaughter, cultured muscle cells do not have the same opportunity to encounter intestinal pathogens such as *E. coli*, *Salmonella* or *Campylobacter* (10), three pathogens that are responsible for millions of episodes of illness each year (19). However, we can argue that scientists or manufacturers are never in a position to control everything and any mistake or oversight may have dramatic consequences in the event of a health problem. This occurs frequently nowadays during industrial production of chopped meat.

Another positive aspect related to the safety of cultured meat is that it is not produced from animals raised in a confined space, so that the risk of an outbreak is eliminated and there is no need for costly vaccinations against diseases like influenza. On the other hand, we can argue that it is the cells, not the animals, which live in high numbers in incubators to produce cultured meat. Unfortunately, we do not know all the consequences of meat culture for public health, as *in vitro* meat is a new product. Some authors argue that the process of cell culture is never perfectly controlled and that some unexpected biological mechanisms may occur. For instance, given the great number of cell multiplications taking place, some dysregulation of cell lines is likely to occur as happens in cancer cells, although we can imagine that deregulated cell lines can be eliminated for production or consumption. This may have unknown potential effects on the muscle structure and possibly on human metabolism and health when *in vitro* meat is consumed (21).

Antibiotic resistance is known as one of the major problems facing livestock (7). In comparison, cultured meat is kept in a controlled environment and close monitoring can easily stop any sign of infection. Nevertheless, if antibiotics are added to prevent any contamination, even occasionally to stop early contamination and illness, this argument is less convincing.

Moreover, it has been suggested that the nutritional content of cultured meat can be controlled by adjusting fat composites used in the medium of production. Indeed, the ratio between saturated fatty acids and polyunsaturated fatty acids can be easily controlled. Saturated fats can be replaced by other types of fats, such as omega-3, but the risk of higher rancidity has to be controlled. However, new strategies have been developed to increase the content of omega-3 fatty acids in meat using current livestock farming systems (23). In addition, no strategy has been developed to endow cultured meat with certain micronutrients specific to animal products (such as vitamin B12 and iron) and which contribute to good health. Furthermore, the positive effect of any (micro)nutrient can be enhanced if it is introduced in an appropriate matrix. In the case of *in vitro* meat, it is not certain that the other biological compounds and the way they are organized in cultured cells could potentiate the positive effects of micronutrients on human health. Uptake of micronutrients (such as iron) by cultured cells has thus to be well understood. We cannot exclude a reduction in the health benefits of micronutrients due to the culture medium, depending on its composition. And adding chemicals to the medium makes cultured meat more "chemical" food with less of a clean label.

Comparison of Environmental Impact With Conventional Farming

Generally speaking, the production of cultured meat is presented as environmentally friendly, because it is supposed to produce less GHG (which is a matter of controversy), consume less water and use less land (this point being obvious) in comparison to conventional meat production (13, 24, 25), from ruminants particularly. However, this type of comparison is incomplete and sometimes biased or at least, partial as discussed below.

Regarding GHG, it is true that livestock, mainly ruminants (i.e., cattle), are responsible for a significant proportion of world GHG emissions, in large part due to methane emissions from the digestive tracts of herbivores. As such, reducing methane emissions (one of the most potent GHG) is presented as one of the more important potential benefits of *in vitro* meat over conventional livestock farming. Cattle farming is, as well-known, associated with the emission of three GHG [especially methane (CH_4), but also carbon dioxide (CO_2), and nitrous oxide (N_2O)]. On the contrary, emissions by cultured meat are mainly CO_2 due to fossil energy use to warm cultured cells. Nevertheless, in carbon equivalent, there is no consensus about GHG emissions of lab-grown meat compared to conventional meat: a first study gave an advantage to cultured meat (25) whereas a second study was inconclusive (26).

In a recent study, Lynch et al. (24) concluded that global warming will be less with cultured meat than with cattle initially, but not in the long term because CH_4 does not accumulate as so long in the atmosphere unlike CO_2. In some cases, cattle systems are characterized by a greater peak warming compared to *in vitro* meat. However, their warming effect will decline and will be stabilized with the new emission rates of cattle systems. On the other hand, warming due to the long-lived CO_2 gas from *in vitro* meat will persist. It will even increase with a low meat consumption, being even higher than that of cattle production in some cases. They concluded that the potential advantage of cultured meat over cattle regarding GHG emissions is not obvious.

Otherwise, some scientists (27) demonstrated that conventional beef production systems in the USA (finished in feedlots with growth-enhancing technology), produce less GHG emissions, and require the fewest animals, water, and land, with a relatively low carbon footprint to produce beef, compared to a -fed systems. Indeed, with the shortest time interval from birth to slaughter, conventional systems require less maintenance energy.

So, the respective impacts of cattle and cultured meat will depend on the availability of systems for energy generation and of production systems that will be in place.

Regarding water consumption, it is claimed in the media that 15,000 L of fresh water are necessary to produce 1 kg of beef. In reality, 95% of this amount of water is used for the growth of crops, plants and forages to feed animals. Much of this water is not saved if farm animals are removed from pastures and land. Thus, different methods give wildly different results for the same livestock product. It is now accepted that the production of 1 kg of beef will require 550–700 L of water as reviewed some years ago (28, 29). This reference point is important for the comparison of water requirements for the production of cultured meat. Unfortunately, the comparison was unfair because it was on 15,000 L. It should be based on 550–700 L. One other issue is the quality of water, which may be not so good from cultured meat factories, if we consider the activities of the chemical industry for the production of the growth factors and hormones required for cell culture. Indeed, waste and spillage of chemical products could occur and these products may be in water discharged into the environment by meat incubators, which is, however, unlikely to occur in highly controlled circumstances.

Regarding land, it is obvious that cultured meat will need less land than conventional meat production, largely based on pasture. However, this does not equate to an advantage for cultured meat. Indeed, livestock plays a key role in maintaining soil carbon content and soil fertility, as manure from livestock is a source of organic matter, nitrogen, and phosphorus. Furthermore, while it is true that the production of feed for farm animals requires 2.5 billion ha of land (i.e., about 50% of the global agriculture area), 1.3 billion ha (of land used for feed production) corresponds to non-arable grasslands, useable for livestock only (30).

Land use is a distorted and unfair comparison between cultured meat and conventional meat. Indeed, in this type of comparison, authors do not take into account the diversity of environmental services and impacts of livestock farming systems (not only GHG emissions and water use, but also carbon storage and biodiversity of plants and of animals as well) (4, 31).

Comparison of Welfare Issues With Conventional Farming

Animal welfare is a major focus of concern in some parts of our modern society. For example, Mark Post observed that there is an increasing trend of awareness of animal welfare among the Western community (16). Therefore, there are some animal defenders who can readily accept the concept of cultured meat and some have labeled cultured meat as "victimless meat" (32). Despite the fact that the process of cultured meat needs muscle samples from animals, the number of slaughtered animals can be reduced significantly (33).

However, nowadays, issues of animal welfare concern mainly cattle feedlots and pig and poultry industrial production units. Indeed, with their very high animal concentrations and associated economies of scale, such industrial units also compete strongly with smallholder farms, which are declining worldwide.

In addition, if livestock are removed and replaced with cultured meat, a number of livestock services will be lost. Indeed, livestock farming systems perform numerous functions: besides supplying proteins for human nutrition, livestock provide income for rural populations and thus support a large part of the world's rural communities. Livestock produce not only meat, milk, and eggs, but also wool, fiber, and leather. They also provide socio-cultural services including tourist events such as transhumance, and products with a local image and sense of *terroir* such as Protected Designation of Origin cheeses and other products (4, 31).

MARKET AND LEGISLATION

A recent review (34) detailed (i) the market for cultured meat, and (ii) identified key consumer, political, and regulatory issues for cultured meat.

Market

The first *in vitro* hamburger was made in 2013 after 2 years in development, by Professor Mark Post from Maastricht University. The price of this innovation was more than $300,000 in 2013. This high cost was explained by the fact that Professor Post used products and compounds (such as hormones and nutrients) traditionally used in medical science. Soon after the presentation of this innovation, Professor Post received further investments and founded a team of researchers to develop *in vitro* meat within a new start-up called *Mosa Meat*. Today, he is suggesting that in 2021, the same hamburger will be worth around US$9, which is still expensive compared to the regular hamburger at $1 (35). Furthermore, *Mosa Meat* has recently announced the development of serum-free medium according to their website's FAQ (36). No cultured meat has yet to reach the stores' shelves and the project needs more research to lower its price.

Livestock farmers are worried about the steady progress made by the aforementioned research. Indeed, the potentially effortless and low-cost production of *in vitro* meat is supposed to make it more economical than regular meat. Moreover, the issue of spoilage and of pathogens are different between cultured meat and conventional meat: keeping contamination out of cultured meat is going to be a challenge when manufacturing is scaled up and one is using a factory and not a laboratory.

Among the solutions, cultured meat is presented as a good alternative (37, 38) for consumers who want to be more responsible but do not wish to change the composition of their diet (10–13).

A recent survey shows that a potential consumer of cultured meat (which is in development) is described as a young, highly educated meat consumer, who is a little familiar with *in vitro* meat and willing to reduce their slaughtered meat consumption (39).

Due to the rise in demand for protein analogs, cultured meat sales may increase in the near future (34). Indeed, some researchers consider this new meat as a vegetarian product—good news for the expanding number of consumers who are incorporating more vegetarian and vegan choices into their diets (40, 41).

For example, Informa Agribusiness Intelligence estimates that by 2021, UK sales of meat analogs will grow by 25% and milk alternatives by 43%; such growth will take the total UK sales of milk alternatives from £149 million (US $208 million) to £299 million (US$400 million) (34). In fact, cultured meat start-ups, as well as farmhouse cheesemakers and charcuterie

producers, will have a wide range of opportunities to create their own product version, leading to additional brand diversity and competitiveness in the market, as well as engaging in higher skilled jobs in a new knowledge economy (34).

In addition, different studies have shown that acceptance of cultured meat will vary substantially across cultures (42), between gender (43) and depending on the amount of provided information about cultured meat (43). Moreover, as said previously, cultured meat is one of the solutions presented as a good alternative for consumers who want to be more responsible, but do not wish to change the composition of their diet.

As with any food product, consumers will not be willing to accept any compromises in terms of food safety or indeed to compromise much on taste or other attributes (42). Indeed, consumers are still highly influenced by the sensory quality of meat. Thus, plant-based meat alternatives have been developing and have improved a lot in terms of sensory traits in recent years, because a lot of progress has been made in mimicking real meat. Therefore, with high sensory/organoleptic quality, these meat substitutes should not be considered as an intermediate step leading to the acceptance and greater consumption of artificial meat. Indeed, sales of meat analogs made from plant-based proteins and mycoproteins may increase more than cultured meat in the near future. These meat substitutes are holding an important market share (19, 43), especially in light of the fact that $16 billion was invested in start-ups and companies offering vegetable meat substitutes ($673 million in 2018), which is much more than investments in start-ups working on cultured meat (about 100 to 200 million since 2015). Therefore, some scientists consider that cultured meat is already obsolete since progress in plant-based meat alternatives is already well advanced (44).

Furthermore, the meat industry of the future will undoubtedly be more complex than the meat industry today, with a greater number of meat products or meat substitutes on the market coming from different sources or processes (19, 43). All protein sources inherently contain both drawbacks and advantages that will affect their ability to be commercialized and accepted by consumers (43). For new products to be successful, they must be commercially viable alternatives to conventional meat production. The success of cultured meat as an alternative, substitute or complement to conventional meat will play an important role, because consumers are likely to refer to products with similar positioning in the market (38, 42, 45). Indeed, if the palatability issues are solved (which is the case today with at least some plant-based meats) and if meat substitutes are competitive in terms of price, consumers will be more open to changing their purchasing habits (43, 46, 47). However, the most technologically challenging alternatives to meat also require moderate to high degrees of social-institutional change (38). A recent study conducted by Van der Weele et al. (38) demonstrates that cultured meat and plant-based meat alternatives both require a moderate degree of social-institutional change (from the current Western dietary patterns), even if they don't require the same degree of technological change, given that, unlike cultured meat, some plant-based products are already being commercialized (**Figure 1**). In brief, to be successful, new beef products (either from the conventional beef industry or from the "*FoodTech*" industry) will need to be competitive and sustainable and in keeping with consumption habits and cultural models.

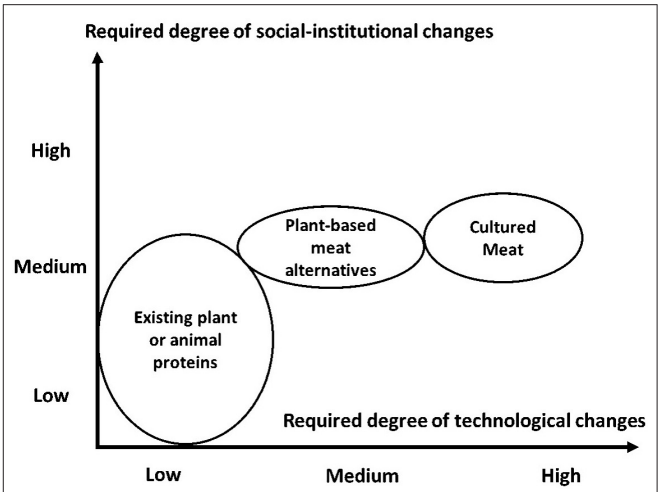

FIGURE 1 | Degree of social-institutional and technological change required for meat alternatives. Adapted from Van der Weele et al. (38).

Indeed, cultured meat requires a high degree of technological change, which may compromise a rise in its consumption. On the other hand, plant-based proteins are present in some products that are already commercialized. Some existing protein sources are either well accepted (beef, pork, meat from poultry, crops, etc.), whereas others are much less consumed or accepted (such as meat from horses, guinea pigs etc.), despite their consumption in some countries.

Legislation

A small but important body of literature exists on the regulation of cultured meat, with Schneider (48) considering regulation in the United States and Petetin (49) considering regulation in the European Union (34).

In terms of status, *in vitro* meat stands at the frontier between meat and non-meat. In April 2018, France had already banned the use of meat- and dairy-related words to designate vegetarian and vegan products. The use of the word "meat" for *in vitro* meat has not been decided yet (50). Livestock farmers in the US are backing a new law in Missouri, which states that for a product to be called "meat," it has to come from a real animal as indicated in most dictionaries. Furthermore, meat scientists differentiate between "muscle" and "meat," with the latter being the result of a natural biological process of muscle aging after slaughter due to the cessation of oxygen supply to muscle cells (51). Should "cultured meat" be called meat? If not, should *in vitro* meat still be regulated in the same way as regular meat? (52).

It is likely that the response on regulation will take time, and it is possible that the definition of "meat" will vary between countries. The Cattle Council of Australia CEO, Margo Andrae, is already warning "cultured meat companies" to avoid repeating a battle over terms as happened with "milk" and "dairy"; her view is that it should "be called what it is, which is lab-grown protein"

(50). Furthermore, the various start-ups have clearly different strategies based on marketing choices, with some of them calling the product "animal protein" and others "artificial meat." The former are driven by the will to tell the truth to consumers, the latter by a desire to be provocative in order to increase consumer interest (43).

PUBLIC PERCEPTION

How consumers perceive and accept or reject cultured meat is largely a matter of controversy (42, 53).

Consumer Perception

Advocates of cultured meat are concerned that the name could put off consumers, with possible connotations of a product that is "fake." Indeed, the lack of consumer acceptance could be a major barrier to the introduction of cultured meat (54). Furthermore, it seems difficult to evaluate consumer acceptance for an earlier stage product, which does not exist yet, as cultured meat.

It is widely acknowledged that the name given to an object or phenomenon can affect subsequent evaluations and impressions of it. In this way, different names which have an influence on consumer attitude were proposed for cultured meat (55, 56). Indeed, "*in vitro* meat," "clean meat," "cultured meat," "lab-grown meat," "synthetic meat" and other names (15) suggest that this innovation is slaughter-free, more responsible toward our environment and a credible alternative to the current intensive farming systems.

Otherwise, some authors have demonstrated (57) that consumers tend to strongly reject the name "*in vitro* meat." Moreover, the term "cultured" is less disliked than the terms "artificial" and "lab-grown" (57). This is confirmed by the Siegrist et al. study (54), which concluded that participants have a low level of acceptance of cultured meat because it is perceived as unnatural. Furthermore, they found out that giving information to participants in the survey about the production of cultured meat and its benefits has the paradoxical effect of increasing the acceptance of traditional meat (54). Bryant et al. (58) and Siegrist and Sütterlin (59) argued that a higher acceptance may be favored by less technical descriptions of cultured meat. This may be explained by the fact that the "high- tech" process is associated with something scientific and unnatural, and therefore negatively affects the product's image. In reality, consumers seem to dislike unnatural food.

In the study of Verbeke et al. (42), conducted in three EU countries, researchers demonstrated that "consumers' initial reactions when learning about cultured meat were initially underpinned by feelings of disgust and considerations of unnaturalness. After thinking, consumers envisaged few direct personal benefits from cultured meat, but they acknowledged possible global societal benefits. Perceived personal risks from eating cultured meat were largely underpinned by considerations of unnaturalness and uncertainty, and therefore inducing some kind of fear of the unknown." Later on, consumers may accept scientific progress and therefore cultured meat, but will require a trusted process of control and regulations to ensure complete safety of the product.

In a recent survey, Bryant et al. (58) asked participants from the USA, India and China about their willingness to try occasionally or to buy cultured meat regularly, to eat cultured meat instead of conventional meat or plant-based meat substitutes. Willingness to try or to eat cultured meat was quite high: 64.6% of the participants being willing to try it, and 49.1% willing to buy it regularly and eat it instead of conventional meat (48.5%). The authors interpreted those results in favor of cultured meat, saying that this "indicates a substantial potential market for cultured meat" with the consequences that cultured meat could replace a significant amount of conventional meat according to Bryant et al. (58). However, this contradicts the results of a survey by Hocquette et al. (60), who found that the majority of more educated consumers from different countries will not buy cultured meat regularly although one-third of the respondents answered "I do not know." Moreover, consumers' vision of cultured meat is likely to change over time through receiving more information.

Ethics

Ethical issues are more and more important in food choices (61), and this encourages the development of social or societal concerns (21). While the potential advantages of cultured meat regarding ethics and environmental issues are acknowledged, many consumers have concerns about food safety mainly due to the unnaturalness perception of cultured meat (42, 53) as discussed previously.

In vitro meat, like any new technology, raises inevitable ethical issues. One of the main purposes of this innovation, according to cultured meat advocates, is to stop the cruel practices endured by animals that are sometimes confined in tight spaces and slaughtered in inhumane conditions. Besides, the usual conditions of life for battery-farmed animals often lead to diseases, infections, behavioral problems, and suffering. However, due to the lack of a nervous system, cultured cells and *in vitro* meat are supposed to be free from any type of pain (62, 63) although biopsies on animals to collect cells may raise some issues concerning animal welfare. Therefore, some scientists consider this new (artificial) meat as a vegetarian product (62, 64, 65).

Thus, cultured meat aims to use considerably fewer animals than conventional livestock farming. Indeed, from an animal welfare perspective this could be attractive to some vegetarians, vegans and those conscientious omnivores interested in reducing their meat intake for ethical reasons (64).

The aforementioned idea would be more accurate if, as some start-ups have claimed, a new type of medium has been developed without the use of FBS from dead calves. Actually, some vegans have been avoiding animal food because of the meat taste. Others would consider eating it if it was produced in a cruelty-free and friendly environment (66).

Otherwise, while many scientific authors recognize the potential ethical benefits of artificial meat, namely an increase in animal welfare, nutrition-related diseases, food-borne illnesses, resource use, and greenhouse gas emissions (32), other authors, as discussed previously, are not convinced that the production of artificial meat will have a low carbon footprint. Nevertheless, it is

clear that the environmental impact of artificial meat is difficult to evaluate because it is currently based on speculative analyses (21).

But it is not that simple. There are certain issues to be considered. For example, at present, animals still have to be used in the production of cultured meat, even in fewer numbers for muscle sampling only. Whether painful or painless, animals must be reared so that their cells can be harvested to produce *in vitro* meat. "Consequently, lab-grown meat still involves animal exploitation, which is what the proponents of artificially grown meat want to avoid" (66).

Naturalness

However, if this description is true for some intensive livestock systems, whereas intensive livestock remains cruel for a lot of people, it is not the case for a significant proportion of livestock in the world, and particularly for many extensive systems in France or some African countries. In a recent review, some authors (67) concluded that sustainable intensification and agroecology could converge for a better future by adopting transformative approaches in the search for ecologically benign, socially fair and economically viable livestock farming systems.

Religion and Meat Consumption

In vitro meat, like any other new technology, raises numerous ethical, philosophical and religious questions. Mainly because of its nebulous status, religious authorities are still debating the following: whether *in vitro* meat is *Kosher* (consumable under Jewish dietary laws), *Halal* (for Muslim consumers, compliant with Islamic laws), or what to do if there is no animal available for ritual practices (Hindu consumers).

Concerning the Jewish religion, rabbinical opinion is divided. Some think that cultured meat can only be considered *Kosher* if the original cells were taken from a slaughtered *Kosher* animal. Others assume that regardless of the source of the cells used to produce the cultured meat, they will certainly lose their original identity. Therefore, the outcome cannot be defined as forbidden for consumption (68).

For the Islamic community, the crucial question is whether the cultured meat is compliant with Islamic laws or not, most commonly referred to as "*Halal* or not." Since meat culturing is a recent invention, the traditional Islamic jurist that Muslims often refer to has never discussed its *Halal* status. Therefore, contemporary Islamic jurists have taken on this mission. The *Halal* status of cultured meat can be resolved through identifying the source of the cells and serum medium used in culturing the artificial meat. Accordingly, *in vitro* meat is considered *Halal* only if the stem cell is extracted from a *Halal* slaughtered animal, and neither blood nor serum is used in the process. Indeed, serum should be avoided unless one can prove that the meat will not be changed as a result of contact with the serum (being potentially unclean) (14).

CONCLUSION

To meet the increasing demand for food by a growing population in 2050, the FAO has concluded that 70% more food will be needed to fulfill this demand. In this context, livestock systems will be a vital element in addressing global food and nutrition security in the world. However, to avoid criticism of livestock farming concerning environmental and animal welfare issues, more efficient ways of protein production are being developed to sustain the growing global population.

One option is to culture muscle cells in an appropriate culture medium, the most efficient so far being a medium containing FBS. The medium should provide nutrients, hormones, and growth factors, so that muscle cells will proliferate before being converted into muscle and hence produce a huge amount of meat from a limited number of cells. Hopefully, thanks to technical advances, FBS has been replaced, at least in research laboratories, but maybe not yet at the industrial level. Furthermore, as hormone growth promoters are prohibited in conventional farming systems for conventional meat production in the European Union, this is still an issue. However, this technique is able to produce disorganized muscle fibers which are far removed from real muscle, and this is a huge limitation in seeking to reproduce the wide range of meats representing the diversity of animal species and breeds, as well as muscles or cuts. Moreover, the role of blood vessels and blood, nerve tissue, intramuscular fats, and connective tissue affect both taste of meat. Indeed, a number of the "good" veggie meat burgers fail on texture and taste from the point of view of being too uniform.

The nutritional quality of cultured meat can be theoretically controlled by adjusting the fat composites used in the medium of production. This is also the case with conventional meat, with newly-developed strategies increasing the content of omega-3 fatty acids in meat with current livestock farming systems. However, controlling the micronutrient composition of cultured meat is still a research issue. Finally, the impact of cultured meat consumption on human health will have to be carefully checked and documented.

Regarding GHG, there is no consensus on the potential advantages in terms of GHG emissions of lab-grown meat compared to conventional meat on a short-term or long-term basis.

Despite its current high price, the production costs of cultured meat will probably decrease in the near future. This may help consumer acceptance, despite a strong rejection of names that refer to "*in vitro*" or "cultured" meat technology. However, cultured meat will be in competition with other meat substitutes already on the market and better accepted by consumers, such as plant-based products.

Ethically, cultured meat aims to use considerably fewer animals than conventional livestock, which makes the product attractive to vegetarians and vegans. However, a few animals will still need to be reared so that their cells can be harvested to produce *in vitro* meat.

Moreover, the religious authorities are still debating; whether *in vitro* meat is *Kosher* (consumable under Jewish dietary laws), *Halal* (for Muslim consumers, compliant with Islamic laws).

In conclusion, it seems clear that research projects on cultured meat have had a limited scope as *in vitro* meat development is still in its infancy. The product will evolve continuously in line with new discoveries and advances

that optimize the production, quality and efficiency of cell division. It remains to be seen whether this progress will be enough for artificial meat to be competitive in comparison to conventional meat and the increasing number of meat substitutes.

AUTHOR CONTRIBUTIONS

SC and J-FH contributed equally in the redaction of this review. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Microcarriers for Upscaling Cultured Meat Production

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Due to the considerable environmental impact and the controversial animal welfare associated with industrial meat production, combined with the ever-increasing global population and demand for meat products, sustainable production alternatives are indispensable. In 2013, the world's first laboratory grown hamburger made from cultured muscle cells was developed. However, coming at a price of $300.000, and being produced manually, substantial effort is still required to reach sustainable large-scale production. One of the main challenges is scalability. Microcarriers (MCs), offering a large surface/volume ratio, are the most promising candidates for upscaling muscle cell culture. However, although many MCs have been developed for cell lines and stem cells typically used in the medical field, none have been specifically developed for muscle stem cells and meat production. This paper aims to discuss the MCs' design criteria for skeletal muscle cell proliferation and subsequently for meat production based on three scenarios: (1) MCs are serving only as a temporary substrate for cell attachment and proliferation and therefore they need to be separated from the cells at some stage of the bioprocess, (2) MCs serve as a temporary substrate for cell proliferation but are degraded or dissolved during the bioprocess, and (3) MCs are embedded in the final product and therefore need to be edible. The particularities of each of these three bioprocesses will be discussed from the perspective of MCs as well as the feasibility of a one-step bioprocess. Each scenario presents advantages and drawbacks, which are discussed in detail, nevertheless the third scenario appears to be the most promising one for a production process. Indeed, using an edible material can limit or completely eliminate dissociation/degradation/separation steps and even promote organoleptic qualities when embedded in the final product. Edible microcarriers could also be used as a temporary substrate similarly to scenarios 1 and 2, which would limit the risk of non-edible residues.

Keywords: cultivated meat, clean meat, bovine myoblasts, satellite cells, bioprocessing, microbeads, cell expansion

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INTRODUCTION

The livestock sector is responsible for 18% of greenhouse gas emissions, 8% of human water consumption and contributes to water, air and soil pollution (1). Taking into account the predicted global population increase for 2050 (2) and the ever increasing meat consumption (3), sustainable alternatives are urgently needed. Since the first laboratory grown hamburger in 2013, research

on cultured meat has taken off all around the world. Its potential to reduce the environmental impact and eliminate the controversial treatment of animals (4) associated with industrial meat production has attracted a vast interest. Different life cycle analyses for cultured meat production have been theorized. Mattick's et al. (5) study presents significant differences between different types of meat. For instance, pork and poultry produced by cellular agriculture technology would lead to higher global warming potential, whereas beef would lead to a lower impact (5). Other long-term, worst case scenario models predict an initially greater peak warming due to cattle as opposed to cultured meat, but a higher warming effect of cultured meat in the long run, due to the different way that CO_2 and CH_4 gases accumulate in the atmosphere. However, these studies use current methods of energy production in their models not taking into account potential energy decarbonization for the next 1,000 years (6). Smetana's et al. (7) study also shows a direct link between environmental impact and method of energy production, as cultured meat processing is highly energy dependent (7). It is therefore possible that innovation in the energy field will result in decarbonization of energy and thus lead to a more sustainable process than the one expected by the less optimistic models. Cultured meat also has the possibility to improve consumer health and nutrition by tailoring product composition (8) and to reduce zoonotic contamination by working under controlled atmosphere, compared to poor handling and hygiene in animal farming (9, 10). However, mainly due to the astronomical production costs, substantial effort is still required to reach sustainable and cost-effective large-scale production.

Several methods of producing cultured meat have been proposed and different cell types have been considered, including embryonic stem cells (ESCs), induced pluripotent stem cells (IPSCs), mesenchymal stem cells (MSCs) and satellite cells (SCs) (8, 11–13), The latter, also called bovine muscle stem cells, seem the most straight-forward, suitable candidates for this purpose. They are mononuclear cells which can be found between the basal membrane and the sarcolemma of nearby muscle fibers in mammalian's skeletal muscles (14). They are involved in skeletal muscle regeneration and have the ability to proliferate while keeping their stemness and, when specific signaling pathways are activated, they can differentiate into muscle cells. As opposed to IPSCs, MSCs, and ESCs which can differentiate into different lineages, SCs can only differentiate into myocytes, thus facilitating the whole bioprocess.

The production of cultured meat from SCs is a simple concept which can be briefly described in four steps: (1) satellite cell isolation (2) expansion, (3) differentiation, and (4) assembly of muscle fibers (**Figure 1**).

Methods and protocols for the identification and isolation of SCs, have already been widely described and only a few milligrams of muscle are now required to isolate a sufficient amount of cells to start a culture (15, 16).

Once SCs are isolated, they need to be expanded *in-vitro* to achieve large cell numbers. SCs are adherent cells, meaning that they need a surface, mimicking an extracellular matrix, for attachment. Flat plastic surfaces coated with a hydrogel are commonly used in satellite cell culture (15, 17). When the required amount of cells is achieved, the differentiation process is initiated. During this step, cells fuse to form myotubes and start expressing proteins characteristic to functional myocytes.

Cell culture with current conventional planar culture systems, presents significant limitations related to their low surface to volume ratio, the lack of pH, gas and metabolite concentration control and is therefore not scalable (18, 19). As a consequence, it is only possible to produce up to 10^{11} cells with these methods (20). Large-scale production requires generation of a significantly higher amount of cells (10^{12}-10^{13} cells corresponding to 10–100 kg of meat) while using limited space, time, amount of resources and requiring minimal handling (21). This review aims to discuss the possibility of upscaling cultured meat production with the use of microcarriers, taking into consideration the specific requirements of satellite cells and the specific requirements deriving from the fact that the product needs to be suitable for consumption. The feasibility of a one-step bioprocess will also be discussed.

SCALABILITY OF SC CULTURE THROUGH THE USE OF MCs

To address the issue of scalability, three techniques are commonly used for the culture of adherent mammalian cells: (1) culture

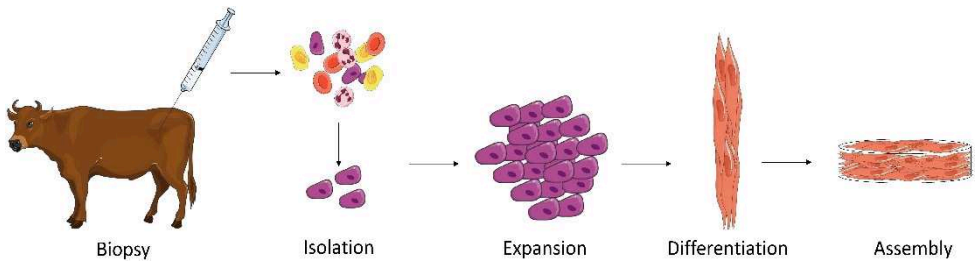

FIGURE 1 | Main steps required for production of cultured meat from an animal biopsy. A muscle biopsy is performed on a living animal. Satellite cells (SCs) are then isolated and subsequently expanded. When a sufficient quantity of cells is obtained, differentiation of SCs is induced. This includes cell fusion and myotube formation. The formed myotubes start producing proteins to form functional myocytes which can be then assembled with known food processing methods (mixing, molding) to form cultured meat (Illustrations have been taken from Servier Medical Art licensed under a Creative Commons Attribution 3.0 Unported License).

in aggregates, (2) culture in fixed bed reactors, and (3) culture on microcarriers (MCs). Culture in aggregates consists in the formation of clumps of cells that grow in 3D and serve as anchors for their neighbors (21), whereas MCs are beads composed of various materials, porosities and topographies which provide a surface for anchorage-dependent cells to adhere to McKee and Chaudhry (22). Although very high achieved cell densities have been reported with aggregates (23–25) and in theory, a 3D environment closer to the native environment of the cells is provided, this technique offers little control of aggregates size, resulting in nutrients' and O_2 gradients inside the aggregates and necrotic cores (22, 26). There are a few reports referring to the aggregate culture of myogenic cells (27–29). However, these were performed with the purpose of sustaining their *in vitro* culture rather than for cell proliferation (doubling times of $>$ 150 h) and were undertaken in static conditions. In addition, Aguanno et al. (30) showed that C2C12 cells cultured in suspension form aggregates that produce extracellular matrix and express markers of quiescent satellite cells, which does not meet the requirement for proliferation (30).

MCs, offering a large surface/volume ratio, are the most promising candidates for upscaling. The suspended microcarriers in the medium offer a 3D culture environment, but the cells still grow on a 2D surface, albeit that the strong curvature of bead surface does affect cell attachment and growth (31–33). Still, the translation from the traditional monolayer culture to a suspension culture is smoother, since the micro-environment of the cells essentially remains the same. They also allow for flexibility in terms of the type of vessel that can be used for scaling-up. Depending on their buoyancy and density, they can be used in stirred-tank, fluidized bed, packed bed and aerated reactors which are commonly used for scaling-up chemical processes and have also been successfully applied to bioprocesses. Microcarrier based bioprocesses also have the advantage of being easier to control and monitor, when compared to fixed bed bioreactor cultures (e.g., hollow fiber or multi-plate), resulting in quality and consistency of the products, as well as cost reduction (34). Lastly, a significant advantage of MC based cultures is that the growth surface provided to the cells can be increased by simply adding new MCs to the culture, as it has been established that cells are able to migrate from bead to bead and populate newly added microcarriers (35–39). This phenomenon, commonly referred to as "bead to bead transfer," can be explained by two main mechanisms: cells detaching from a confluent MC and reattaching onto other MCs, or cells forming bridges between MCs upon collision (40). It has been shown that successively transferring a small proportion of near-confluent MCs (10–25%) into a new vessel loaded with fresh MCs leads to a decrease of lag phase and an increase of the overall yield (35, 38). Even if using MCs can lead to the formation of cell-loaded MCs aggregates that can inhibit proliferation, adding fresh MCs in combination with adapted agitation have been shown to reduce MCs aggregation (41–43). Although different techniques, such as intermittent stirring, have been implemented to enhance cell transfer and limit clumping of MCs, no robust method for SCs has been reported so far. Since satellite cells do not produce that much ECM as MSCs, which are the cells typically to be reported to be cultured on microcarriers, aggregation of microcarriers is not expected to be a major issue when culturing satellite cells on microcarriers. In our hands, aggregation of microcarriers was less of a problem in the case of satellite cells than has been reported for MSCs (unpublished observations) and indeed, Verbruggen et al., only report aggregation occurring in a microcarrier based culture of satellite cells, when the cell density reaches confluence; when new surface area is introduced to the culture by new microcarrier addition, aggregation abates (39).

Since the first introduction of the MC concept for the culture of adherent cells in 1967 (44), many MCs have been developed and commercialized. Many of them have been discontinued for various reasons, an up to date list with the currently commercially available ones is presented on **Supplementary Table 1**. MCs have been mostly used for the expansion of cells producing molecules of interest (e.g., monoclonal antibodies, vaccines, proteins) (45) but usually not with the purpose of using the cells as the final product. However, with the latest progress in the field of cell and gene therapy, many efforts (46–51) have been invested in developing MCs for the culture of human stem cells for cell therapies. However, none have been specifically developed for myoblast expansion or meat production.

Microcarriers to be used for meat production should comply with food regulations while also offering an optimal topography and surface chemistry for the target cell type, in this case bovine myoblasts. Ideally, they should also be animal-free to serve the purpose of eliminating the use of animal products throughout the production of cultured meat.

MCs could also serve as nutrient carriers. Essential and/or unstable growth factors, amino acids and nutrients could be loaded and controllably-released from the MC's core, to meet SCs' nutrients demand. This would help reduce the number of medium exchange steps and thus the risk of cell loss or contamination. Perez et al., have succeeded in loading sol-gel derived bioactive glass MCs with basic fibroblast growth factor (FGF-2) and cytochrome c protein, which were sustainably released over a period of several weeks. Mesenchymal stem cells adhered and proliferated to significantly higher levels on the FGF-2 loaded microcarriers when compared to the control (52). Micro-encapsulation and sustained release of bioactive molecules is a field vastly researched for food applications (53, 54) and the same principles can apply for microcarrier based cell culture for meat production. Temperature and pH cues can also be applied to control the *in vitro* release kinetics from loaded microcarriers (55, 56).

BASIC REQUIREMENTS FOR SC ADHESION AND PROLIFERATION ON MCs

Like most mammalian cells SCs are anchorage dependent, hence, cell attachment onto MCs' surfaces is a prerequisite. Cell attachment is a crucial parameter which influences the whole process as a low attachment efficiency will lead to a low expansion yield (57). Cell attachment involves interaction between several cell adhesion molecules (CAMs) and substrates on the surface of the microcarrier (**Figure 2**) (58).

The integrin family is the main surface receptor family regulating cell adherence (19). They are heterodimeric glycoproteins composed of α and β subunits, each with numerous isoforms (59) and, depending on the subunits expressed, integrins bind to different proteins; for instance $\alpha_1\beta_1$ has a specific affinity to collagen, $\alpha_5\beta_1$ to fibronectin and $\alpha_v\beta_3$ to vitronectin (60). SCs express on their basal surface different integrins, including $\alpha_7\beta_1$ integrins that bind specifically to laminin (61). In order to enhance cell attachment and proliferation, many efforts have been dedicated to the modification of MCs properties and seeding optimization. Four main strategies are shown in **Figure 3**.

Coating of the MC surface with extracellular matrix (ECM) proteins, such as collagen, laminin, fibronectin or vitronectin (62) is a widely applied method for the enhancement of cell attachment on MCs. These proteins contain a specific amino acid sequence, so called RGD (for Arginine-Glycine-Aspartate), which is one of the main domains responsible for cell adhesion (19, 63). Using ECM proteins not only has the advantage of enhancing cell attachment, but also provides a more *in-vivo* like environment, resulting in the maintenance of cell functionality and differentiation capacity (19). Wilshut et al. compared attachment, proliferation and differentiation of porcine SCs on several adhesion proteins including Matrigel, gelatin, collagen-1, fibronectin and laminin. Fibronectin and laminin were shown to be more effective in enhancing cell attachment, while laminin and Matrigel provided optimal proliferation and differentiation (64). Dodson et al. performed a similar work with ovine SCs on gelatin, collagen-1, collagen-4, fibronectin, laminin, poly-L-lysine and poly-D-lysine. Best attachment was obtained with fibronectin, whereas optimal proliferation and differentiation were obtained with gelatin (65). Laminin has also been shown to promote cell migration (66) and myoblast proliferation (67). Besides, it has been shown that *in vivo*, the SCs are in contact with the basal membrane of skeletal muscle cells which consists of type IV collagen, laminin, entactin, fibronectin and glycosaminoglycans, such as perlecan (68). Taking into consideration these results and the fact that SCs express laminin (61) and fibronectin receptors (69), the use of laminin or fibronectin as a coating would be promising for enhancing cell attachment as well as proliferation and differentiation. Likewise, the use of other proteins containing the RGD peptide are also promising. Instead of protein coated MCs, conditioning of uncoated microcarriers in a protein containing medium before inoculation can also be effective through adsorption of the protein molecules on the MCs surface (70, 71).

Modification of the MC's surface properties, such as surface charge and hydrophilicity can be achieved by incorporating chemical groups, e.g., amino groups (-NH_2) or carboxyl groups (-COOH) (19). How surface charge and hydrophilicity influence cell behavior has not been studied in depth, but there is empirical consensus that these factors significantly affect cell attachment and behavior (72). The surface of mammalian cells is known to be negatively charged (73), and therefore, modifications leading to a positively charged surface seem promising. Indeed, Chen et al. (62) observed a lower attachment efficiency of hESCs onto negatively charged compared to positively charged

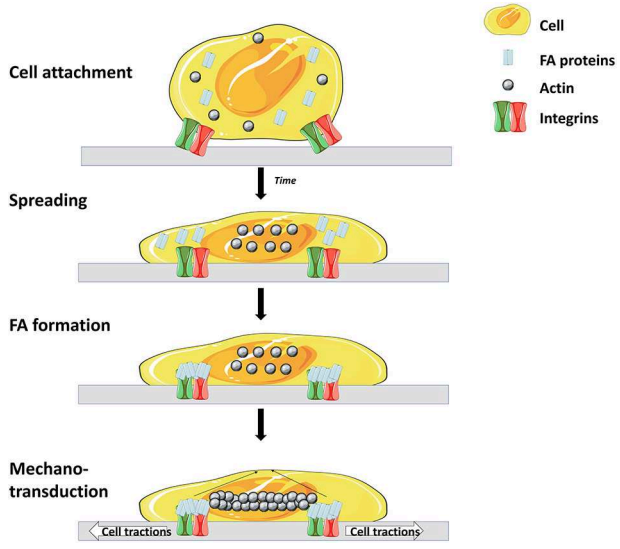

FIGURE 2 | Main steps and molecules involved in cell adhesion to matrix. Cell surface receptors (mostly integrins) interact with specific molecules of the matrix, leading to attachment and spreading of the cell. Attachment is then enhanced through the interaction of focal adhesion (FA) proteins and integrins. Finally, a rearrangement of the cytoskeleton occurs which leads to spreading of the cell over the surface (56) (Illustrations from Goldmann et al. have been recreated with Servier Medical Art, licensed under a Creative Commons Attribution 3.0 Unported License).

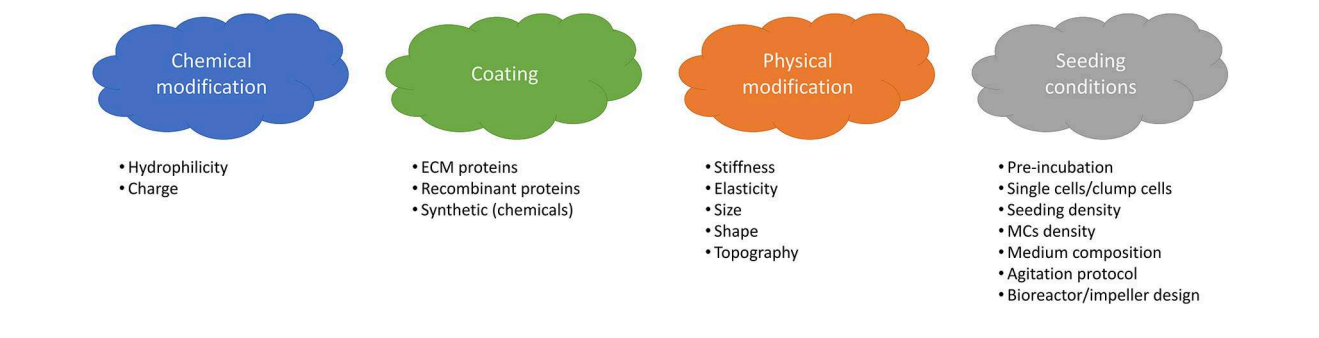

FIGURE 3 | Main variables affecting cell attachment and growth onto MCs.

MCs. Similarly, a better attachment on positively compared to negatively charged surfaces was observed by Schneider et al., Lee et al. for a variety of cell types (74–76). Satellite cells have also been shown to successfully attach and grow on positively charged Cytodex 1 microcarriers (39).

Regarding hydrophilicity/hydrophobicity, it is well-established that slightly hydrophilic surfaces lead to better cell attachment (76–79) than hydrophobic (>90°) and superhydrophobic (>150° contact angle) surfaces that have been shown to inhibit mammalian cell adhesion (80). This is mainly due to the fact that hydrophilic surfaces allow for better protein adsorption (81, 82). A super hydrophilic surface of <10° contact angle has also been reported to support CHO cell attachment, however since protein adsorption to superhydrophilic surfaces is very low, the cell attachment in this case can happen only if the cells can directly adhere to the surface chemical groups (83, 84). It becomes clear that, when evaluating the surface chemistry for cell attachment, cell-surface, protein-surface as well as protein-cell interactions should be carefully investigated. For example, Papenburg et al. have observed a better proliferation of C2C12 cells on a more hydrophilic surface, however neither direct correlation between surface wettability and total protein adsorbed nor between total protein adsorbed and cell attachment has been reported. More specifically, cell attachment might be indirectly affected by the wettability through a specific protein ratio adsorbed as well as the conformation of the adsorbed protein. Surfaces presenting both hydrophilic and hydrophobic domains might be preferable for adsorbing different groups of proteins, whereas a mono-phase surface might select a specific kind of protein (85). The degree of adsorption should also be carefully controlled as a weak protein adsorption could result in a lack of binding sites for cell interaction and a strong protein adsorption might affect their conformation. As a general conclusion, it has been shown that surfaces with a moderate hydrophilicity lead to optimal protein adsorption in terms of amount and conformation, and thus optimal attachment and proliferation (84). There is no reason to suspect that satellite cells would behave very differently from other mammalian cells in this context, and therefore, the use of a positively charged with moderately hydrophilic MC surface should favor satellite cell adhesion.

Modification of the physical properties of MCs, such as shape, size, stiffness, elasticity, topography and roughness can also be tuned to enhance satellite cell attachment and proliferation. The definition of an optimal range of these physical properties for this specific cell type is challenging. Stiffness, for instance, is a critical parameter for adherent cell as it can influence cell adhesion (86), protein expression, cytoskeleton modification (87) as well as cell viability (88). Gilbert et al. observed higher engraftment efficiency of SCs when cultured on a poly-ethylene glycol (PEG) gel of muscle-like stiffness (∼12 kPa) compared to tissue culture plastic (89). Boonen et al. also observed higher growth rates and sustained proliferation of primary myoblasts on a surface with an elastic modulus of 21 kPa when compared to softer (3 kPa) or stiffer (80 kPa) surfaces (90). It has also been reported that increasing the stiffness from 0.5 to 2 kPa leads to activation/proliferation of mouse myoblasts, whereas at 18 kPa differentiation was induced (91). These findings are in accordance with reported results of 11.5 ± 1.3 kPa stiffness reported for undifferentiated C2C12 cells by Collinsworth et al. (92) and therefore, results from the literature suggest that MCs with a muscle-like stiffness of 2–12 kPa could be beneficial for satellite cell expansion. In order to achieve a desired stiffness, tunable hydrogels have been developed (71) which can offer solutions for controlling satellite cell attachment and proliferation.

Surface topography is another important parameter that affects attachment, proliferation and differentiation of muscle cells. C2C12 cells cultured on a micropatterned surface including pillars showed better cell attachment whereas proliferation and spreading were higher on non-patterned surfaces (85). Better proliferation of C2C12 cells on a randomly oriented nanofibers was observed than on aligned ones, nevertheless, a better fusion and alignment of myoblasts was observed on the latter (93). There is consensus that nanofibrous surfaces, by mimicking the extracellular matrix (ECM), are promoting cell attachment and proliferation (94). In addition to topography, curvature should also be carefully defined since it has been shown to affect the speed (32) as well as the direction and the persistence of hMSCs migration (33). Although other studies have also reported effects of curvature on several cell types including fibroblasts (31, 95), osteoblastic cells (96, 97) and MSCs (31–33), there is a lack of information regarding satellite cells, thus further investigation is still needed. With the increasing development of tools for the fabrication of micro-curved surfaces, more systematic and precise studies should be possible (98).

The size of MCs has also been shown to affect cell behavior. Schmidt et al. reported better cell attachment on larger MCs (1,500 and 3,000 μm) compared to smaller MCs (500 μm). In contrast, a higher growth rate was observed on smaller MCs due to increase in shear stress on the larger ones (99, 100). Nevertheless, the MC diameter should not be <100 μm as most adherent-dependent cells fail to develop their normal morphology and multiply well on sharply curved surfaces (62, 101). It is worth noting here that the development and tailoring of MCs properties for a specific cell type can be very challenging, as traditional material characterization methods used for stiffness, elasticity, topography and roughness measurements are difficult to translate from planar systems to spherical microparticles (19, 100).

Lastly, optimization of the seeding conditions (inoculum and operating parameters) is another way of improving cell attachment (19). The inoculum can be either in the form or single cells or cell clumps, but a few groups have reported that the use of cell clumps leads to heterogeneous distribution of cells onto MCs, resulting in variability in attachment yields (102, 103). There are also discrepancies in the literature (104–107) regarding the optimal cell number per MC to be seeded, that might be traced back to differences in cell types, similar to differences in optimal seeding density on planar systems. On planar culture systems, myoblasts have been cultured at different seeding densities, ranging from 100 to 10,000 cells/cm^2, with the latter leading to higher growth rate (108). It has to be noted that MCs are often seeded at a slightly higher density

than on planar systems, to account for potential losses due to non-attachment (71).

Operational parameters during inoculation also affect cell attachment. The use of dynamic conditions showed positive effect on cell attachment and distribution by increasing cell-MCs contacts (19, 48). However, the seeding density as well as initial MC concentration should be carefully assessed, as cell growth can be negatively affected due to particle collisions (109) and nutrient concentration gradients cause by diffusion limitations (110). Implementing intermittent stirring has also been reported as an efficient strategy and has been widely used for the expansion of stem cells (111–114). Lastly, the bioreactor should also be designed in such a way that shear stress is limited and mixing maximized.

For large scale production, the efficiency of the microcarrier culture, measured as volumetric productivity is an important parameter that needs to be taken into consideration. As microcarriers come in different sizes, shapes and materials, they provide different surface areas per weight and swelling properties. This results in different values of maximum surface area per mL of medium that can be reached with a given MC, defining the maximum volumetric productivity, which is the ultimate efficiency parameter to be carefully considered when up-scaling.

SCENARIO SPECIFIC CONSIDERATIONS

Potential attributes of MCs to be used for meat production are reviewed below and are divided based on three different scenarios (**Figure 4**): (1) MCs are serving only as a temporary substrate for cell attachment and proliferation and therefore they need to be separated from the cells at some stage of the bioprocess, (2) MCs serve as a temporary substrate for cell proliferation but are degraded or dissolved during the bioprocess, and (3) MCs are embedded in the final product and therefore need to be edible.

Scenario 1: Temporary Microcarriers for SC Proliferation

When microcarriers are used for as temporary substrates for SCs expansion, they need to be removed at the end of the process. There are two important prerequisites in this case: MCs need to (1) provide a high detachment yield (2) and also be easy to separate from the cells.

Dissociation

The dissociation of SCs from microcarriers has been shown to be challenging (39, 115). Strategies based on chemical, mechanical and thermal principles have been developed to detach other cell types from MCs while maintaining cell viability, proliferation and differentiation capacity. Chemical detachment consists of enzymatic and non-enzymatic dissociation of cells. The enzymatic detachment is based on proteases, which have the ability to split bonds between amino acids involved in cell attachment, also a very commonly dissociation process used in planar cultures. Proteases are generally used in combination with chelating agents for Ca^{2+} that reduce the ionic strength required for cell binding. The specifics of this protocol are highly dependent on the microcarriers, cells and enzymes used (116, 117). To achieve an animal-free production process, animal-derived proteases can be replaced

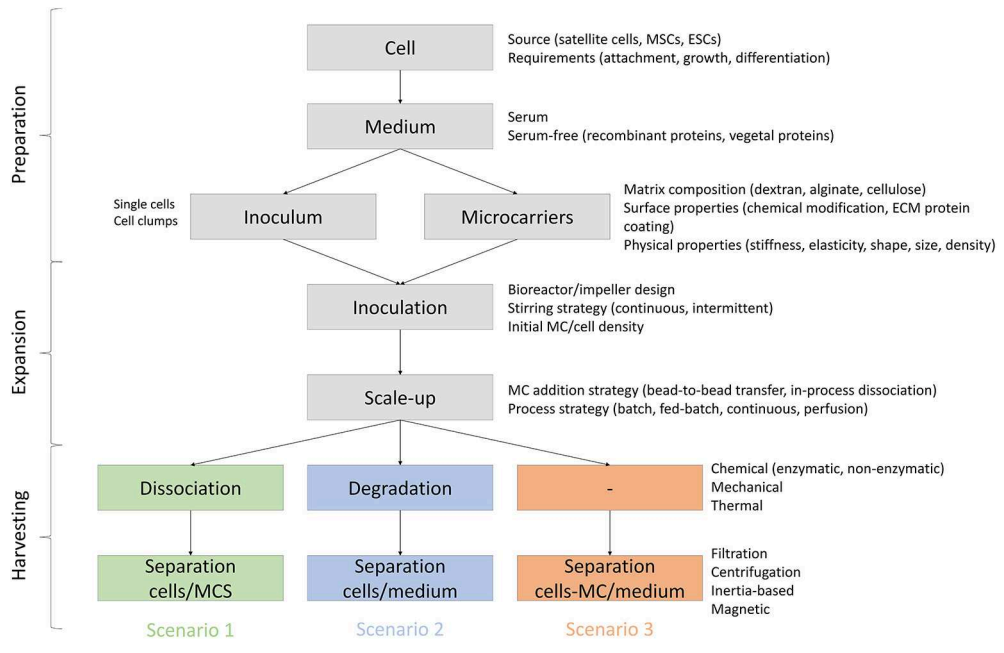

FIGURE 4 | Process requirements and variables for MC based bioprocesses in three scenarios: (1) MCs are serving as a temporary substrate for cell attachment and proliferation and therefore they need to be separated from the cells at some stage of the bioprocess, (2) MCs serve as a temporary substrate for cell proliferation but are degraded or dissolved during the bioprocess, and (3) MCs are embedded in the final product and therefore need to be edible.

by recombinant enzymes which have already been proven to efficiently recover cells with high viability while maintaining proliferation and differentiation capacities (118, 119). However, the use of proteases may also lead to proteome (120) and chromatin structure modification (121) which could impact cell stability and subsequent differentiation. For this reason, non-enzymatic techniques have also been researched. Non-enzymatic dissociation agents, such as dextransulphate, N-acetyl-L-cysteine and dithiothreitol, are mimicking enzyme activity that cleaves or degrades MCs coating, if present and have been successfully used for cell detachment from microcarriers.

Mechanical forces are also being used for the detachment of cells from MCs. Katayama et al. showed that pipetting can lead to detachment of epidermal basal cells from Cytodex 3 without the need of trypsin. Rafiq et al., also demonstrated the efficiency of combining the use of trypsin-EDTA with high agitation for the detachment of hMSCs cultured on P102-L MCs (71). Following this, Nienow et al. developed a detailed dissociation protocol based on the Kolmogorov's microscale of turbulence which dictates that to avoid cell damage during dissociation, the size of the biological entity (either the MC size when the cells are attached or the cell size when the cells have detached) has to be smaller than the Kolmogorov scale (λK) (122). With this method, they successfully detached hMSCs from two types of MCs using different bioreactors, medium and enzymes (123). Spier et al. on the other hand, have demonstrated successful detachment and a 90% cell recovery using a vessel with a vibrating plate, which facilitates cell detachment (124). If those techniques would be applicable to SCs is unknown.

The thermal responsivity that certain materials exhibit has also been used to optimize cell detachment from MCs. Thermo-responsive materials have the ability to undergo a discontinuous phase transition and/or morphological modification in response to a variation of temperature (125). By decreasing temperature below the low critical solution temperature (LCST) of the material, the MCs surface becomes very hydrophilic (contact angle < 10) leading to cell detachment (126, 127). Many thermo-responsive materials have been used in 2D culture of cells including pluronic (128), an elastin-like polypeptide (129, 130), methylcellulose (50, 126, 131), xyloglucan (132) and hydroxybutyl chitosan (133–136). However, due to its quick phase transition and its LCST at around 32°C, Poly(N-isopropylacrylamide) (PNIPAAm) has retained the most attention so far for temperature induced cell detachment from beads (49, 137–140). Although some researchers report that this detachment method can be time-consuming or less efficient than enzymatic methods (49), better cell viability and ECM protein secretion, as well as better reattachment (141) have been observed.

MCs could also be developed to be fully (core and shell) thermo-responsive. For example, below its LCST (32°C), PNIPAAm is soluble in water and can undergo a gel transition phase above its LCT (126), thus it is theoretically possible to release cells from MCs by collapsing the PNIPAAm gel into a liquid solution.

In a similar way to thermo-responsive polymers, the unique properties of pH, photo or electric current responsive polymers can be harnessed to create smart microcarriers for cell detachment from MCs, however, research on those materials is still at early stages and sometimes difficult to combine with cell culture, thus MCs with such responsivity have not been reported yet (142).

Mechanical and thermal techniques present the advantage that they do not require the use of any dissociation agents which could potentially complicate regulatory requirements. Moreover, chemical techniques require several washing steps before and after dissociation which leads to higher processing times and extensive manipulation of the culture. Usually, a combination of two or even three of the above-mentioned techniques results in lower processing times and tends to limit the side-effects of each of these methods. However, still more in—depth research is required to determine the hydrodynamic conditions in which satellite cells can be detached from MCs without being damaged, as well as to define an optimal combination of techniques for their dissociation.

Lastly, liquid/liquid systems where the cells grow in the interface of the two continuous phases has been demonstrated and present the advantage of facile cell recovery after culture. Several groups (143, 144) showed that mammalian cells can grow in the interface of a fluorocarbon liquid and growth medium. The formation of the two-phase system is based on mutual insolubility and density difference between the phases. Perfluorocarbons have also been successfully shaped in microbeads in stirred-tank bioreactor systems. After the culture period, the cells can be collected from the liquid interface, by inducing coalescence of the emulsion droplets, by removing the proteins that accumulate on their surface, or by centrifugation, thus avoiding the use of proteolytic enzymes. Additionally, fluorocarbon fluids can be oxygenated, allowing for better oxygen transfer in high cell density cultures (145). However, the stirring speeds required to initially prevent the perfluorocarbon particles from coalescing and to resuspend them after sedimentation (needed to replace medium for example) might be prohibitive for some stem cell types.

Separation

Once cells have been detached from MCs, they then need to be separated from them. Although many cell/medium separation systems have been developed, only a few are meant to specifically separate cell/MC suspensions. Commercial separation systems are usually based on one of the following four principles: filtration, centrifugation, inertia and magnetism. Dead-end filtration systems have been widely used at small scale, for example nylon filters with mesh sizes of 40–100 μm (111, 146, 147) and have also been developed for relatively larger scale (up to 200 liters): However, as dead-end filtration is generally limited by clogging of filters as the scale increases, more sophisticated systems have prevailed at large scale application. Tangential flow and alternate tangential flow filtration and as well as continuous centrifugal separators are the most used systems, currently. Recently, Moloudi et al. developed an inertia-based device for cells/MCs separation. However, with a filtration rate of 30 ml/min, more efforts are still required to reach an industrially relevant scale (148). Since all of these systems are based on MC

size, specific gravity and shape, MCs need to be able to retain their physical properties (integrity, shape, size, density) throughout the culture period.

To overcome issues related to MCs heterogeneity or potential loss of integrity during culture, magnetism can also be used as a separation method. This requires the incorporation of magnetic particles (made from iron, nickel, cobalt or their alloys) into the MCs core. After dissociation of the cells from the surface of the MCs, the introduction of a magnetic field separates the MCs from the cells. This type of microcarriers have not yet been extensively studied and their application has only been reported at a small scale (50 mL culture) (149), however they do seem promising in increasing control over medium exchanges and cell recovery yields, as the challenge of efficiently separating MCs from cells still remains. At present, usually high cell loss percentages are reported by the end of the process, ranging from 15 to 25% (150). On top of that, the risk of foreign material remaining in the retrieved cell pellet and ending up in the food product is high, as commonly, commercial MCs present quite a high variability in size and densities and it is possible that they lose their integrity during the bioprocess, rendering size exclusion methods unsuitable.

The use of a liquid/liquid system or thermally induced collapsing MCs offers significant advantages regarding separation, as it simplifies cell recovery and purification, which can be achieved through repeated washing and centrifugation steps.

When MCs can be completely separated from cells, they will serve as food contact materials, still requiring them to be sufficiently inert so as to not affect consumer health or food quality. Complete separation of non-edible, stable MCs could lead to re-cycle or re-use strategies resulting in reduced waste material and production costs.

Scenario 2: Non-edible, Degradable Microcarriers

MCs can also serve as a temporary substrate for cell proliferation but instead of being separated at the end of the process they can be degraded at a prior stage. In this case, the dissociation step can be replaced by a microcarrier degradation step to obtain the single cell suspension. Degradation refers to a chemical process that affects chemical composition as well as physical parameters including chain conformation, molecular weight, chain flexibility and cross-linking of a polymer (151). Since the first dextran-based MC, diverse degradable materials have been used for MC production, including polystyrene, cellulose, collagen, gelatin, alginate, chitosan, poly (lactic-co-glycolic acid) (PLGA), polylactide (PLA), or poly(ε-caprolactone) (PCL). Polymers used for their production can be either from natural or synthetic origin, and depending on their properties, they can be degraded in several ways. Degradation can be classified in five categories, based on the factors inducing the process: thermal, chemical, mechanical, photo and biological degradation (151). Bio-chemical and thermal degradation of polymers have been largely investigated in tissue engineering and drug delivery systems, whereas mechanical or photo degradation compatible with cell culture have not yet been reported in the literature.

In the context of cell recovery, degradation of MCs needs to be carefully controlled. The method should be selected in order to be robust, quick (<few hours) and prevent any damage or interaction of the SCs with the degradation products. In addition, MCs' physical properties should remain stable during the expansion phase, as premature degradation of the material will affect proliferation, gene and protein expression of cells as well as the overall control of the bioprocess (152).

SCs have been successfully cultured in many biodegradable hydrogels including alginate (59, 153), fibrin (154, 155), PEG (91, 156), collagen (157) and polyacrylamide (158), however, none of these studies were focused on developing a fast stimulus-degradable material. Usually, a degradation rate matching the tissue skeletal muscle regeneration rate (4–6 weeks) is aimed for (159). Nevertheless, MCs composed of these materials, or of their combinations, could be proven suitable temporary substrates for relatively short duration, large-scale expansion of SCs and stimulus-induced degradation. Accelerated degradation can be achieved with the use of concentrated enzymatic solutions, pH and temperature shifts, with or without concomitant application of mechanical forces (151, 160).

Up to date, only one MC now commercialized by Corning, has been developed with the purpose of being totally and rapidly degraded for cell harvesting (161). It is made of crosslinked polygalacturonic acid (PGA) and can be easily dissolved within 10–20 min using an EDTA solution, which destabilizes the PGA crosslinking in combination with pectinase that digests the polymer. Many other polymers including dextran, cellulose, collagen, pectin or gelatin could be theoretically enzymatically digested in a similar way. For instance, the dextran-based MC Cytodex 1 has not been specifically developed to be degradable, however Lindskog et al. have reported complete dissolution of Cytodex 1 MCs using dextranase while maintaining high cell viability (161). Similarly, the degradation of alginate, which is generally slow *in vivo*, can be accelerated by the use of non-enzymatic chemicals, such as citrate or phosphate. Specifically Voo et al. have shown complete *in vitro* dissolution of 2% and 6% w/v alginate beads in 0.1 M phosphate-buffer solution (pH 7.4) at 37°C after 80 and 240 min, respectively (162). Thermo- and pH responsive degradable beads have also been developed in the context of drug delivery (163, 164). Steinhilber et al. have developed pH-degradable beads, composed of polyglycerol and PEG, which are stable for 2 weeks at 37°C, pH 7.4 and 5% CO_2 and can be easily degraded in the course of 3 days by lowering the pH to 6.0 while releasing encapsulated NIH3T3 cells with high viability (165).

Of course, the stimuli applied for MC degradation should be compatible with SCs culture requirements, to retain cell function. For instance, Ren et al. reported a dextranase extracted from the marine bacterium *Catenovulum* sp. which presents satisfactory activity (above 80%) at a temperature range of 30–50°C and at a pH ranging from 7.0 to 8.5 (166). Commercially available dextranases, usually fungi derived, are mostly active at acidic pH (5.0–6.0) and higher temperatures (50°C), thus less compatible with cell culture.

Thermal and photo degradation, are also likely to be less suited for cell culture. The high temperatures required to thermally degrade polymers, as well as ultraviolet radiation that is needed to induce photolytic, photo-oxidative and thermo-oxidative reactions (167), resulting in photo-degradation, are also known to cause protein and DNA denaturation and damage (especially UVC: 200–280 nm and UVB: 280–320 nm) (168).

Mechanical forces can be used in combination with chemical degradation (enzymatic or non-enzymatic) to facilitate/accelerate the degradation process and reduce the concentration of enzymes. Increased stirring speeds, shaking or fluidization could serve as such. However, the shear stresses exerted on the cells should be meticulously investigated in order to ensure that cell viability and integrity are maintained.

Overall, results in the literature suggest that there is a variety of materials suitable for degradable MC production, which can be tuned to be stable for the expansion phase and can be *in situ* degraded when a certain stimulus is applied, to allow for the further processing of cells in the differentiation step.

Slowly degrading materials compatible with SC culture could also be used. MCs made of materials that have been developed with the purpose of being bio-chemically degraded *in vivo* in the context of skeletal tissue engineering and drug delivery systems become more relevant in this case (169–172). For instance, Zhou et al. have developed an alginate-fibrin microbead that starts to degrade and release cells 4 days after injection in a calcium phosphate cement scaffold (173).

PLGA and chitosan are also interesting candidates as their degradation rates can be controlled by adjusting the ratio of lactic to glycolic acids and de-acetylation degree, respectively (46, 174). Almeida et al. have developed a curcumin-loaded dual pH and thermo responsive MC. They used pectin, a bio-compatible, biodegradable and non-toxic polysaccharide with pH-responsive properties in combination with PNIPAAm (163). Similarly, Işilkan et al. have developed a pH and thermo-responsive chitosan coated pectin-graft-poly(N,N-diethyl acrylamide) MC (164).

The use of degradable MCs eliminates the need for separation, simplifying the process and resulting in increased cell recovery. Thus, the cell suspension can be washed and used directly for downstream processing. However, it has to be noted that when using degradable MCs, cells are usually released as a sheet/cell-clump, therefore proteolytic enzymes may be additionally needed to promote dissociation into single-cell suspension (161). Depending on the downstream processing of the SCs, however, a single-cell suspension may not be necessarily required and aggregates may be permitted.

Scenario 3: Edible Microcarriers Embedded in the Final Product

MCs can also be composed of edible materials and be embedded in the final product. As opposed to the previous cases where MCs are considered as a food contact material, in this scenario they should comply with regulations for use as a food ingredient or additive. Indeed, besides supporting cell growth, an edible MC would also be part of the final product and might affect the sensory attributes of the meat product, such as taste, color or texture.

Edible polymers that can be used as substrates for cell expansion are classified into four categories: polysaccharides (e.g., starch, alginate, carrageenan, chitosan, cellulose, carboxymethylcellulose, pectin), polypeptides (e.g., collagen, gelatin, gluten), lipids (e.g., paraffin, shellac), and composites/synthetics (e.g., PGA, PEG) (175). They have been widely used in the food industry as stabilizers, thickeners, coatings and emulsifiers. Cellulose, chitosan and alginate could be good candidates for large-scale expansion of SCs, as they are the most abundant natural polymers and are known for their biocompatibility and biodegradability (176, 177). However, in order to enhance attachment of SCs, incorporation of RGD-containing proteins is required (178), which have not yet received approval for use in food. One patent describing an edible and animal-free MC for engineered meat, proposes the use of pectin coupled with cardosin A, an RGD-containing polypeptide (179). Thus, the use of polypeptides as collagen or gelatin could be more suitable as the tripeptide motif is already naturally present. Using lipid-based MCs could also be an interesting way to bring fatty flavors to the product.

In order to eliminate or limit the effect of the MCs on the sensory profile of the meat, the cells can still be detached and separated from the edible MCs, however, a higher threshold for MCs being present in the recovered cells can be set, allowing for better harvesting yields. Less stringent separation methods, such as separation through sedimentation or centrifugation become more relevant in this context. Edible MCs with controllable degradation properties can also be used and be partially degraded, remaining in the cell harvest for further processing. It should be noted here though, that it is unknown whether remnants of partially degraded MCs could interfere with the differentiation process and would impede the ability of the cells to remodel their environment and fuse into myotubes if seeded in the differentiation scaffold.

The dissociation step can be omitted completely if the MCs are edible. In such case, the edible polymer to be used as cell substrate during the proliferation stage, can also be designed to enhance or introduce desired properties, such as texture, taste or color. For instance, the texture of the final product could be regulated through MCs stiffness. A microcarrier incorporating a hydrogel with specific water retention capacity at high temperatures could be used to enhance juiciness of the cooked product. Additives for a smoked or herb flavor as well as beneficial polyunsaturated fatty acids can also be incorporated through microcarriers. The color of the final product could also be adjusted through the addition of natural food colorings. However, when using MCs that will remain present throughout the process, care should be taken so that their presence doesn't interfere with further processing steps.

Regardless of which polymer is used for MC production, it is essential that its production and processing are well-controlled and comply with food standards regulations. From cross-linking to surface modification of MCs, diverse physical and chemical techniques are used, each one presenting advantages and drawbacks. For instance, physical cross-linking of polymers lead to lower toxicity of the cross-linked material when compared

to chemical methods (180). However, toxic compounds are commonly used in many stages of food production and processing, such as the use of pesticides in agriculture, or the use of solvents for oil extraction and the manufacture of food additives. For all edible products, though, including cultured meat, toxicity is based on remaining concentrations in the final product, thus it should be carefully analyzed to meet food grade standards and be safe for human consumption.

Summarizing, edible MCs could be either used as temporary substrate which is either separated or degraded during the process or purposefully used as part of the product that could bring additional sensorial properties. Natural polymers, physically cross-linked seem to be more promising for cultured meat applications as they maintain a better biocompatibility and low toxicity compared to synthetic chemically cross-linked polymers (181).

IS A ONE-STEP PROLIFERATION/ DIFFERENTIATION BIOPROCESS FEASIBLE?

In the vast majority of the literature, microcarriers are used for the expansion phase of cell culture, as achieving high cell numbers and specific cell productivity are the ultimate goals for the production of an advanced medicinal product. However, for meat production, the differentiation of SCs into myotubes and subsequently into myofibers is an integral part of the process, which usually happens in a subsequent, separate step. The differentiation phase demands very distinct conditions in terms of nutrients and physical environment. The idea of a simplified bioprocess though, where the same culture system can be used for both phases is very attractive, as it would minimize capital investment in equipment, processing times and cell manipulation. The necessary nutrients can be provided through a switch from a "proliferation medium" to a "differentiation medium," but providing the physical environment that the cells need in order to differentiate is more challenging. The substrate requirements for the proliferation and differentiation phases are typically different in terms of surface chemistry and topography (88, 91, 93). Stiffness requirements on the other hand shouldn't be difficult to combine for the proliferation and differentiation phase. Although softer substrates are known to retain SC stemness better than stiffer ones which are known to promote differentiation (89), observations on optimal stiffness for proliferation and differentiation often overlap. For example, Engler et al. has shown that culturing mouse myoblasts on a polyacrylamide gel of muscle-like stiffness (∼11 kPa) led to better myotube maturity (182), while Boonen et al. have demonstrated better proliferation on a 21 kPa substrate (88). A muscle-like stiffness therefore, in the range of 11–21 kPa could apply for both phases in the presence of other cues.

Torgan et al. have attempted to grow and differentiate SCs on MCs in a stirred-tank bioreactor hypothesizing that simulated microgravity environment would affect the myogenic differentiation. They reported that SCs cultured onto MCs in a microgravity bioreactor expressed less myogenin transcription factor as well as myosin and tropomyosin compared to SCs cultured in a "normal" gravity bioreactor suggesting that mechanical forces affect SCs differentiation (183).

Mechanical stimuli can be transduced by cells via transmembrane proteins into biochemical signals (184) and are also essential to promote protein synthesis and organization into contractile units (8). To promote mechanical stimuli, cells are usually cultured in a gel between anchor points which simulate tendons, thus creating a passive tension which leads to protein production when the tissue compacts (185, 186). This suggests a specifically designed morphology of MCs that allows a similar tension development during tissue formation. In combination to passive forces, different techniques to enhance protein synthesis including application of cyclic stretch (187–189) and electrical stimulation (190, 191) have been attempted. Although Boonen et al. and Kook et al. have not reported any positive effect of cyclic stretching, passive tension seems to be a minimum requirement to promote maturation of muscle cells. Mechanical stimulation of skeletal muscle cells through fluid generated shear stress has been reported in some cases to promote the differentiation process (192–194). High shear stress (5–10 Pa) has been shown to be detrimental to cells, however the effect of lower shear stress ranges (1–1,400 mPa) have been investigated and found to positively influence mechano-transduction in muscle cells. Naskar et al. reported a higher expression of myogenic marker and longer myotubes formed at 16 mPa and a better alignment of cells at 42 mPa (194). Therefore, shear stresses generated during a microcarrier based dynamic culture could be tailored to meet the stimulus required for differentiation, through the tuning of operational parameters, or the design of MCs that allow for the culture of cells onto regions of controlled shear stress, as has been recently reported by Wu et al. (195). Micro-patterned MCs providing topographies favoring myogenic differentiation, such as aligned patterns (93) could also potentially support a one-step bioprocess.

Thus, to support consecutive proliferation and differentiation in one setup, the material used for MCs production should be either tunable *in situ* to meet physical environment requirements for each phase (coating, stiffness, topography and shear stress) or less specific but adapted to both phases. In any case, the use of a non-edible and non-degradable MCs seems unlikely applicable in this situation because, a dissociation and separation step would be needed, which in the case of myotube/myofibers would be more challenging than for individual cells. Following differentiation, MCs could be degraded (or not depending on if the material used is edible), and the produced myofibers (or myofibers-MCs) can be assembled with classic food processing techniques to obtain a product comparable to traditional minced meat.

Besides combining both proliferation and differentiation requirements in one microcarrier, a one-step bioprocess also demands an easy way to maximize productivity of the bioreactor used while maintaining cell performance.

CONCLUDING REMARKS

Based on the MCs physical and chemical properties, several production scenarios for SCs proliferation and differentiation at large-scale are conceivable. Optimization of cell adhesion and

expansion on the MCs however, remain a common prerequisite for all scenarios.

Up to date, no MCs have been developed specifically for SCs expansion. The materials for such a MC, as well as the medium composition should be chemically defined to comply with GMP and HACCP standards. Adsorption or coating with recombinant proteins specifically binding to SCs' integrins, such as laminin and fibronectin, tailored substrate stiffness (2–12 kPa) as well as surface properties of the MCs to imitate the SC niche and activate cell proliferation should be taken into account when designing an MC for the expansion of SCs. Chemical modifications to add positive charge to the MC surface, render it moderately hydrophilic or functionalize it with amino groups, would probably be beneficial for SCs attachment. Robust protocols for SCs culture on MCs need to be developed and optimized since the impact of seeding conditions, such as seeding density, type of inoculum and stirring have not been systematically investigated. From current SC culture practices on monolayer, it seems that a positively charged surface with moderate hydrophilicity, protein and peptide coatings and muscle-like stiffness substrates seem to promote the attachment and proliferation of SCs.

In the case of non-edible and non-degradable MCs, cells need to be subsequently detached and separated from MCs. Enzymatic methods have been the most widely used so far for MC cultures and represent the golden standard in cell detachment. However, considering the potential cell damage occurring with this method and the risks associated with cell loss at large-scale production, physical and thermal techniques, based on smart materials have started to be developed and are likely to outperform the use of enzymatic treatments in the future. Following detachment, cells still need to be separated from MCs, and the challenge of achieving high separation yields without MCs residues in the cell pellet, still remains. Although sophisticated single-use filtration systems are already being used in the biopharma industry, for food applications a more straight-forward approach is required to limit production costs. Magnetism, fluidization, vibration and inertia-based separation are currently at early stages of development, but significant work needs to be done for these to be translated into robust devices reliable for production.

In the second scenario, where a degradable MC is used for cell expansion, the dissociation and separation steps can be replaced by a degradation step. Most degradable materials developed so far were designed for *in vivo* degradation and drug release purposes, thus presenting a very slow degradation rate. For large scale bioprocessing of satellite cells, a quick, stimulus induced degradable MC is more applicable.

Edible MCs can also be used, obviating the need to dissociate/separate and degrade the MCs, thus facilitating the production process. Indeed, this third scenario appears to be the most promising for cultured meat production. The use of an edible microcarrier would at least limit the dissociation/degradation/separation steps and can even be tailored to promote organoleptic qualities if embedded in the final product. In the case where microcarriers are not compatible with the differentiation process, edible microcarriers could also be used as a temporary substrate similarly to scenarios 1 and 2 which would limit the risk of non-edible remaining residues. Abundant, cheap, edible and degradable materials, such as alginates, pectins and celluloses seem to be promising candidates for this purpose.

Apart from serving as a passive substrate for cell expansion, MCs can also be engineered to serve as nutrient carriers to the cells or to encapsulate flavors or other substances to enhance the sensorial and nutritional attributes of the final product. Ideally, proliferation and differentiation should be combined in one-step, by providing necessary topographical, mechanical and other cues for differentiation, preferably in a temporal sequence following proliferation.

AUTHOR CONTRIBUTIONS

VB wrote the manuscript. PM wrote, edited the manuscript, and provided intellectual input. MP edited, provided intellectual input, and approval for publication.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest: VB is employed by Mosa Meat, B.V., a company that aims to commercialize cultured meat. PM is employed by Mosa Meat B.V. MP is co-founder and shareholder of Mosa Meat, B.V.

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Sensorial and Nutritional Aspects of Cultured Meat in Comparison to Traditional Meat: Much to Be Inferred

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Cultured meat aspires to be biologically equivalent to traditional meat. If cultured meat is to be consumed, sensorial (texture, color, flavor) and nutritional characteristics are of utmost importance. This paper compares cultured meat to traditional meat from a tissue engineering and meat technological point of view, focusing on several molecular, technological and sensorial attributes. We outline the challenges and future steps to be taken for cultured meat to mimic traditional meat as closely as possible.

Keywords: post-mortem metabolism, texture, flavor, color, nutritional composition, cultivated meat, clean meat

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INTRODUCTION

In 2013, the first cultured meat prototype in the shape of a hamburger was presented in the media (1). The hamburger was based on 10,000 strips containing myotubes engineered in a hydrogel. However, the engineered muscle-like tissues also required the addition of colorants (beetroot juice), flavors (saffron and caramel), and texturizers (bread crumbs and a binder) to make the patty similar in appearance to a hamburger (2). Producing a high-quality hamburger from traditional meat does not require the addition of these ingredients, suggesting that the intrinsic characteristics of the cultured cells differed significantly from traditional meat. The tasting panel commented that the burger tasted a little dry due to a lack of fat, but no profound quality or sensorial assessment was performed. The only other, modest, sensorial test on cultured cells reported in scientific literature, dates back to the early years of cultured meat experimentation and included smelling and observation, but no tasting (3). In addition, several review papers briefly discussed the potential sensorial characteristics of cultured meat (or derived products) (4, 5), but most of the information provided was based on indirect assumptions and on knowledge of the current *in vitro* production capabilities. To our knowledge, a scientific and technological comparison between cultured meat and traditional meat has not been published thus far. This relates to the fact that cultured meat is currently not available in sufficient quantities to conduct, such assessments. Still, based on the currently available state of the art concerning the production process of cultured meat, important considerations with regard to the technological, sensorial and nutritional characteristics of cultured meat can be inferred.

Cultured meat aspires to be biologically equivalent to traditional meat (6). If cultured meat is to be consumed, sensorial characteristics (texture, color, flavor) are of utmost importance. These sensorial properties are derived from the molecular characteristics of the product, such as the content and nature of the proteins, the presence of myoglobin, the composition of volatile compounds, etc. In addition to sensorial attributes, the nutritional quality of cultured meat should also resemble its traditional counterpart as closely as possible. Traditional meat is a nutritionally

dense food containing high-quality proteins, vitamins, minerals, and other important nutrients (7, 8). It is of interest to note that many compounds that accumulate in the muscle are not produced in the muscle but derive from animal feed components which have been digested and modified by non-muscle organs. Unless specifically added to the culture medium and taken up by the cells, these compounds would be absent in cultured meat, influencing processes determining flavor, texture, color and nutritional aspects.

POST-MORTEM METABOLISM

When a farm animal is slaughtered, muscles are transformed into meat through a complex biochemical process. The lack of oxygen supply results in a metabolic shift toward anaerobic glycolysis, by which glycogen present in the muscle cell is converted to lactate. This results in an intracellular pH drop from around 7 (in the living animal) to ∼5.4–5.8. Due to calcium release from the sarcoplasmic reticulum, muscle contraction is initiated. As the ATP concentration in the cell drops, muscle contraction ceases at a state in which actin and myosin heads closely interact (*rigor mortis*), forming the permanent actomyosin complex (7, 9, 10). This muscle contraction and complex formation significantly influence the properties of the meat. On the one hand, tenderness and water holding capacity decrease (9). The formation of the actomyosin complex necessitates the use of phosphate, releasing the bonds between actin and myosin, in the production of many processed meat products (11, 12). On the other hand, pH decline and other changes in intracellular conditions activate enzymes responsible for tenderization and formation of aroma precursors, as discussed below.

With respect to cultured meat, due to the lack of cultured meat available for scientific study, there is no information available on whether and to which extent such transformations occur (4). Future studies on cultured meat should shed light on glycogen content and pH evolution after harvest to assess the (dis)similarities to traditional meat. Isoforms of actin and myosin in cultured muscles were found to be predominantly neonatal or embryonic, rather than adult (13). This may alter the proteins' response to a potential post-mortem transformation. If these transformations are absent, then muscle is not transformed into meat, which is biochemically dissimilar (14). If *rigor mortis* would be less strong or no actomyosin complex would be formed, this may have a positive effect on the product quality with respect to tenderness and water holding capacity in comparison to traditional meat, while on the other hand, it may change the further aging process.

After slaughtering, meat is aged for tenderization and formation of flavor precursors (15). The aging period depends on the type of meat. In beef, in which a low amount of proteases are present, aging takes ∼14 days. The tenderization process is complex, involving many proteolytic enzymes and has been studied for many years but is not entirely elucidated. Calpain, a protease complex present in the sarcoplasm, is thought to play a central role in the process (9). Calpains degrade several myofibrillar proteins, but not actin and myosin (10). Several other enzymes, such as proteasome, caspase (9, 10), or the lysosomal enzyme cathepsins (7, 16) are also involved. The extent to which these enzymes act strongly depends on the microenvironmental conditions, such as pH, ionic strength and oxidative and nitrosylation status of the cell (10). Intracellular conditions in cultured meat may substantially differ from traditional meat, which will influence the rate and extent of tenderization and flavor development.

STRUCTURE AND TEXTURE

The technological challenges with respect to cultured meat texture are strongly dependent on the type of meat or meat product that is produced. The challenges to creating an appealing texture in producing cultured meat mimicking fresh meat are by far greater than challenges involved in the preparation of ground or finely minced meat products. It is acknowledged that the production of a full-sized cultured product similar to steak or pork chops is challenging and may not be feasible within the near future (4, 5, 17, 18). Due to the absence of blood, providing nutrients and oxygen, and diffusion limitations, only a few cell layers can be produced using currently available culture techniques (19). The production of thicker meat pieces would require a perfusion system allowing medium with nutrients and oxygen to be distributed throughout the tissue. Assembly of a vascular-like system lined by endothelial cells may be a way to allow such perfusion (20). In traditional meat, the texture depends on the myofibrillar structure as affected by *rigor mortis* and aging, the amount and structure of connective tissue present in the endo-, peri-, and epimysium of the muscle and the amount and composition of fat in the muscle (7, 21). Closely mimicking these properties would require co-culturing of myoblasts with fibroblasts and adipocytes (22). However, co-culture of several cell types is technically challenging, since each cell type grows and differentiates in specific media. When several cell types are cultured in the same medium, these conditions may be sub-optimal for one or more cell types (23). By medium additions, cells can be directed toward increased deposition of extracellular matrix, changing the mechanical properties of the tissue (24). On the other hand, instead of inducing a structure through complex cell co-cultures, a connective tissue structure can also be created by means of an edible (non-cellular) matrix. Such matrix (also called "scaffold") could be based on connective tissue when made of structural proteins, such as collagen and elastin.

The production of ground cultured meat products, such as hamburgers, is more feasible, as proven by the cultured meat prototype demonstration in 2013 (2). Traditional hamburgers of high quality are produced by grinding meat (beef) using a 3–6 mm blade. The final structure still includes tissue fragments. Binding of these fragments occurs mainly through meat proteins that are extracted by adding a small amount of salt (7). In the patty produced from cultured meat in 2013, 10,000 muscle fiber strips of ∼1 mm in diameter were used (1), hence the tissue fragments were significantly smaller. In order to bind these strips and to provide the product with the texture needed, breadcrumbs, egg white powder and binders were needed (18).

The texture of the resulting product is therefore expected to resemble industrially processed burgers (which are minced more finely and also contain these additional ingredients), rather than fresh high-quality burgers, which only contain salt as an ingredient.

Other processed meat products, such as cooked sausages, are even more finely minced. In these products, meat is minced to such extent that no cellular structures remain (25). This could reduce the complexity of cultured meat production for this purpose. For example, the use of edible scaffold material for cell culture could be omitted (26). Structure formation in these products strongly relies on the techno-functional properties of the dissolved proteins, more specifically the gelation of the myofibrillar proteins actin and myosin during pasteurization. In addition, if a fat fraction is added (which is the case in cooked sausages), the proteins stabilize the fat by forming an interfacial protein film around the fat globules (7, 25). Hence, the gelling and emulsifying characteristics of meat proteins are of paramount importance in the production of finely minced meat products. It has been suggested that the biochemical composition of cultured meat is expected to be very close to that of regular meat, since both contain muscle fibers (5). However, muscle fibers formed through the currently available *in vitro* methodologies contain only small amounts of predominantly embryonic or neonatal isoforms of actin and myosin (13). Electrical and/or mechanical stimulation increases myofiber diameter, enhances myotube structure and increases myofibrillar protein content (27, 28). It remains to be determined whether such stimulation is scalable, economically feasible and whether the resulting protein content and techno-functional quality would be sufficient to provide the gelling and emulsifying properties needed in the production of such meat products. If not, additional structure forming ingredients would be needed, such as other proteins, hydrocolloids, starches, fibers, etc. Many currently available meat alternatives, commonly based on plant proteins, also contain considerable amounts of structure-forming ingredients in order to correct for their inferior techno-functional properties. However, this addition may lower the product attractiveness to consumers, who demand clean label products.

From a textural point of view, it can be questioned whether entire muscle cells are needed for the production of *in vitro* produced finely minced meat products, as no cellular structures remain after the mincing process (25). The use of synthetic meat proteins produced through fermentation could be a more feasible alternative (29).

COLOR

The red color of meat is mainly attributed to the presence of myoglobin, a heme containing protein. Cultured muscle tissues generally have a pale color due to the absence of myoglobin, since myoglobin expression is suppressed at ambient oxygen conditions (18, 20, 30). Several approaches have been suggested to increase the myoglobin content of cultured meat.

A first approach is to increase myoglobin expression by adaption of culturing conditions, for example by culturing muscle fibers under low oxygen conditions (18, 31). However, more research is needed to determine if hypoxic conditions alone are sufficient to increase myoglobin expression (32, 33) and evaluate the impact of low oxygen conditions on the culturing efficiency. Increased glucose consumption and lactic acid production has been reported under hypoxic conditions, suggesting better efficiency (34). However, this may result in medium acidification, which could damage the cells (14, 35). Expression of myoglobin could also be stimulated by presence of media additives, such as lipids or acetic acid (34). In addition to myoglobin protein synthesis, color development also requires the presence of sufficient amounts of iron in the cell. Myoglobin contains heme, which has iron in the center of its structure. Basal media for cell culture contain no iron (e.g., IMDM, RPMI1640) or only a low amount of iron in the form of ferric nitrate non-ahydrate (DMEM: 0.1 mg/L) or ferrous sulfate heptahydrate (Ham's media 0.8 mg/L). Supplementation of the cell culture medium with extra iron results in an increase of iron content of the cells, although only part of the iron is taken up, suggesting there might be a limit to the amount of nutrients the cells can incorporate (36). Uptake is dependent on transferrin, a protein which binds iron and mediates transport in the cell (34, 35). The extent to which iron is then incorporated into heme (necessary for good iron bio-accessibility) and myoglobin (necessary for color development) remains to be studied (18).

A second approach to increase the myoglobin content in cultured cells is the direct addition of myoglobin to the medium. In a recent study by Simsa et al. (31), the addition of metmyoglobin was shown to increase the cell proliferation capacity and resulted in an increased myoglobin content in the cultured cells. However, myoglobin contents were still much lower compared to beef, and the resulting color was brown, resembling cooked beef rather than fresh beef which was due to the use of metmyoglobin (the oxidized form of myoglobin).

Failure to incorporate sufficient amounts of myoglobin in the cultured cells would necessitate the external addition of myoglobin or other colorants at a later stage in the production process. This would only be possible for processed meat products. In this regard, an artificial colorant, soy leghemoglobin, produced via a genetically engineered *Pichia pastoris* (37), recently obtained FDA approval for incorporation in a plant-based burger, giving it the color and taste of a natural beef burger (38). However, it is not clear whether soy leghemoglobin could be applied in the context of cultured fresh meat. Finally, it must be noted that red meat has been associated with increased incidence of several types of cancer (39). While the exact mechanisms are not completely understood, the potential role of heme iron has been pointed out in this respect (39). Therefore, from a health perspective, the use of alternative colorants instead of heme might be pursued for cultured meat.

FLAVOR

Fresh, uncooked meat has little flavor. It tastes rather bloody (15, 40), which is attributed to its relatively high iron content. As discussed in the previous section, the iron content in the cells

can be increased to some extent by using iron-fortified medium. Other compounds contributing to the taste are lactate (sour taste) and inosine 5′-monophosphate (IMP, umami taste), both formed during post-mortem metabolism (15). Upon heating, complex thermally-induced reactions result in the formation of enormous numbers of volatiles, some of which (but not all) contribute to the typical meat flavor. The main reactions involved are the Maillard reaction and lipid degradation reactions, as well as interactions between both (15, 40).

Maillard reaction involves a reaction between an amino compound (free amino acids or peptides) and a reducing sugar (mainly ribose and ribose 5′-phosphate, which are a breakdown products of IMP). In traditional meat, substantial amounts of these precursors are formed during post-mortem metabolism. It is unclear to what extent these flavor precursors will be present in cultured meat, in which the prevalence of post-mortem metabolism has not been studied due to the lack of cultured meat currently available.

Lipid degradation upon cooking occurs even in very lean meat and meat products, due to the presence of intracellular lipids and especially phospholipids from membranes, which generally contain a higher amount of polyunsaturated fatty acids that are more susceptible to oxidation (15, 40). When higher amounts of fat are present, the contribution of these volatiles to the overall flavor increases (40). While oxidation products contribute to the desirable aroma of meat, they can also cause off-flavors (e.g., warmed-over-flavor) and are often the cause of meat spoilage (15). When considering the presence of fat in cultured meat, again the distinction between fresh meat and processed meat is necessary. On the one hand, in fresh meat, fat is known to contribute significantly to the taste of the product, as well as the texture and juiciness. Adding a fat fraction to cultured meat may require co-culture of muscle cells with adipocytes. On the other hand, in finely minced meat products, fat (in most cases pork back fat) is often added as a separate raw material (7). Analogously in cultured meat, fat may be added at the end of the culture process and alternatives, such as (separately) cultured fat or plant-based fat may be used instead of animal-derived fat. From a technological point of view, the addition of alternative fats in finely minced meat products is well-described (41, 42).

In case the culturing process itself does not result in a product with satisfactory flavor, addition of artificial flavor compounds akin to those currently used in plant-based meat substitutes (22) might be an option.

It can be added that in cultured meat, some specific problems related to off-flavors that occur in some traditional meats can be avoided. An example is boar taint, an off-odor present in uncastrated male pigs, related to the presence of androstenone, indol, and skatol (43).

NUTRITIONAL COMPOSITION

Meat is generally considered as a nutritious product due to the presence of highly digestible proteins with excellent amino acid composition, vitamins, and minerals. With regard to proteins, some considerations have already been given in section Structure and Texture. It is not clear to what extent the protein content and composition of cultured cells resembles that of traditional meat.

Scaffolds composed of naturally occurring polymers are commonly used as a way to organize cells in a 3D environment (14). In current tissue engineering approaches, a hydrogel of such polymers is often used as this facilitates cell-induced contraction and tissue alignment. The hydrogel volume typically largely exceeds that of the cells, even after prolonged culture time (27); therefore macronutrient composition of the overall product will also be affected by the scaffold material. Proteins, such as collagen or fibrin are already used in muscle tissue engineering approaches. Collagen contains mainly non-essential amino acids (44) but also a moderate amount of lysine, which is considered a limiting amino acid in diets devoid of meat (45). However, lysine in collagen of connective tissue is to a varying degree post-transcriptionally modified to hydroxylysine which cannot be used in protein synthesis (46). Therefore, it will be of interest to determine the amount of lysine vs. hydroxylysine in the collagen, which will be dependent on the source (different types of animal collagen or recombinant collagen). In lean meat, collagen makes up only a small fraction, but in the case of processed meat products, it can be added to constitute up to 25% of total protein (47). To avoid animal-derived components, polysaccharides, such as alginate, cellulose or chitosan (derived from algae, plants, and fungi, respectively) could be used as scaffold material, providing a source of dietary fiber, which has numerous health benefits and is underrepresented in western diets (48).

From nutritional point of view, fat in meat can be characterized by its percentual content and fatty acid composition. These characteristics are influenced by variables, such as livestock species and breed, age, type of feed, and meat cut (49). While overall fat content impacts mainly the caloric density of the product, fatty acid composition influences the dietary value in more complex ways (saturated or unsaturated fat, ratio of polyunsaturated fatty acids, trans-unsaturated fats). Addition of fatty acids can be pursued by co-cultures of adipocytes derived from adipose stem cells, which can synthesize various saturated and unsaturated fatty acids (50). However, essential fatty acids (mostly linoleic and α-linolenic acid) and some other nutritionally valuable compounds (e.g., conjugated linoleic acid, synthesis of which depends on biohydrogenation occurring in ruminants) present in meat (49) may still be missing in the co-culture approach. More research is needed to determine whether the fatty acid composition of adipocyte culture can be manipulated for instance by directly adding essential fatty acids to the media without disrupting growth and lipogenesis (51). Alternatively, end-stage addition of (plant-based) fats in cultured meat products may be economically and technically more feasible compared to *in vitro* co-culture with adipocytes.

Meat is also a significant dietary source of minerals, such as iron, zinc, and selenium. In muscle tissue, iron is either present as a part of a heme group in myoglobin (and to lesser extent hemoglobin) or stored in complex with ferritin in a non-heme form (52). From a nutritional standpoint, it is advantageous to consume iron in the heme form, because it is absorbed more easily than the non-heme form and its absorption

is not hindered by chelating agents naturally occurring in some foods (53). Increasing myoglobin content would therefore improve nutritional characteristics in addition to color and taste properties. Other minerals, such as zinc and selenium are either not present in basal cell culture media (e.g., DMEM, RPMI1640) or in very low concentrations (Ham's media contain zinc sulfate heptahydrate and IMDM contains sodium selenite) and thus need to be supplemented to support cell growth. Thus far, nothing is known about the uptake of these minerals in cultured meat.

In most diets, meat provides a large share of various B-group vitamins, especially B12 (8). The latter vitamin is synthesized exclusively by microorganisms (bacteria and archea) and then absorbed and utilized by animals, while plants rarely contain considerable amounts of B12 (54). Hence, people following plant-based diets need to take vitamin B12 supplements in order to fulfill their dietary demands (55). If cultured meat is to be regarded as a substitute for traditional meat, it is vital that it contains vitamin B12. With regard to tissue engineering, vitamins are necessary in the media for optimal cell proliferation (56), but it is not clear whether the uptake from media results in levels of vitamins in cultured meat comparable to traditional meat. Furthermore, uptake of B12 requires a binding protein (transcobalamin II) enabling transport across the cell membrane (55, 57, 58). This can potentially present an additional challenge to achieving adequate levels of B12 in cultured muscle tissue. Further research is needed to determine if spontaneous vitamin uptake mechanisms are sufficient to achieve nutritional parity with traditional meat. An alternative approach would be the post-culture addition of vitamin B12 to the meat (product). Similarly, many currently available plant-based meat alternatives contain added vitamin B12 in order to enhance their nutritional value.

Aside from crucial nutrients, such as vitamins, minerals, and essential amino and fatty acids, meat also contains numerous bioactive compounds beneficial to human health. Taurine is a free amino acid playing a vital role in many metabolic processes (59). In humans, it is partially obtained from diet, but internal synthesis of taurine, occurring mainly in liver and brain, is sufficient in healthy humans (60). However, high dietary intake has been associated with a protective effect against cardiovascular diseases (61), and increasing the taurine content of cultured meat might therefore be beneficial. Furthermore, the potential of cultured meat as an ingredient in pet food is currently being explored (62), since pet food creates 25–30% of the total environmental impact from animal production in the US (63). Taurine is an essential nutrient in cats and conditionally essential in dogs (64), making taurine addition necessary for this application, considering general cell culture conditions are taurine deficient. Taurine treatment enhances the differentiation of myoblasts to myotubes (65), therefore addition of taurine to the cell culture media may increase efficiency of the production process, in addition to its nutritional benefits.

Creatine, a substance widely known to accumulate in muscle where it provides an instantaneous source of energy for contraction, is synthesized mainly in liver, kidney and pancreas. Dietary supplementation has been extensively studied and found to be beneficial for gain of muscle mass and to a certain extent also improvement in cognitive function in healthy adults and the elderly (66). Moreover, addition of creatine to the cell culture media improves myoblast differentiation (67) and could therefore be used to improve cultured meat production. However, increasing the creatine content might also have an accidental adverse health effect. As a result of the Maillard reaction during cooking, creatine in traditional meat forms carcinogenic heterocyclic amines (68). Other compounds in traditional meat products, such as N-nitroso compounds and heme iron have also been associated with increased cancer risk (39). It remains to be seen whether the levels of these compounds could be lowered in cultured meat without compromising sensorial and nutritional aspects.

CONCLUSION AND FUTURE CHALLENGES

Due to technological challenges related to its production, cultured meat prototypes are currently not available for independent technological, sensorial and nutritional assessment. Based on the available state of the art regarding production processes, it can be inferred that cultured meat currently differs significantly from traditional meat in its technological, sensorial and nutritional properties. Revealing the extent to which post-mortem processes occur in cultured meat is crucial to understand its impact on sensorial and technological properties. Production of cultured meat resembling fresh, unprocessed meat entails the biggest challenges with respect to texture, color, flavor as well as nutritional composition. Ideally, this would entail co-culturing of myoblasts with fibroblasts and adipocytes. In addition, electrical and/or mechanical stimulation may be needed to improve the techno-functional quality of the meat proteins. However, the technological and economic feasibility of these solutions, especially at large scale, can be questioned (13). With regard to nutritional value, we illustrated the long trajectory of additional research that is needed before the composition of cultured meat could resemble traditional meat, as well as the complexity of the medium composition needed to achieve this. This will not only add to the cost of the medium, but also increase the environmental footprint of the entire process. In processed meat products, most of the challenges mentioned above may be overcome by the simple addition of texturizing ingredients, colorants, flavorings and nutrients in order to remedy the sensorial and nutritional properties. However, this decreases consumer acceptability. Further, in the absence of a defined and openly communicated production process, it is currently impossible to gauge all potential issues related to sensorial aspects and nutritional value of cultured meat products entering the market in the forthcoming years.

AUTHOR CONTRIBUTIONS

IF initiated the sections on structure, texture, color, and flavor from the perspective of a meat technologist. MK initiated the section on nutritional composition. LT and HV added the information throughout the manuscript relating to muscle tissue engineering and edited in all sections. All authors had contributions throughout all sections, read, and approved the final manuscript.

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Cultured Meat and Australia's Generation Z

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This exploratory study of Gen Z consumers ($n = 227$) examines perceptions and opinions about cultured meat of young adults residing in Sydney, Australia. It uses an online survey and describes the findings quantitatively and through the words of the study participants. The results show that the majority (72%) of the participants are not ready to accept cultured meat; nonetheless, many think that it is a viable idea because of the need to transition to more sustainable food options and improve animal welfare. When faced with a choice between different alternatives to farmed meat, a third of the participants reject cultured meat and edible insects but accept plant-based substitutes finding them more natural. Concerns about masculinity and betraying Australia as a country of quality animal meat are also raised. A significant number of young people (28%) however are prepared to try cultured meat. Environmental and health concerns may influence a broader section of society to embrace this novelty. With its power as the emerging new consumers, Gen Z is putting the future of cultured meat under scrutiny.

Keywords: cultured meat, disgust, environmental, Gen Z, masculinity, meat alternatives, sustainability, Sydney

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INTRODUCTION

There is increasing awareness about the negative impacts of the current levels of meat consumption on the natural environment and the planet's ecosystems (1). For example, the disproportionately large appropriation of land for grazing and production of animal feed, is a major contributing factor for the highest rate of biodiversity loss and species extinction in human history (2). Further destructive consequences from excessive consumption of animal-based foods are manifested with the precarious increases in greenhouse gas emissions, freshwater use, deforestation, land, and water pollution. In 2019, there have also been significant social unrest and protests across the globe by groups concerned about climate change as well as the exploitation of animals for human consumption (3, 4). This has resulted in confrontations between those who believe that meat is an essential component of the human diet equated with good nutritional qualities, strength and masculinity (5), and those who argue that the long period of dependence on livestock as food should be assigned to the past (6). The creation of cultured meat leading to cellular agriculture is the way of resolving these tensions emerging after 20 years of scientific research and recent investment waves (7).

Cultured Meat

Producing animal meat without livestock (8) is being described as a tissue-engineering technology which uses live cells taken painlessly from the animal's body to be proliferated and grown independently from its organism. This cultured meat, also referred to as *in-vitro*, cell-based, lab-grown, cell-cultured, fake and clean meat, is perceived to have animal welfare, environmental

and health advantages over traditional meat. As cultured meat is still in its infancy, any claims about its energy or water efficiency are yet to be fully substantiated (9). An anticipatory life-cycle analysis (10) suggests that cultured meat would require smaller agricultural inputs and less land, however it would be more energy-intensive compared to livestock. Although the term meat continues to be used to indicate the animal origin of the product, it defines a new conceptual line of producing cruelty-free food. Van der Weele and Driessen (11) describe cultured meat as an ethical framework that allows people to continue to consume animal proteins while also having a meaningful relationship with farm animals.

Irrespective of the technological and production advances, a major factor for the adoption of cultured meat is its acceptance by the consumers. There is a large number of factors that shape consumer preferences ranging from sensory experiences to psychological predisposition, health considerations, environmental concerns and marketing influences (12). Age and gender also impact on people's food choices (13). In this study, we explore the attitudes of the young generation of Australians in relation to cultured meat by surveying Generation Z Sydney residents.

There have already been other studies investigating the acceptance of cultured meat in places, such as USA (14, 15) and the Netherlands (16); comparisons have been made between consumers in USA, China and India (17); the USA and the UK (18); and China, Ethiopia and the Netherlands (19). Systematic literature reviews highlight the demographic and geographic variations between consumers (20, 21) as well as the need for further research.

Generation Z

This is the first study to explore the acceptance of cultured meat amongst Australian youth with a specific focus on Generation Z living in Sydney, Australia. Sydney is Australia's best-known iconic city which has a distinctive dynamic and multicultural atmosphere with vibrant cultural and artistic life. It is also Australia's business hub with innovation, knowledge-based industries, services, health and medical care and tourism flourishing. Sydney is consistently being ranked as one of the most liveable cities in the world characterized with economic and population growth as well as numerous opportunities supported by its modern infrastructure and competitive advantages (22). This study explores a particular section of Sydney's population, Generation Z or Gen Z—a demographic cohort following the Millennials (23) and preceding the latest Generation Alpha (24).

Generation Z (born between 1995 and 2010) represents around 20% of the current Australian population or 5 million young people (24). Its share in the world is larger at almost 30% or 2 billion people in 2020 (24). This generation grew up with digital technologies, the Internet and social media. Some argue that Gen Z is defying the reputation of entitlement characteristic for the Millennials, is "prematurely mature" [(25), p. 59] and is already exhibiting qualities, such as social generosity and environmental responsibility (26).

There is a limited number of studies specifically examining the consumer attitudes of this newly emerging world power (23, 25, 27), and particularly around their relationship with meat alternatives and cultured meat (28, 29). Although yet to be properly understood, Gen Z is not only defined by technology but is also the future economic, decision-making, political and social change driving force. They are ready to intervene and break the status quo, charged with a mission of social and environmental responsibility. Most of the knowledge, concepts and ideas this highly technologically advanced and diverse digital generation grasps from the net, exploring the vast opportunities of Google, YouTube and other social media (30). Globally aware and no strangers to social activism, the technologically empowered Gen Z wants to make a difference and to leave a mark of significance.

Gen Z has already demonstrated their strong views about the world, their own future and the need for rethinking the relationships between people and with the planet through the voices of Malala Yousafzai—the Pakistani activist for women's rights, Greta Thunberg—the Swedish environmental activist and climate change campaigner, and Billie Eilish—the American vegan singer-songwriter. This generation wants its voices and opinions to be heard, to be actively engaged in political conversations, to become influencers, to be involved and bring positive changes. They are smart, challenging, adventurous, active decision-makers (25) who are not to be underestimated with their tech-savvy skills and expertise in easily finding information. This generation is armed with learning from humanity's previous missteps, environmentally aware and tends to rally spontaneously behind global causes that resonate with them.

Despite living in a wealthy country with a prosperous economy, Australia's Gen Z is apprehensive about the world and the many environmental problems they are inheriting, with climate change being their biggest concern (31). They however believe in the power of knowledge, research, science and technology with almost half of them (49%) trusting the university sector to deliver solutions for the world's urgent and pressing challenges—a much higher share than amongst their global counterparts (31). From this point of view, it is very interesting how the Australian Gen Z in particular responds to the emerging cultured meat. However, our 2019 survey covered only adult Gen Z representatives who in that year would have been at least 18 years of age as they are already economically independent and can make their own food-related decisions. Hence, the Gen Z sample covered in this research is of Sydney residents born between 1995 and 2001. None of the participants has tasted cultured meat and their responses are based only on perceptions and the information they have had prior to the survey.

Section Materials and Methods outlines the methodology of the study before we present and discuss the results from the qualitative survey carried out in Sydney in 2019. The final section closes the discussion by reflecting on the dynamically changing circumstances that may speed up consumers' decision about cultured meat. Theoretically, the process of cultured meat production could efficiently supply enough products to satisfy the global demand for meat; the reality however will depend on the existing institutional and international arrangements as well as the deployment and availability of infrastructure and the

political and socio-economic environment (7). People's attitudes will play a major role in this and the paper provides insights from Sydney's Gen Z.

MATERIALS AND METHODS

This exploratory survey of adult Gen Z is based on an online questionnaire containing some quantitative and some qualitative questions. We chose to conduct the survey online because of the target group's familiarity with using the internet. The survey offers an opportunity to explore the views of young people using inexpensive, completely voluntary, interactive, without data restrictions and open in nature method. Tailored to the main characteristics of the target population, the research aimed to develop a first-hand understanding about a specific group faced with a particular situation which we believed was worth exploring but about which there was no prior knowledge (32). With Gen Z being the first all-digital generation, we took an open-mind approach without any explicit expectations to try to understand what excites and affects them (33) in relation to cultured meat.

The quantitative part of the questionnaire requested demographic data about the participants while the qualitative components focused on collecting their opinions related to cultured meat. There were five sections in the questionnaire:

(1) Demographic data related to age, gender, employment status, profession, and income level;
(2) Dietary preferences based on frequency of meat consumption, ranging from daily to a few times per week, occasionally and never;
(3) Opinion about cultured meat and whether it is normal and necessary to accept and if available, consume cultured meat;
(4) Preference for different meat alternatives, namely insects, plant-based options and cultured meat;
(5) Factors and reasons which may influence people to embrace new meat alternatives.

All participants were recruited randomly, electronically from a pool of 30,000+ names registered in a database established by the researchers. They used a checkbox on the questionnaire to indicate their informed consent to take part voluntarily in the survey. An ethics approval was obtained from Curtin University Human Research Ethics Committee. The response rate to the survey was 75% which is relatively high. Such a response rate is considered good to eliminate potential bias from young people who have responded and those who have chosen not to respond (34).

The data gathered from the qualitative sections were in the form of free verbatim comments and direct quotations. As with most qualitative studies, we continued collecting additional data until a saturation of results was achieved, meaning the data no longer provided any further clarity or insights related to the explored topic (35). The collected data were analyzed both manually using researcher discretion and with the help of the computer-assisted qualitative data analysis software NVivo11 (36). Frequently occurring expressions and themes were coded to produce manageable categories related to the topic of cultured meat.

RESULTS

Conducted in 2019, this exploratory study of adult Gen Z in Sydney, Australia covered 227 ($n = 227$) participants born between 1995 and 2001. Below we present the demographic and dietary description of the sample followed by the respondents' opinions about cultured meat.

Description of the Sample

In total, 227 representatives of Sydney adult Gen Z participated in the study through voluntary self-selection (see **Table 1**). The share of male participants (55% or 125 men) was higher than that of female respondents (45% or 102 women). This gender difference in favor of male participants is relatively small and although it was not deliberate, it was important to capture sufficiently men's views as previous research shows that they more frequently opt for meat options (5). There were no major differences in the opinions presented by the male and female participant groups and therefore, the analysis to follow does not apply a gender lens.

The sample is statistically representative at a 95% confidence level with an acceptable margin of error within 5.2%. **Table 1** shows the break-down of the participants by age. The average age of the sample is 21.4 while the median age is slightly higher at 22.

Overall, the sample consists of relatively young adults, the majority of whom have transitioned to being economically independent with 50% being in full-time employment, 42% working part-time and only 7% studying (see **Table 1**). The

TABLE 1 | Demographic characteristics of Sydney Gen Z sample.

Demographic parameters	Category	Total number ($n = 227$)	
			%
Gender	Male	125	55%
	Female	102	45%
Age	18 years	18	8%
	19 years	19	8%
	20 years	39	17%
	21 years	33	15%
	22 years	40	18%
	23 years	42	22%
	24 years	36	13%
Household income	Under $50,000	62	27%
	$51,000 to $74,000	77	34%
	$75,000 to $100,000	52	23%
	$101,000 or more	36	16%
Employment	Full time	114	50%
	Part Time	96	42%
	Study	17	7%

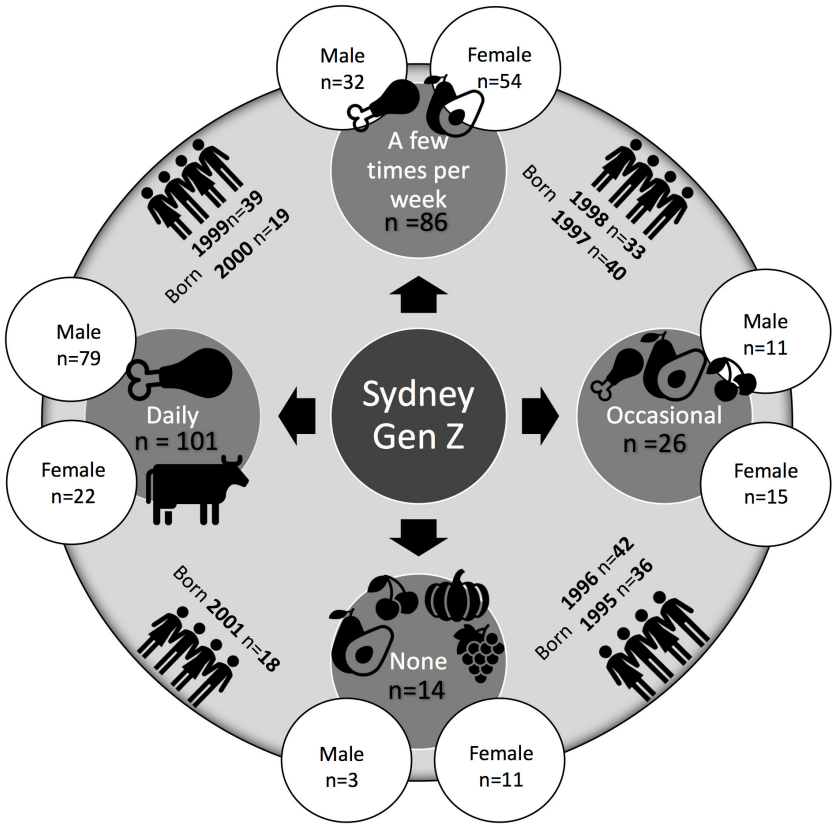
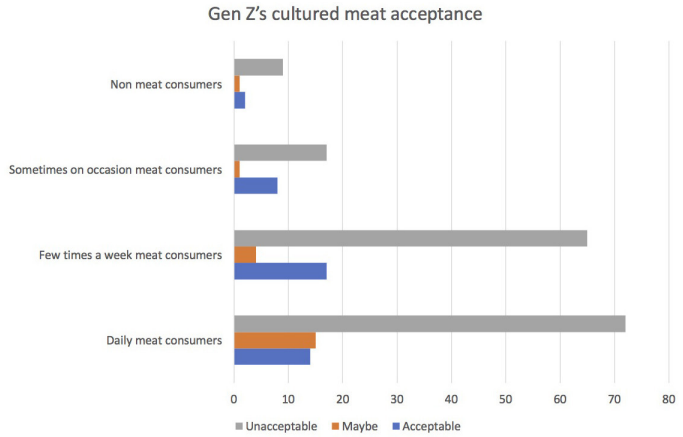


FIGURE 1 | Meat consumption of Sydney Gen Z sample.



	Acceptable	Maybe	Unacceptable
Daily meat consumers	14	15	72
A few times per week meat consumers	19	4	65
Occasional meat consumers	8	1	17
Non-meat consumers	2	1	9
Total	43	21	163
Percentage of total	19%	9%	72%

FIGURE 2 | Acceptance of cultured meat by Sydney Gen Z.

average household income of the Sydney adult Gen Z sample is estimated at around $71,000 per annum.

The levels of meat consumption varied significantly amongst the study's participants (see **Figure 1**). However, there was explicit preference for meat in line with the general trends in Australia which has one of the highest per capita meat consumptions in the world (37). The majority of the respondents, namely 44%, consume meat on a daily basis, followed by those who eat meat a few times per week—38%, occasionally—10%, and never—6%. A 2019 study of the Australian population shows that the food of 12.1% of Australians (or 2.5 million) is all, or almost all, vegetarian (38). Our sample shows a lower percentage of strict vegetarians (6%) but the share of those who eat meat occasionally or have excluded meat completely from their diets, is higher at 16%. Previous research shows that vegetarianism is also constructed as a social category with some vegetarians occasionally consuming meat (39). Hence, a possible explanation for the lower rate of full vegetarianism in our sample is the fact that we asked about frequency of meat consumption rather than how people self-identify. These considerations allow us to conclude that the survey sample is not that different from the overall food trends in Australia.

Only 97 participants (or 43% of the sample) believed that there is a need to replace traditional meat with other food alternatives, including plant-based options and cultured meat. This shows a relatively low level of awareness amongst Australian youth about the negative environmental and other impacts of livestock. Furthermore, cultured meat is not seen as an attractive alternative with only 19% (or 43 participants) accepting it as a food option and a further 9% (or 21 people) being hesitant. The remaining 72% (or 163 participants) were categorically of the opinion that cultured meat is not acceptable to them (see **Figure 2**). What are reasons for these low levels of acceptance of cultured meat are discussed in the qualitative analysis below.

Attitudes Toward Cultured Meat

Concerns dominated Gen Z's attitudes toward cultured meat. They included personal concerns, related to anticipated taste, health and safety, as well as societal considerations related to whether we need to accept the need to consume cultured meat and whether it is more sustainable. Socio-cultural impacts, such as perceptions about masculinity and animal meat being an Australian pride, were also raised. Some were concerned about the animals themselves. Finally, there were those who saw cultured meat as a conspiracy orchestrated by the rich and powerful and were determined not to be convinced to consume it.

This wide range of opinions is discussed below using excerpts verbatim from the survey respondents. It appears that there were

TABLE 2 | Sydney Gen Z's disgust and willingness to try cultured meat.

	Disgust	Willingness to try
1	"*I am more pro meat than pro other meat alternatives including in vitro. When I think about is, I feel disgusted. It makes me really sick*." (daily meat eater, barista, age group 18–20 years)	"*I don't know much about in vitro meat. Humans pretty much eat anything. If it's dressed up nicely and appetizing, I'm all for it. I love to try cultured meat. Life's too short to eat bland and uninspiring, lol*". (daily meat eater, project manager, age group 21–24)
2	"*What is so clean about this meat, nothing clean. It's full of chemicals and looks disgusting. It's really disgusting. I won't eat it*." (daily meat eater, hospital attender, age group 21–24 years)	"*Many of my peers will say it's not normal to eat cultured meat, but I am open to give it a try. It looks good. You can say something is not good if you never try it*." (daily meat eater, assistant accountant, age group 21–24 years)
3	"*Definitely not normal for me and my family. I feel sick even to think about eating meat produced from stem cells. Totally sick*." (daily meat eater, pizza maker, age group 18–20 years)	"*Meat has been linked many times to several diseases. I believe trying in vitro option that is clean from these diseases could be good for the humanity.*" (daily meat eater, boarding flight assistant, age group 21–24)
4	"*Cultured meat, insects are not normal, even disgusting to some extent. We won't eat it and the idea of it is sending messages of pure disgust to me*." (a few times per week meat eater, technical assistant, age group 21–24 years)	*Cultured meat has been there for a while, but still in development stage only, not on the market. We never try it and we don't know what its taste [is] like. I am interested to try it and I reckon the more we know about the process behind cell-based meat production the better acceptance we may have*." (a few times per week meat eater, university student, age group 18–20 years)
5	"*Cultured meat seems weird and a bit disgusting, not because of its look, but because the way it's made.*" (a few times per week meat eater, personal assistant, age group 21–24 years)	"*We will need lots of adapting before putting cultured meat into our mouth. Maybe if it looks like the real thing and if we don't know what it is we will be all willing to eat it*." (a few times per week meat eater, investment coordinator, age group 21–24 years)
6	"*…I don't think we will be able to overcome our aversion to laboratory-grown meat. It's disgusting to grow something from a piece of tiny cell*." (a few times per week meat eater, finance officer, age group 21–24 years)	"*I have no knowledge about cell-cultured meat to comment on it. But if it is helping with animal welfare, I believe people that consume meat should be open to try it. They can reduce the animal burden to die to feed them*." (non-meat eater, bank cashier, age group 21–24 years)
7	"*In vitro meat is much less natural than normal meat. It looks really disgusting and the thoughts we should eat it in the future are making it even more revolting and provoking high unacceptance and dislike.*" (a few times per week meat eater, DJ and Uber driver, age group 18–20 years)	"*Lab grown meats. I am open to trying as they sound interesting*." (daily meat eater, drafter, age group 21–24 years)
8	"*I always try to be open minded, but these alternatives are not so appealing, I have no problems with veggie-based meat, but with the larvae and crickets, and cultured meat I may vomit. Sorry*." (a few times per week meat eater, club team leader, age group 21–24 years)	"*I will try cultured meat if available and of curiosity, but I usually will go with real meat*." (daily meat eater, supply chain coordinator, age group 21–24 years)

overwhelming negative attitudes although some were prepared to give cultured meat a try.

Personal Concerns

Gen Z expressed many personal concerns related to cultured meat. They included disgust about the anticipated taste and eating experience as well as health and safety concerns.

Disgust

When it comes to food, and novel food in particular, people's first reaction is related to the anticipated taste. The study's participants were divided in their reaction. A large majority of the Sydney Gen Z respondents ($n = 163$, 72%) associate cultured meat with a feeling of uneasiness and discomfort. This is expressed with statements, such as: "It makes me really sick," "it's really disgusting," and "I may vomit. Sorry" (see **Table 2**). Some ($n = 64$, 28%), however are more intrigued: "I am open to try it," "if it's dressed up nicely and appetizing" and "the more we know about the process behind cell-based meat production the better acceptance we may have" (see **Table 2**).

Health and safety

Gen Z is very much nutritionally aware and these young people are committed to healthy eating and wellness (40) while open to exploring new food choices which often were not available to previous generations in Australia. It is not surprising then that the Sydney Gen Z is casting doubts whether cultured meat is a healthy and nutritional dietary option with a third of them ($n = 73$, 32%) believing this not to be the case. They described cultured meat as being "far too chemically processed," associated with "engineering and modifications." Others, however, are of the opinion that "it should be healthy and nutritious if they get it right." There are also those who frankly admit: "I have absolutely no idea… whether these alternatives… are nutritious like real meat" (see **Table 3**).

Although sometimes it is difficult to strictly draw the boundary between food quality and food safety, the latter refers to the way the products we consume are being handled and whether there are any negative health consequences from consuming them. Technically, cultured meat is expected to be produced within a clean, sterile and highly controlled environment to prevent any food-related risks. Nevertheless, Gen Z are not

TABLE 3 | Sydney Gen Z's perceptions about healthiness and safety of cultured meat.

	Healthiness	Safety
1	"*In vitro meat is overly processed. In our society, at school, uni, media, magazines, articles, everywhere we are told to limit the consumption of processed food*…." (daily meat eater, sports coach, age group 18–20 years)	"*Cultured meat is an interesting option. Experimentation can lead to great findings for broadening humanity's ever developing knowledge. Although, I think it could be a game not with good end and lots of adverse effects for the humanity, because of our greed for meat*." (non-meat eater, yoga teacher, age group 21–24 years)
2	"*There is a trend now people to become flexitarian and to eat meat alternatives, but all these including cultured meat are not healthy. I prefer to reduce meat intake but will not eat these modern things*." (daily meat eater, university student, age group 18–20 years)	"*If we think about the future food security of the planet, we have to be ready to accept anything. But I believe engineered and chemically processed food are not good for human to consume. I even think these will counteract in the opposite direction and contribute to human non-communicable diseases*." (occasional meat eater, nurse, age group 21–24 years)
3	"*You can't have ribs, steaks etc. out of fake meat and it's not appealing. Even in the future the scientists can grow these, it will be far too chemically processed to be normal and healthy thing to consume*." (daily meat eater, mathematics tutor, age group 18–20 years)	"*Maybe there are more health benefits to not eat meat than eating cultured meat. They could be some future side effects to human from eating it. It's good that it is not mass market produced yet*." (non-meat eater, solicitor, age group 21–24 years)
4	"*No idea how normal meat will be sourced from a lab instead of a farm. More likely not good and unhealthy for us to consume. I will incline toward opting it out*." (daily meat eater, university student, age group 18–20 years)	"*Artificial growth cells and hormones to make it edible in vitro meat thanks god that is still an underdeveloped technology. No one knows what this meat will be lacking and what will be the side effects for us*." (daily meat eater, high school sports aid, age group 18–20 years)
5	"*Not sure why we should think of meat substitutes as healthy. They never will be healthy and good for you like plain fruit and veggies. See the cultured meat, plant-based engineered burger. People will always associate them with engineering and modifications*." (a few times per week meat eater, hairdresser, age group 21–24 years)	"*Not normal, maybe the good thing about it is that humans created some emerging modern technology but multiplying cells to grow meat for human is wrong. It's against the nature and if we consume it, we will pay sooner or later for this*." (daily meat eater, university student, age group 18–20 years)
6	"*Necessary with respect to the environment and the animals, but it's unknown how healthy cultured meat is for humans to consume on a regular basis like meat. More likely not that healthy having in mind the way it's produced*." (a few times per week meat eater, office assistant, age group 21–24 years)	"*Scientists created in vitro meat cultivation because of their interests to advance in technologies, but this doesn't mean what they created is good for human consumption without any future negative effects*." (daily meat eater, trading operations analyst, age group 21–24 years)
7	"*A replacement for meat with in vitro – the scientists are trying hard to replicate real meat, so it should be healthy and nutritious if they get it right*." (a few times per week meat eater, administrator, age group 18–20 years)	"*Need scientifically proven information about cells-made meat before trying it. It could have some unhealthy side effects*." (occasional meat eater, graphic designer, age group 21–24 years)
8	"*In vitro mimic the taste, texture and protein content of meat. Honestly, I have no idea how good it is for you. I have absolutely no idea whether these alternatives are having similar iron, zinc and magnesium content to say if they are nutritious like real meat. I'll say they are fake and not healthy for us to eat."* (a few times per week meat eater, office administrator, age group 21–24 years)	"*We don't know yet if we are going to eat cultured meat. It's still in early stage of its development and far away from the natural meat appearance. It can't be possible to not have some future negative effects on human*." (daily meat eater, physiotherapist, age group 21–24 years)

TABLE 4 | Sydney Gen Z's perceptions about need for cultured meat.

N	Necessary	Unnecessary
1	"*With the population increase it will be very, very necessary to eat meat substitutes and clean meat. I hope there is not going to be a food war*." (daily meat eater, assistant manager, age group 21–24 years)	"*For me is totally unnecessary to push ourselves to eat artificial, cultured meat. Don't we have access to plenty of meat at the supermarket?! Why should we invent the already invented?*" (daily meat eater, community services, age group 21–24 years)
2	"*Solution for meat replacement like lab meat, insects, plant-based are weighty. Availability is important for something to become necessity*." (daily meat eater, swimming coach, age group 18–20 years)	"*I feel like it makes sense for us to move away from consuming meat now that we hear of its negative effects on the environment, but with growing lab meat we will still continue to eat meat. What's the point of lab-growing it? It's not needed.*" (daily meat eater, human resources officer, age group 21–24 years)
3	"*I'll see it necessary to grow meat from cells when there are not enough cows to graze in Australia. Right now, we are enjoying meat and what scientists are up to is something that we will deal with when we reach the point to need it*." (daily meat eater, early childhood teacher, age group 21–24 years)	"*Products that are simulating meat-based dishes but are made from alternate, possibly more sustainable, sources are fine, but I am not sure yet about in vitro meat. It's unnecessary, and sounds very scary*." (daily meat eater, teacher, age group 21–24 years)
4	"*Cultured meat is produced heavily with chemical engineering, but there is no doubt that things like it will be very necessary for the future when there maybe will be not enough meat for everyone*." (daily meat eater, customer service officer, age group 21–24 years)	"*It will be a necessity in the future to consume some substitutes as we will face lack of resources, but in vitro meat is unwanted. It is artificially produced.*" (a few times per week meat eater, online coordinator, age group 21–24 years)
5	"*I think it is necessary to produce different type of meat – cultured, plant-based etc. If we want to reduce our footprint on the planet and the harm to animals, we should accept it*." (daily meat eater, disability support worker, age group 21–24 years)	"*The space and resources needed to farm meat will be under significant pressure in the future, and ethical obligations will prevent factory style farming. But cultured meat farming is a wrong thing to do. It's like a Frankenstein creation, with the difference that we are going to eat it. Sounds not right."* (a few times per week meat eater, 24 traffic control, age group 21–24 years)
6	"*Increasing demand for food and decreasing space will push out appetite for alternative food, so anything on offer cultured meat, plant-based things will be fine as long as they're affordable and available. Alternatives are required to feed people in the future*." (a few times per week meat eater, personal assistant, age group 21–24 years)	"*We are worried and focussed in the future. It is absolutely necessary the humanity to think of new alternatives to meat, but I am not sure why we should focus on creating more processed meat if this is what we have to reduce from our plates. Can we just eat fruits and veggies?*" (occasional meat eater, IT programmer, age group 21–24 years)
7	"*Necessary because of climate change and because of animal suffering. It is good that scientists are making clean meat without using the animals*." (a few times per week meat eater, sales assistant, age group 18–20 years)	"*The meat industry is one of the greatest contributors to global warming - something that needs to be addressed. In vitro meat grown from cells is not a cool option. It's another way to produce more meat."* (non-meat eater, massage therapist, age group 21–24 years)
8	"*I personally believe that using more meat alternatives, mostly plant-based even insects and in vitro meat will have a positive outcome for the environment, humanity and animals. These are all needed for securing future food*." (a few times per week meat eater, retail operator, age group 18–20 years)	"*Even we do have enough information, if we want to save the planet and reverse the climate change, we don't need to grow more meat, especially artificial like in vitro as we can eat veggies and be happy*." (occasional meat eater, dog groomer, age group 21–24 years)

convinced that it will be safe for consumers. A major worry for them are the possible unknown "adverse," "negative," "hidden side effects" of cultured meat (see **Table 3**). This resembles some of the concerns expressed in relation to the consumption of insects in our previous study (29).

Societal Concerns

There are two broader societal themes that emerged in the respondents' answers. One is related to food availability and the other to the environmental impacts of the current meat production. This comes against a background where only a third ($n = 74$, 33%) of the Gen Z participants are willing to change their meat-related behavior, with that share amongst daily meat consumers being much lower ($n = 18$, 18% of the group of daily meat eaters).

Food availability

When it comes to the question about food availability, opinions within the survey sample were divided. Many ($n = 57$, 25%) saw the need to accept cultured meat because of population growth or inability to produce enough livestock-based meat. Expressions, such as "not enough meat for everyone" and avoid "a food war" (see **Table 4**) were used. On the other hand, there were many voices which similarly recognized the need to look at human diet but did not see cultured meat as an option to feed the world or reduce the food's impact on the environment. Examples include: "*in-vitro* meat is unwanted," "it's like a Frankenstein creation" and "can we just eat fruits and veggies?" (see **Table 4**).

Environmental impacts

The need for switching to meat alternatives and more sustainable food choices was highlighted by a large number of study participants ($n = 93$, 41%). They were however unsure whether cultured meat is better and was described as "resource consuming" and not being "environmentally friendly" (see **Table 5**). It is also interesting to note that these concerns were not raised by those who consume meat on a daily basis.

Socio-Cultural Concerns

Two main socio-cultural considerations emerged from the survey. The first is related to the perceptions about masculinity and that meat is the men's choice, while the second is about Australia priding itself as a producer of quality animal-based foods, such as beef.

TABLE 5 | Sydney Gen Z's environmental concerns related to meat.

1	"*Livestock producers must make sure that livestock is environmentally sustainable. Ideas like growing meat on a plate under shelter is quite unsustainable*." (a few times per week meat eater, business owner, age group 21–24 years)
2	"*With the projected rapid decline in meat availability because of climate change, it's important to be substituted with some meat alternatives but not cultured meat. You can't ensure livestock and environmental sustainability with producing extra meat which is the cause of the problem."* (a few times per week meat eater, bartender, age group 21–24 years)
3	"*In vitro meat and other alternatives are important as it can help to reduce greenhouse emissions, save animals and focus on health*." (a few times per week meat eater, installer, age group 21–24 years)
4	"*Lab meat could minimize the associations with the environmental impacts and ethical issues, but it is still resource consuming. Think about how much energy is put into it being under constant light and in a special environment. It's not a sustainable option*." (a few times per week meat eater, remedial massage therapist, age group 18–20 years)
5	"*We need to look after the environment. Lab-meat is environmentally better than livestock produced meat and better for the animals*." (occasional meat eater, acrobatics coach, age group 21–24 years)
6	"*I believe that at the rate our planet is going, we will all have to consider eating less meat. Eating more alternatives for a sustainable diet, like even adopting Meatless Monday, including less meat in our diets or eating more plant-based options. I can't see easily cultured meat fitting into this*." (occasional meat eater, receptionist, age group 21–24 years)
7	"*I'm concerned for the environment and our resources. But I rather eat fruit and veggies than cultured meat or other men-made meat substitutes*." (occasional meat eater, youth worker, age group 21–24 years)
8	"*I don't consume meat but in principle meat alternatives should be sustainable and environmentally friendly (which meat-free alternatives does not necessarily equate to). I don't think cultured meat is any of those*." (non-meat eater, finance officer, age group 21–24 years)

TABLE 6 | Sydney Gen Z's perceptions of masculinity related to meat.

1	"*I can't abandon my meat for other food, even grown from the real animal tissue. A man like me prefers to rip meat from the bone with teeth, to feel the taste, the smell, the blood before I cook it on a barbie, the goodness of the real juicy meat*." (daily meat eater, project officer, age group 21–24 years)
2	"*Man, like me needs good nutrients. Fake meat does not have the same amount of nutrients, minerals, vitamins the actual meat has. Would the protein be the same? I doubt it. I will stick with the manly meat diet*." (daily meat eater, brick layer, age group 18–20 years)
3	"*There is a strict men's rule saying real men eat meat, not artificial meat and I will stick to this rule*" (daily meat eater, IT programmer, age group 21–24 years)
4	"*There are no bones sticking out of lab meat. It's blended and super fake. No one maintaining his masculinity will want to eat a fake meat without a bone*." (daily meat eater, university student, age group 18–20 years)
5	"*In vitro meat is not a food for genuine men... I think you should eat manly things as part of a well-balanced masculine diet and in vitro is absolutely not one of them*." (a few times per week meat eater, clinical associate, age group 21–24 years)
6	"*…Also as a man, I don't think is appropriate for me to eat cultured meat if there is a real, bloody tasty meat around*." (a few times per week meat eater, song writer/installer, age group 21–24 years)
7	"*Forget about edible insects, lab meats, processed plant burgers and other nonsense they try to introduce us to eat. I feel only the juicy meat is natural for my body and health, so I eat it regularly and I feel pretty bloody manly*." (a few times per week meat eater, personal assistant, age group 21–24 years)
8	"*Not interested in these in vitro or other processed plan-based foods. They aren't natural, nutritious, they are not masculine food for Australian men or other men in the world*." (daily meat eater, registrar, age group 21–24 years)

Masculinity

The majority of the male daily and a few times per week meat consumers ($n = 58$, 52% and $n = 58$, 26% of the sample) found cultured meat threatening to their manly traits. Similar concerns have been expressed in previous studies (28, 29), indicating that many men are not ready and unwilling to contemplate changing their dietary preferences (5, 41). **Table 6** shows the expressions such men use, e.g., "rip meat from the bone," "real men eat meat," and "I don't think is appropriate for me to eat cultured meat if there is a real, bloody tasty meat around."

Australian pride

Pride in Australia being a producer of "superb quality" and "best in the world" meat (see **Table 7**) where it is widely available and affordable was expressed by some participants ($n = 29$, 13%). Many stress that "meat is plentiful in Australia" and "we produce lots of good meat the nation is proud of" (see **Table 7**). These participants see cultured meat as a disloyalty to Australian meat and betrayal of their country.

Concerns About Livestock Animals

Concerns about animal welfare and dignity were expressed by some of the participants in the Sydney study. They saw cultured meat as a way to avoid animal suffering but others also raised concerns that using animal cells for growing meat in a lab is unethical from the point of view of the animal.

Animal welfare

Improved animal welfare because of a switch to cultured meat was seen as an advantage by some participants ($n = 37$, 16%). They described this as "this way we are not harming the animals," "stop exploiting them" (see **Table 8**). Such voices were raised mainly by those who consume meat less frequently.

Animal dignity

Another interesting nuance of the ethical use of animals for food production was the issue about animal dignity raised by some participants ($n = 26$, 11%). These participants represented different dietary practices and they spoke about "the permission of the cow" to use its cells, being "really unethical and painful" (see **Table 9**).

Conspiracy Concerns

Concerns were raised by Gen Z whether cultured meat is part of some hidden agenda, including those who have funded its development wanting to see return on their investment. The participants were divided in two groups—pro and against the sponsors of cultured meat (see **Table 10**). Those who are against the development of cultured meat describe this as "another thing our generation to worry" and explain that "there must be serious interest from people who created it." By comparison, those

TABLE 7 | Sydney Gen Z's pride with Australian meat.

1	"*Coming from a meat-eating nation with one of the best superior quality meats in the world, I feel we should be quite cautious not to betray our beautiful meat for this artificial meat*." (daily meat eaters, shop assistant, age group 18–20 years)
2	"*I believe in vitro meat and other plant-based meat are not that essential, meat is plentiful in Australia and one among the best in the world. We don't need to worry too much*." (a few times per week meat eater, legal secretary, age group 21–24 years)
3	"*In Australia we produce lots of good meat the nation is proud of and more lab-grown meat is unnecessary*." (non-meat eater, yoga instructor, age group 21–24 years)
4	"*Aussie meat is the best and part of our culture, no any other meats, even lab meat, can replace its quality*." (daily meat eater, electrician apprentice, age group 18–20 years)
5	"*We love Aussie meat. The best in the world and I can't replace it with gross lab meat*." (daily meat eater, planner, age group 21–24 years)
6	"*Australia grows naturally exceptional livestock and produces the best meat cuts worldwide. Not clear how the lab meat is grown, what chemicals, preservatives they put into it to prevent it from rotting or to maintain its taste, texture*." (daily meat eater, clients' relations, age group 21–24 years)
7	"*Not normal for me to eat some fake, synthetic meat, especially living in Australia where the meat is with no doubt the best in the world*." (daily meat eater, pastry chef, age group 21–24 years)
8	"*Right now, it is not natural at all to consume lab meat. It looks yuck, patty not like meat. It seems quite artificial and can't even compete and beat the Australian meat which is number one*." (daily meat eater, laborer, age group 21–24 years)

TABLE 8 | Animal welfare concerns by Sydney Gen Z.

1	"*Extremely necessary as we have not enough resource to sustain the planet and this way we are not harming the animals, but they are still helping us to eat using their cells to grow meat*" (a few times per week meat eater, recruitment agent, age group 21–24 years)
2	"*Very necessary for climate change and for the animal suffering. I believe scientists [have] done it with all these considerations in mind."* (a few times per week meat eater, assistant manager, age group 21–24 years)
3	"*With in vitro you don't need to kill animals to source your meat. This makes people feel good about the animals*." (a few times per week meat eater, administrator, age group 21–24 years)
4	"*In vitro is good for animal welfare viewpoint. Other than that, it's still an imitation*." (a few times per week meat eater, kindergarten aid, age group 18–20 years)
5	"*It's good for the animals not to be exploited for human food, but actually if the humans reduce their consumption of meat there is no need of a huge exploitation. We don't even need inventions like cultured meat, just change of our diet will sort the issue*." (a few times per week meat eater, café staff, age group 18–20 years)
6	"*It's needed source of meat without harming animals. I believe it's humane way to produce lab grown meat instead of real meat*." (occasional meat eater, carpenter, age group 18–20 years)
7	"*I don't like in vitro and plant-based meats as I care about the animal welfare and don't want to consume anything that resembles meat*." (occasional meat eater, project officer, age group 21–24 years)
8	"*Cultured meat is not applicable to my diet. We have to be ethical to animals and stop exploiting them, not artificially multiplying them.*" (occasional meat eater, community support worker, age group 18–20 years)

TABLE 9 | Animal dignity concerns by Sydney Gen Z.

1	"*Artificial meat substitutes are unnatural. You could figure it out with a simple Google search. And clean meat is not even that clean as it is against the animal dignity to be grown from a cell*." (daily meat eater, engagement coordinator, age group 21–24 years)
2	"*If these are environmentally and ethically produced, they will be good to eat and when people have more knowledge, they could consume them on a regular basis. The problem is that they are not ethically produced with the permission of the cow…*" (daily meat eater, university student, age group 18–20 years)
3	"*I am skeptical about lab-meat. I think we need to work hard to mimic the real meat, but in an ethical for the animal way.*" (daily meat eater, soccer coach, age group 18–20 years)
4	"*I hope it is not becoming necessary to eat only cultured meats. I read that the cells are drawn from live animals and then manipulated chemically to grow. It's quite strange thing to do. It is sad thing to do and really unethical and painful*." (a few times per week meat eater, waitress, age group 18–20 years)
5	"*Humans need to look at new protein sources but not to cultured meat. It is unnatural meat made without even asking animal for a consent*." (non-meat eater, assistant IT software engineer, age group 21–24 years)
6	"*Not exactly made from animals, but from animal cells. This sounds really scary and as vegetarian and animal activist I think this is also another way to be unethical to animals, creating their own counterparts like AI, robots*." (non-meat eater, office worker, age group 18–20 years)
7	"*Humanity must meet the future generation's needs. In vitro is giving new perspectives to grow meat without the animal and with respect for the animal*." (non-meat eater, dancer, age group 18–20 years)
8	"*I have never eaten cultured meat. I think it is still very expensive to produce and not yet fully developed as a technology. Plus, the technology itself is creating synthetic, artificial products using animal cells with no respect for the animal dignity*." (a few times per week meat eater, dental nurse, age group 21–24 years)

who support this innovation describe it as "money… invested for a good cause," "a smart move" by people who are "pretty advanced thinkers."

The Future of Cultured Meat

The respondents were also asked their opinion about the future place of cultured meat in human diets through three different perspectives: first, how they see its future; second, in relation to other meat alternatives; and third, what would make them accept alternatives to traditional animal meat. Their answers are presented in turn below.

Prospects for Cultured Meat

Lack of information about the way cultured meat is created, the substrates and the processes used combined with it not being yet available in Australia, was making it an undesirable food option for many of the respondents ($n = 103$, 45%). This was an interesting observation given the fact that Gen Z is accustomed to be using the web for communication and thrives in the social media. A further large group ($n = 88$, 39%) was indecisive about the future of cultured meat. The remaining participants ($n = 36$, 16%) saw a good value in cultured meat, believing that if it is done right it could work and become one of the "future food trends." **Table 11** presents some examples of the way the participants described cultured meat becoming a normal food

TABLE 10 | Sydney Gen Z's attitudes toward investors in cultured meat.

	Negative	Positive
1	"*When I first heard media reporting something about billionaires like Gates and Branson supporting cultured meat R and D, I remember I said to myself this is another thing our generation to worry. We are not interested how much they put into it. We've seen enough of this 'business deals' and fake 'benefits' for us, the normal humans. I worry a lot*." (daily meat eater, university student, age group 18–20 years)	"*I'm not sure. I don't have enough information and I am not a specialist. But I read that scientists and rich people like Bill Gates and Richard Branson are investing in cultured meat and it must be good as these people are not investing their money for something that is not going to work and them to profit from it.*" (daily meat eater, cashier, age group 21–24 years)
2	"*Rich people like Branson and Gates have invested in this type of fake alternatives and now they are looking to have a return on investment and are marketing cell-based meat as good replacement of meat. Australians have enough good cuts of meat to consider eating cultured meat. No FOMO [fear of missing out]. None of my friends will do*". (daily meat eater, army force soldier, age group 21–24 years)	"*We have to be open to new food sources because we have no idea what food we will have available in the future. If we learn how to produce meat from animal cells, can you imagine how much meat we can produce from a single cow? We can even produce only the best cuts*." (a few times per week meat eater, cinema manager, age group 21–24 years)
3	"*I am not trying cultured meat even for free. Who came up with the idea for this thing? Who stays behind this? There must be serious interest from people who created it, in a similar way like pharmaceutical companies*." (daily meat eater, veterinary clinic assistant, age group 21–24 years)	"*I think it's positive that high profile people invest in cultured meat. I think their moneys are invested for a good cause*." (a few times per week meat eater, carpenter, age group 18–20 years)
4	"*Lab meat is a huge bioengineering animal cells made thing and not for a good cause. There are people with big money behind lab meat and obviously want to profit from it. I am sick of people not seeing the proper root cause of our dietary problems. We need not another meat but reduction of what we eat right now*." (a few times per week meat eater, client liaison officer, age group 21–24 years)	"*Meat from cow tissues and cells is a fascinating concept closer to the sci-fantasy movies than to our reality. I know many wealthy people invested in the technology to develop this kind of meat, but the normal people are not familiar with it yet. I think it's a good thing to do as humanity needs to use the advancement of the technology*." (a few times per week meat eater, project officer, age group 21–24 years)
5	"*It's a new technology still and it doesn't mean that in vitro meat is good to consume if it doesn't involve real livestock. The people that financed it claimed it as a huge discovery for the future humanity food security. Same way soon we will start growing humans from cells. What a hypocrisy*." (daily meat eater, landscaper, age group 21–24 years)	"*People think they are natural and good because we don't kill animals. I need more information about lab grown meat is kind of needed. I feel the people that gave the money for this lab meat are pretty advanced thinkers*." (a few times per week meat eater, office manager, age group 21–24 years)
6	"*I heard and believe clean meat is part of big corporations' vast interests. It has nothing to do with meat scarcity or environment issues. It's part of another big scheme, like the production of drugs*." (a few times per week meat eater, business development assistant, age group 21–24 years)	"*I think making alternatives like lab meat, plant-based that are not harming the environment and the animals is a smart move. I think lab meat was sponsored from Bill Gates*." (occasional meat eater, practice manager, age group 21–24 years)
7	"*Cultured meat is another food part of some billionaire's interests. I think this was backed by Sergey Brin not long time ago. People with money think they can buy everything, but they can't buy us or make us eat their fake meat investments*." (a few times per week, business project coordinator, age group 21–24 years)	"*We are so cruel to animals and I think this is why rich philanthropic people, scientists are creating the lab meat to save them from suffering*." (occasional meat eater, human resources officer, age group 21–24 years)
8	"*Despite the ambition of Microsoft Gates and Google Sergey Brin I don't think people will consider cultured meat as a normal meat from real animals. I am wondering whether something unpleasant behind its production is holding it from mass consumer release*." (a few times per week meat eater, technician, age group 21–24 years)	"*As people are becoming more socially aware of the consequences of eating meat, in vitro meat and other alternatives will become popular. This is what I think will happen, especially from my generation point of view. It's good that they have stable finance providers and supporters*." (non-meat eater, university student, age group 18–20 years)

choice or continuing to be perceived as abnormal, unnatural and "produced against nature."

Cultured Meat vs. Other Alternatives

When asked to express their opinion about different alternatives to livestock-based meat, namely edible insects, plant-based meat and cultured meat, according to their acceptance and preferences, the Gen Z participants were divided into five groups (see **Table 12**). One of the groups (n = 38, 17%) rejected all alternatives, including cultured meat. They were seen as "chemically produced," "heavily processed," and "not what our generation needs." Another group (n = 25, 11%) rejected all alternatives in favor of increased consumption of fruit and vegetables: "I will stick to pure veggies" and "why… not eating normal veggies, we know they are good." A larger group (n = 79, 35%) rejected cultured meat and edible insects but accepted plant-based alternatives because they "sound more natural" and are "normal." Cultured meat was acceptable or possibly acceptable to the fourth group (n = 64, 28%) "if we can master it" as this will be "new forms of protein." The fifth group (n = 21, 9%) accepted edible insects but rejected cultured meat because "it's too artificial and not a natural food like insects" and "innovations can try to change the meat industry but can't easily change the consumers who prefer natural stuff."

Embracing Meat Alternatives

What could make Gen Z embrace alternatives to traditional animal meat, including cultured meat, is an important question related to the future of food. The question allowed multiple choices and **Figure 3** presents the frequencies for each indicated answer. Broader sustainability concerns, including

TABLE 11 | Sydney Gen Z's perceptions about cultured meat being normal/not normal in the future.

	Normal	Not normal
1	"*Cultured meat is not natural for us to eat but in the future, this could be our only option to secure food. It could contain good nutrients as it is scientifically made, and everything is additionally added to make it taste better*." (daily meat eater, community worker, age group 18–20 years)	"*It is absolutely not normal to consume cultured meat. It's out of my food comfort zone. I even don't want to try it*." (daily meat eater, office assistant, age group 21–24 years)
2	"*Not sure if it is normal to eat now, but if no any other alternatives are available maybe people will start eating cultured meat*." (daily meat eater, executive assistant, age group 21–24 years)	"*Meat substitutes are normal and quite popular even fashionable lately among young people like me. But lab meat is purely food-based biotech. Very abnormal for me and I will never eat these foods*." (a few times per week meat eater, assistant, age group 21–24 years)
3	"*It is not normal for my diet right now to eat cultured meat, but with more information and with practice things could change and could become normal in the future.*" (daily meat eater, executive assistant, age group 21–24 years)	"*Meat alternatives are emerging and trendy now, but except some of my friends that are eating plant-based I don't think anyone who knows the taste of real steak to be willing to eat anything else including cultured meat although it is supposed to be a duplicate of a real meat*." (a few times per week meat eater, retail team leader, age group 21–24 years)
4	"*The food of the future when there will be not enough food – if people like eating cultured meat I see no issue with the food shortages or with the future acceptance.*" (daily meat eater, netball coach, age group 18–20 years)	"*I think cultured meat is an unnatural nonsense*." (occasional meat eater, shop assistant, age group 18–20 years)
5	"*Unfamiliar with cultured meat. I heard it's very expensive to produce and energy consuming. If cultured meat is made to exactly replicate meat in couple of years nobody will question from where the meat is coming from. People don't care much about it anyway*." (a few times per week meat eater, administrative assistant, age group 21–24 years)	"*In vitro is completely unnatural thing. The meat gains its flavor from the animal, the amount of fat content, the marbling. It is a reflection of the way the animal is grown - the pasture it's grazed on, the food it's being fed. These all bring the flavor to the actual meat. While cultured meat is an artificially produced and flavored, not natural*." (a few times per week meat eater, library services, age group 18–20 years)
6	"*Not now as they are not suitable for consumption, but I think cultured meat could become the new food social norm when it becomes more natural and accessible for humans to adopt it*." (a few times per week meat eater, bookkeeper, age group 21–24 years)	"*I wouldn't eat stem cell based artificial meat from a lab. Animal stem cells, muscles are used to create a piece of meat. It's not normal. It's totally sick. It's really scary to think about, not even to consume it*." (a few times per week meat eater, office assistant, age group 18–20 years)
7	"*Lab-Grown Meat as meat substitute will become normal if it is culturally accepted as now it is more looked as an artificial, engineering creation*." (a few times per week meat eater, administrator, age group 21–24 years)	"*Chemically produced cell grown food can't be normal to consume. They can mimic the meat nutrition, but actually they are not as nutritious as meat. Marketers can say anything, but I am sure they serve someone's agenda*." (few times a week meat eater, business development officer, age group 21–24 years)
8	"*Now cultured meat seems strange, but with the time when engineers improve the prototypes, it can look and taste and bleed like a real meat. Engineers almost done it with the plant-based meat*." (a few times per week meat eater, case worker, age group 21–24 years)	"*I rather go vegetarian or vegan than eating cultured meat. It's not natural or normal….*" (a few times per week meat eater, finance officer, age group 21–24 years)

environmental impacts and contribution to climate change, was the most preferred answer (59%, $n = 135$), followed by specifically resource depletion (44%, $n = 101$). Other reasons are: health concerns (43%, $n = 97$), population growth (40%, $n = 90$), animal welfare (24%, $n = 55$), and fashion trend (22%, $n = 50$). These results confirm that Gen Z is concerned about the natural environment and is likely to adopt an eco-friendlier lifestyle (42).

DISCUSSION

Some of the findings from this exploratory study of adult Sydney Gen Z were expected and in line with previous research while others are unexpected. They all offer insights into this new generation and potential consumers of novel foods.

Sydney Gen Z's attitudes are very similar to the consumers' concerns in the West summarized by Bryant and Barnett (20) when it comes to the unnaturalness, safety, healthiness, anticipated taste and appearance of cultured meat. The residents of the largest Australian multicultural metropolis are used to a diversity of food options but the artificial and technology-based nature of cultured meat is making it difficult for the majority of them to accept this as a future choice in their diets. Some of their objections are grounded in the lack of sufficient information, but also in the almost instinctive feeling of disgust—a food-related emotion which plays a major role in building cognitive-affective linkages that guide behavior (43).

A 2020 study conducted in USA (44) shows a much higher acceptance of cultured meat amongst general consumers than what Sydney Gen Z indicates. Assuming that the price of cultured meat is the same as animal-based meat, 53% of the American consumers are prepared to make the substitution. Price considerations do not appear to be of any concern to Sydney's young adults but still only 28% of them consider cultured meat acceptable. Furthermore, 56% of the American consumers are prepared to substitute farmed meat for plant-based alternatives (44), while this percentage is lower at 35% for Sydney's Gen Z. These comparisons however should be treated with caution as the surveys conducted in the US and Australia use different descriptions of cultured meat, as well as different question formats, which can affect the responses.

Another study conducted in the USA shows that younger people in particular are more inclined to try cultured meat with

TABLE 12 | Cultured meat vs. alternatives according to Sydney Gen Z.

Pro cultured meat	Pro plant-based alternatives, against cultured meat and edible insects	Pro insects, against cultured meat	Against all meat alternatives, including cultured meat	Against all new alternatives in favor of traditional vegetarian diet
"*If we can master it and grow it out of bones, maybe cultured meat products will help us to change our attitude to animals and we can save the planet*." (a few times per week meat eater, project coordinator, age group 21–24 years)	"*…. Except plant-based, the rest of the alternatives, cultured meat especially and the insects to some extent, are a bit odd food to eat… I am keen to follow the plant-based trend, but not the cultured meat*." (daily meat eater, childcare educator, age group 21–24 years)	"*In the future, it will be necessary to eat edible insects because food and meat require lots of land to produce but I don't think that cultured meat will be something that people will like eating. It's too artificial and not a natural food like insects*." (a few times per week meat eater, coordinator, age group 21–24 years)	"*Not considering in vitro or plant-based meats as natural as they are chemically produced to imitate meat*" (a few times per week meat eater, councilor, age group 21–24 years)	"*It is necessary as we are consuming vast quantities of meat and this is harmful for both the environment and our health, but honestly I wouldn't eat any clean meat or other plant-based as they are too processed. I prefer becoming a vegetarian, but not eating these meat mimicking 'meats'*". (a few times per week meat eater, teachers' aid, age group 21–24 years)
"*One day when we no longer have space to farm animals like cows it may be necessary to consume new meat alternatives and in vitro meat. We should start preparing ourselves as these lab meats could be our only chance*." (a few times per week meat eater, IT analyst, age group 21–24 years)	"*Plant-based products to me sound more natural, the in vitro option does not seem natural.*" (daily meat eater, pharmacy assistant, age group 18–20 years)	"*You can't change our traditional diet with lab innovations. I rather eat bugs and other natural stuff than lab meat. Innovations can try to change the meat industry but can't easily change the consumers who prefer natural stuff.*" (daily meat eater, IT network, age group 21–24 years)	"*All plant- and lab-based meat alternatives are heavily processed to mimic meat. Maybe they are claimed good for the animals, for the environment, but how good lab meat could be if it is grown in an artificial way?*" (occasional meat eater, assistant, age group 18–20 years)	"*Why we should tolerate heavily processed stuff like lab meat and plant-based things to experience new food but not eating normal veggies, we know they are good? I don't get it*." (a few times per week meat eater, roster coordinator, age group 18–20 years)
"*Investigating new forms of protein like lab meat are a good option and alternative for the growing humanity.*" (a few times per week meat eater, assistant operation manager, age group 21–24 years)	"*Plant based alternatives are normal, but the rest – cultured meat and the crickets and larvae are totally sick*" (daily meat eater, digital analyst, age group 21–24 years)	"*Cultured meat is not appealing, I feel it is the worst option even compared to crickets and other bugs we could have for our future food security*." (non-meat eater, kitchen hand, age group 18–20 years)	"*Innovative solutions to replace meat with artificial meat and plant-based meats are not what our generation needs. We want clean, natural food*." (occasional meat eater, university student, age group 18–20 years)	"*I wouldn't eat cultured meat, crickets and larvae. I can't put them into my mouth. I will stick to pure veggies.*" (occasional meat eater, human resources trainee, age group 21–24 years)

51% of those aged between 18 and 29 willing to do so (45). This share is again much lower at 28% amongst the Sydney Gen Z. A study in Germany (46) shows that children and adolescents are more willing to consume a burger made of cultured meat than of insects but it also confirmed the negative influence of neophobia. Similar attitudes were also manifested among the participants in our survey; however the feeling of disgust was quite pronounced in Australia whilst missing in Germany.

Cultural dimensions related to perceptions about masculinity and Australia's pride in producing high-quality animal meat products, are adding additional weight to the objections Gen Z has against alternatives to farmed meat. Although they do not explicitly express any considerations specifically about farmers (20, 47) or concerns about how farmed meat is produced (48), the way others have done previously, Gen Z seems to value Australia's reputation about being a supplier of quality livestock-based meat.

In 2019, many Gen Z students and young people actively participated in the climate strikes in Sydney raising their voices against greenhouse gas emissions, continuing deforestation and biodiversity loss. The issue about meat consumption was not part of the activists' agenda despite ample scientific evidence about the livestock's high environmental footprint. It seems that Gen Z has not been exposed enough to reliable information about farming practices in Australia, such as the use of antibiotics in poultry farms and the ecological footprint of the Australian beef. Only 41% express environmental concerns associated with the way meat is being currently produced. It is therefore important to educate young people about the environmental impact of farmed meat production.

If cultured meat is to replace livestock-based proteins, it will have to emotionally and intellectually appeal to the Gen Z consumers. It may be through its physical appearance, but what seems to be more important is transparency about its environmental and other benefits.

This generation has vast information at its fingertips, but is still concerned that they will be left with the legacy of exploitative capitalism that benefits only a few at the expense of many. They have witnessed such behavior resulting in climate change and are now afraid that a similar scenario may develop in relation to food, particularly as investors are pursuing broader adoption (48). The conspiracy theory identified as a major concern in relation to edible insects (29), also rings alarm bells in the case of cultured meat. Conspiratorial ideation related

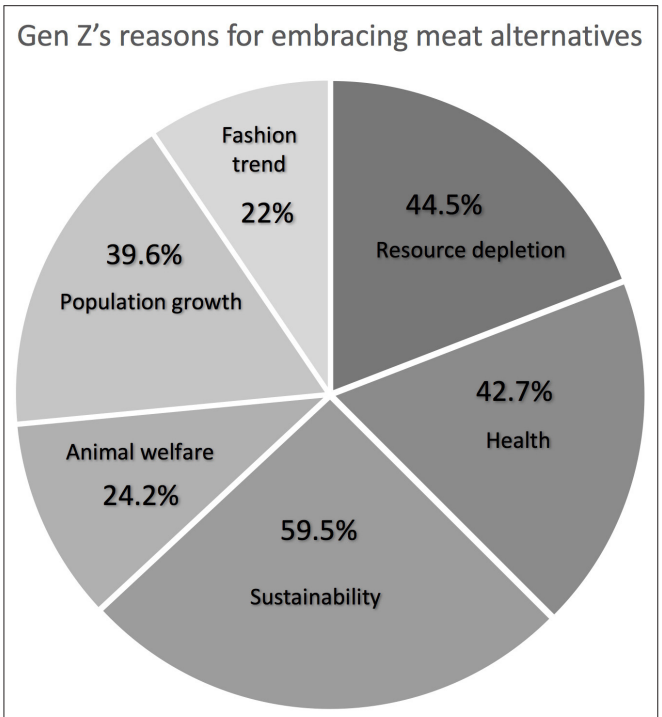


FIGURE 3 | Reasons for embracing meat alternatives by Sydney Gen Z.

to rejection of cultured meat was also identified in the study by Wilks et al. (49).

With the exception of 19% ($n = 43$), the remaining participants did not share proper conceptual knowledge about the way cultured meat is made. As explained by Madden (30, p. 193), this characteristic is a result of Gen Z being "brokers of information rather than knowers of content." They value the opportunity to access current information when needed, but not memorizing the content for future use. In this sense, it is likely that when cultured meat becomes available at the Australian market, Gen Z will start looking for answers in order to understand the "why" behind the "what" (30).

Devoted to their true technological nature some of the participants find fascinating the technological advancement of humanity to create *in-vitro* meat. They also see this as an opportunity to prevent further animal suffering and reduce the environmental impacts of livestock production. This generation appears to be opening the door to the miracles of technology for the achievement of broader societal benefits with 28% ($n = 64$) already accepting cultured meat. Focussing on the benefits of the final product, not the way it is produced (50), may increase acceptance.

Shaped with increased global awareness, Gen Z is particularly cautious about the impact of their product choices. What matters the most is the ethical side of production (30) and this was demonstrated through their concerns about animal dignity in the process of cultured meat. If the produced outcomes are environmentally and ethically good, it will be worth considering.

The participants indicated that they do not have a "fear of missing out" (30) when it comes to cultured meat. They do not prioritize concerns about establishing competitive advantages, the anticipated price of cultured meat or whether the process will be properly regulated and controlled. Living in a prosperous and stable economic environment with ample food availability, Sydney Gen Z values higher its freedom of choice, be it to opt for the traditional fruit and vegetables, than being the early innovators.

These young adults have also been exposed to the Covid-19 pandemic which is challenging human relationships with food from different angles (51). Large sections of natural habitat have been destroyed for the purpose of producing meat and growing animal feed, threatening further the survival chances of thousands of species. As our respondents indicated, many people are likely to seriously consider the environmental impacts of their food choices and opt for better options. Ironically, during the spread of the pandemic many abattoirs, meat and poultry plants in Australia and around the world became clusters for coronavirus cases (52). Cultured meat may soon be perceived as safer and not posing health risks, because it is produced in a virus-free sterile environment where no antibiotics are used. When faced with such choices, people may soon find the cultured meat decision more attractive.

While the future of cultured meat is still hanging in the balance, Gen Z consumers are similarly in the waiting. They are also open to be convinced. Being an exploratory qualitative study, it provides new insights about a demographic population that has not been studied previously and opens up opportunities for further statistically significant explorations.

CONCLUSION

According to Stephens et al. (7), whether cultured meat would really become clean, ethical and with a low environmental impact will depend on the efficiency of the production processes and the motivations of the companies and their funders. For the time being, Gen Z is not ready to embrace cultured meat with 72% finding it not acceptable. Emotional feelings of disgust dominate their individual attitudes toward the anticipated taste of cultured meat. Many are prepared to opt for plant-based meat alternatives and even the traditional fruit and vegetables instead. Australia's Gen Z is not concerned about the price, regulations or controls surrounding cultured meat, however some doubt the motivation behind investing in these technological developments. A major concern for Gen Z are the healthiness of cultured meat and its unnaturalness with 32% being of the opinion that it is not a healthy option.

Nevertheless, 28% of Gen Z are prepared to try cultured meat and some (25%) find it an option which can help with population growth. As 41% already acknowledge the need to switch to more sustainable food choices, further information related to the environmental implications, animal welfare and health impacts of farming livestock may sway these young people's opinion. Any persuasion however should be logical, with a clear purpose

and no hidden messages as Gen Z are independent thinkers, conscientious about their food choices and prefer to find the answers themselves. Being digitally savvy and highly connected, they will also have to address the challenges the previous generations have thrown at them, including climate change, environmental health and new emerging zoonotic diseases.

It is not surprising that Gen Z's value system is penetrating its food choices. As each generation makes its mark on the world, it may well be that Gen Z is the one putting cultured meat to the test and deciding its future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors on request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Curtin University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DB and DM conceptualized the study, analyzed the data, and contributed equally to all the sections in the article. DB conducted the survey. All authors made substantial contributions throughout all sections, read, and approved the final manuscript for publication.

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REVIEW
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Scale-Up Technologies for the Manufacture of Adherent Cells

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Great importance is being given to the impact our food supply chain and consumers' food habits are having on the environment, human health, and animal welfare. One of the latest developments aiming at positively changing the food ecosystem is represented by cultured meat. This form of cellular agriculture has the objective to generate slaughter-free meat products starting from the cultivation of few cells harvested from the animal tissue of interest. As a consequence, a large number of cells has to be generated at a reasonable cost. Just to give an idea of the scale, there were billions of cells just in a bite of the first cultured-meat burger. Thus, one of the major challenges faced by the scientists involved in this new ambitious and fascinating field, is how to efficiently scale-up cell manufacture. Considering the great potential presented by cultured meat, audiences from different backgrounds are very interested in this topic and eager to be informed of the challenges and possible solutions in this area. In light of this, we will provide an overview of the main existing bioprocessing technologies used to scale-up adherent cells at a small and large scale. Thus, giving a brief technical description of these bioprocesses, with the main associated advantages and disadvantages. Moreover, we will introduce an alternative solution we believe has the potential to revolutionize the way adherent cells are grown, helping cultured meat become a reality.

Keywords: cultured meat, scale-up cells manufacture, bioprocessing, continuous bioprocessing, adherent cell manufacture, bioreactors

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INTRODUCTION

In the last 20 years there have been considerable advances in disciplines such as biology and biotechnology each generating important breakthroughs in tissue engineering and regenerative medicine. As a result, considerable progress has been made in different fields leading to the development of multiple cell-based therapies, new and more effective biologics as well as improved approaches to regenerate damaged tissues. Moreover, this state-of-the-art knowledge fostered the development of new fields such as cultured meat (1, 2). Indeed, this form of alternative protein production relies upon applying and manipulating cutting edge technologies in cell culture, tissue engineering and bioprocessing to achieve the *in vitro* production of slaughter-free meat. In addition, this new but rapidly developing field demands a strong interdisciplinary effort spanning from molecular and cell biology to engineering.

Scientists working in the field of cultured meat are facing numerous challenges, largely the scale and type of problem depends upon the approach they are taking to generate their final products–lab grown meat (3, 4). One of the most critical decisions each manufacturer must make is which

scale-up bioprocessing approach they should take. As in other fields such as allogeneic cell therapy, there is the necessity to efficiently generate large numbers of cells (5, 6). For instance, production of cultured meat will require the producers to culture billions of cells (10^{12}-10^{13} cells to generate ∼10–100 kg of meat) while aiming at using limited space, time, and resources to keep the costs down (7). To give a general idea of the scale, to satisfy only 10% of the world meat consumption (∼30 × 10^6 t/y), we would need at least 2 × 10^6 m^3 bioreactor volume (corresponding to ∼200,000 × 100 m^3 bioreactors). Growing this number of cells is extremely challenging since scalability for adherent cells has never being proven at such high scale.

Thus, choosing the right scale-up process is essential not only to meet the required cell demand, but also to limit the costs of manufacturing. As an example, when Professor Mark Post took on the exceptional challenge and created the first "cultured burger," adherent cells were grown upon a surface made of thousands of layers of tissue culture plastic stacked on top of each other, ramping production costs to around €250,000 for that single burger (1). Indeed, this culture system has significant limitations in terms of scalability (currently limited to the production up to 10^{11} cells), with unfavorably low surface to volume ratio, as well as lacking control over pH, gas, and metabolite concentrations (8).

A major scale-up challenge is for those cells that are anchorage-dependent, commonly referred to adherent cells. These are the most common form of animal cell and are widely used in all fields (i.e., regenerative medicine, cell therapy, to produce biologics etc.), including the production of cultured meat (mesenchymal stem cells, muscle satellite cells, and induced pluripotent stem cells are just some examples) (1, 9). These cells need to adhere to a surface in order to remain viable and proliferate. Thus, for an efficient *in vitro* cell expansion system, there is an urgent need for improved bioprocesses which enable a more favorable surface to volume ratio, tighter control over critical growth parameters, better optimized dissociation from the growth surface and more efficient final cell harvest. In order to improve on the surface to volume ratio, two strategies are employed typically: (i) adapt the cells to grow as anchorage-independent (suspension) cells or (ii) use suspension culture systems (such as microcarriers) where cells are attached to and proliferate upon carriers that are constantly agitated to remain in suspension (**Figure 1**). Adapting adherent cells to grow as suspension cells is often laborious as it can take months to achieve and ultimately can often be unsuccessful as not all cells are capable of fully adjusting to this new growth condition (10). Moreover, if the adaptation step is successful, it remains important to closely monitor the system and regularly dissociate cell aggregates to prevent spontaneous differentiation and the formation of necrotic cores within the aggregates. On the other hand, more common is the use of suspension culture systems like microcarriers since they can be used in different bioprocesses and offer an adhesive surface whilst their mass is small enough to be suspended in the cell culture media under stirring (**Figure 1A**).

We are aware that there might be studies and strategies exploring the production of cultured meat using cells adapted to grow in suspension. However, bioprocesses to scale-up suspension cells are less challenging than for adherent cells as the need for specialized growth surfaces for the cells to adhere to is removed. Moreover, the footprint and the complexity of the cell collection step are reduced and are well-established within the industry (**Figure 1B**).

In light of this, within this review paper we have decided to focus on the manufacture of adherent cells highlighting existing and future technologies to their scale-up.

KEY PARAMETERS AND CONSIDERATIONS ON SCALE-UP

Before starting to list and technically describe all the different scale-up technologies, it is important to highlight what key parameters need to be considered when designing a bioprocess that aims to successfully manufacture a large number of adherent cells.

Availability of Key Elements

Oxygen, carbon dioxide and nutrients need to be added to the media in order to support cell growth within the expansion system (11). Oxygen can be added either in the form of aeration through sparges within the bioreactor, or upstream to ensure the media is saturated with dissolved oxygen. Bubble aeration through sparging is traditionally used to supply oxygen in large scale bioreactors, however alternative bubble-free aeration methods exist such as use of gas permeable silicone tubing for feed piping, or an external media aeration device. When the oxygen falls below the cell metabolism requirement level, the speed of respiration slows down, negatively impacting cell growth and consequently product quality (12).

Depending on the buffer used to maintain pH 7.2–7.4 within the bioreactor, provision and maintenance of carbon dioxide concentration may be required. In large-scale culture systems, high concentration of carbon dioxide (CO_2) is often considered undesirable (13). When CO_2 is above a certain level, cell growth can be inhibited, and the product quality compromised since cell-derived polysaccharides (N-glycans) can be affected due to disruption of the intracellular pH environment (14, 15). In light of this, sensors to monitor and feedback control systems on these key elements are critical.

Regarding the availability of nutrients, the most common strategy to feed the culture medium into the process is the fed-batch system. Fed-batch is an operational technique used in a variety of biotechnological processes where one or more nutrients are fed (supplied) to the bioreactor during the culture period and in which the product(s) remain in the bioreactor until the end of the run (16, 17). The culture medium is typically added through perfusion leading to less variation in nutrients and better cell yields (15). Perfusion of the culture medium allows for monitoring and control of the process conditions, which, as mentioned previously, is critical in the development of a reproducible manufacturing process.

Shear Stress

On one hand, the dynamic culture in bioreactors enhances nutrients transport and waste removal, but on the other it

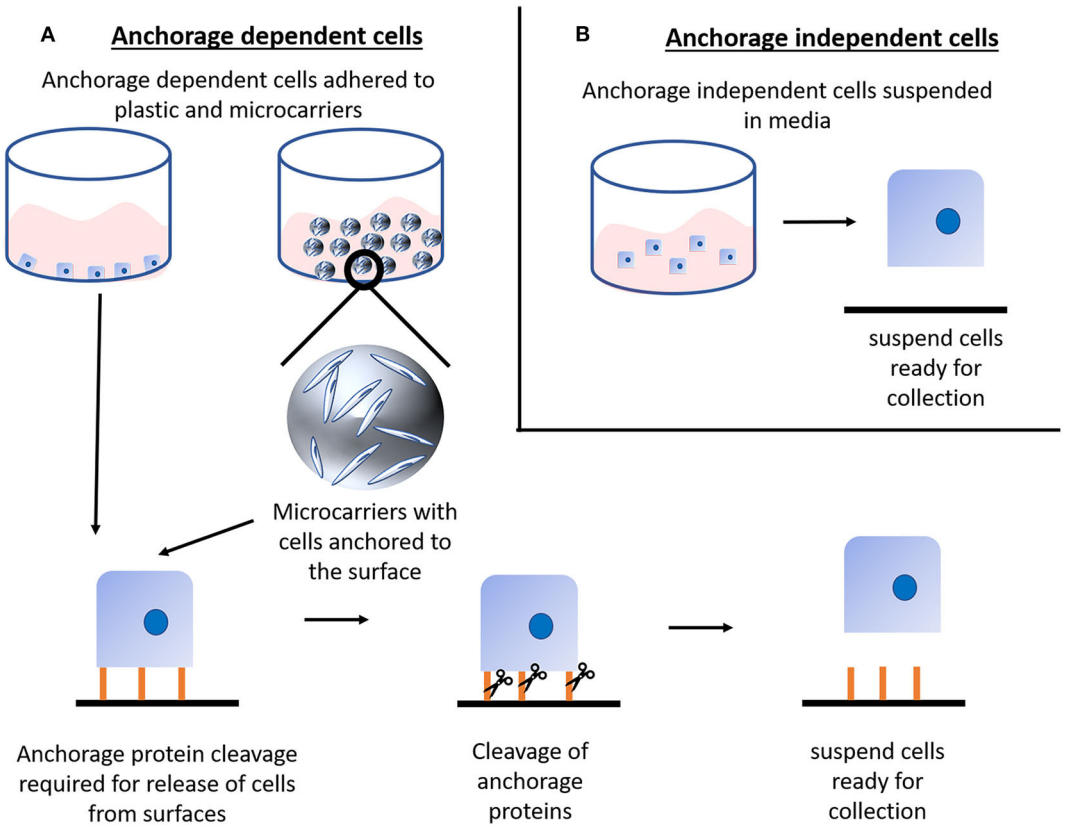

FIGURE 1 | Cell harvesting: **(A)** Anchorage dependent (adherent) cells require adherence to a surface for sustained and healthy culture, this anchorage can be to either common tissue culture plastics or to microcarriers. Although microcarriers themselves are in suspension it is important to note that the cells are still anchored to the microcarrier and thus require the same cleavage of anchorage proteins as cells anchored to tissue culture plastics. Classically, anchorage proteins are cleaved either via enzymatic or mechanical means. Such cleavage releases the cells from the surface and into suspension for collection. Anchorage dependent cells typically cannot survive long in suspension conditions, hence the requirement for batch cleavage. **(B)** Anchorage independent (suspension) cells do not require surface adherence to be viable and proliferate, thus they are readily available for collection and easily adaptable to bioprocessing.

is exposing the cells to increased fluid shear stresses (18, 19). Cells that are grown under these conditions respond to these external *stimuli* in different ways, depending on the cell type (19). Considering that bioreactors aim at recreating an *in vitro* environment that is very similar to the *in vivo* condition, shear stresses can be modulated *ad hoc* depending on the cell type and on the application (15). For instance, osteoblasts and mesenchymal stem cells (MSCs) have been shown to directly respond to shear stress (20). Indeed, mechanical stimulation through fluid shear stresses seems to promote bone differentiation and mineralization (21). However, there are cases when these forces impact negatively cell viability, growth, and cell behavior (22, 23). In this regard, the pharmaceutical and cell therapy industries have raised concerns and are still looking to minimize and optimize the stirring method to reduce the impact of fluid shear stress on the cells within the bioreactor.

Footprint

Regarding the equipment involved in scale-up processes, it is important to consider the physical space occupied by a certain machine, aka its footprint. The size, type and number of bioreactors will have an impact on the environment, overall costs, energy consumption, resources, handling, product quality, and reproducibility (24, 25). Large footprints are generally more associated with the expansion of adherent cells, since they are required to adhere to a substrate (26). Currently, the most common technologies aiming at reducing the footprint during the expansion of adherent cells are based on cultures using microcarriers-based and hollow-fiber bioreactors (that will be discussed in the following chapters).

Traditional and Intensified Processes

A traditional bioprocess consists of expanding an initial cell aliquot starting from a small vessel and then progressively increasing the vessels' size every time the cells reach confluency (**Figure 2** top). This process can take 3–4 weeks and requires frequent and multiple manual operations to generate a sufficient cell number to progress to the next stage. Tao et al. proposed an alternative system to both speed-up the time and reduce the number of steps during the scale-up process by producing high density (HD) cell banks (27) (**Figure 2** bottom). Traditional vials contain 1–4 million cells, whilst each of these cell banks generally

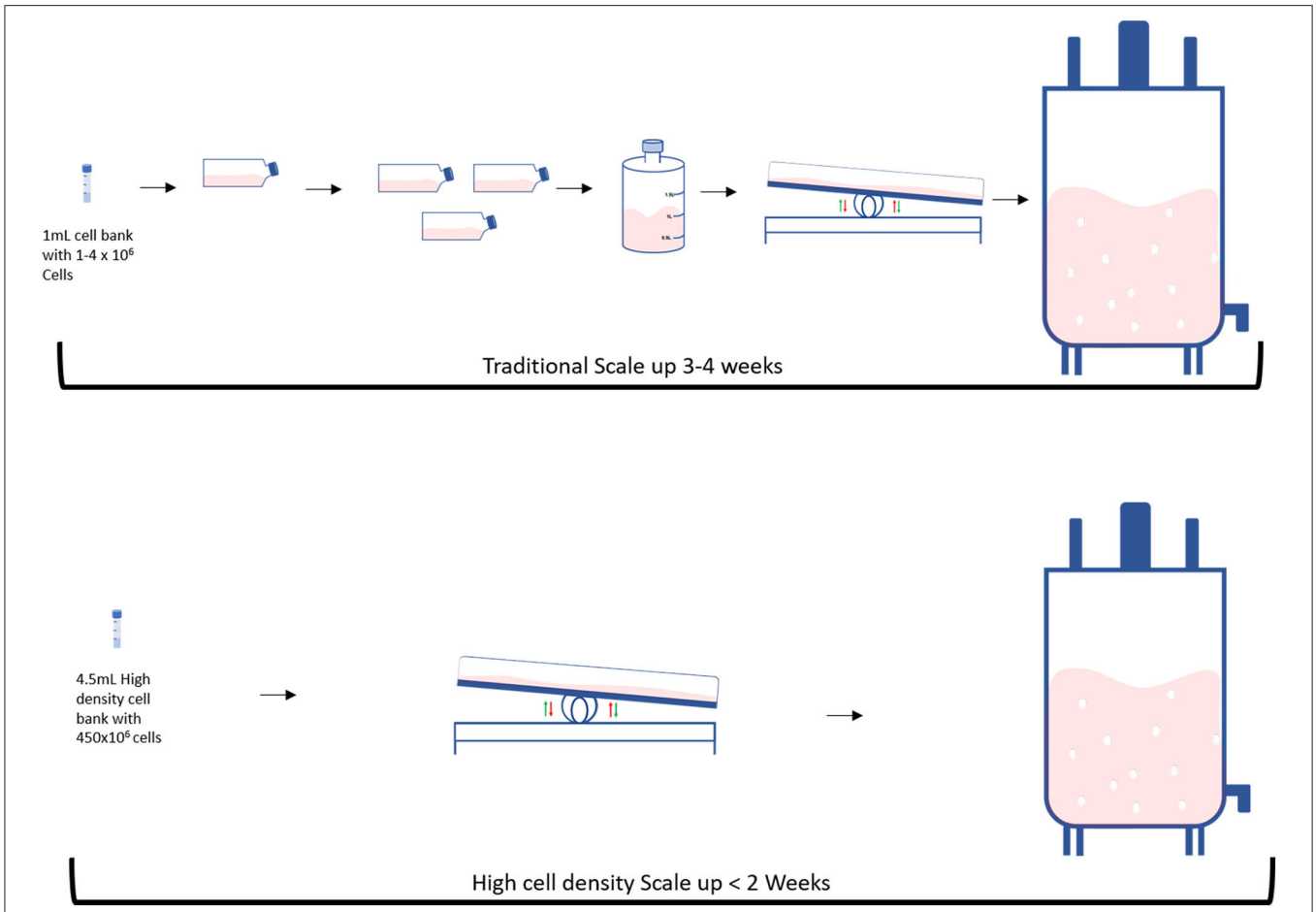

FIGURE 2 | Differences between traditional and High intensity scale up: **(Top)** Traditional and **(Bottom)** High intensity methods.

contain 450 million cells. Such HD cell bank vials are then used to inoculate several rocking motions (wave movement) bioreactors, eliminating several intermediate expansion steps in shake flasks (**Figure 3**). In this way, the manipulations in the laminar flow hood are significantly reduced, decreasing the associated labor and the potential risk of contaminations. This strategy is capable of reducing process time up to 9 days and improves operational success in seeding expansion steps.

Scaling-Up and Scaling-Out

In the biotechnological and bioprocessing industries, scale-up and scaling-out are two widely employed strategies to generate large numbers of cells. Scale-up systems progressively increase the surface area/culture volume as the cell number raises (28). Scale-out systems are based on the use of multiple culture vessels/bioreactors working in parallel (**Figure 4**). There are advantages and disadvantages associated with each approach. For instance, compared to scale-up processes, scaling-out can better deal with changes in product demand and improves process performance, however reproducibility can be difficult to achieve. Instead, scaling-up processes are more difficult to handle and control due to the high working volumes involved, but it can lower the costs of goods in the long term (28, 29).

Monitoring Systems

Bioreactor monitoring systems can be divided into three types: "offline," "at line," and "online." Offline monitoring can be defined as a manual operation consisting in removing a sample from a bioreactor and processing it in the laboratory. The at line system differs from the offline in that the sample, despite being removed from the bioreactor, is being tested right next to it. However, an online system provides the opportunity to test samples both *in situ* and *ex situ*. In the *in situ* system, an in line analyser tests the sample and then returns it back into the bioreactor; while in the *ex situ* approach the sample does not return to the bio-analyser after been measured (30). While in line analytical methods to monitor the pH, dissolved oxygen and temperature are already available, other parameters like the substrate density are still being measured offline through laborious and error prone methods (31). An example in which components of a bioreactor can be monitored offline, with the help of biomass separation methods, is the High-Performance Liquid Chromatography (HPLC) system. The

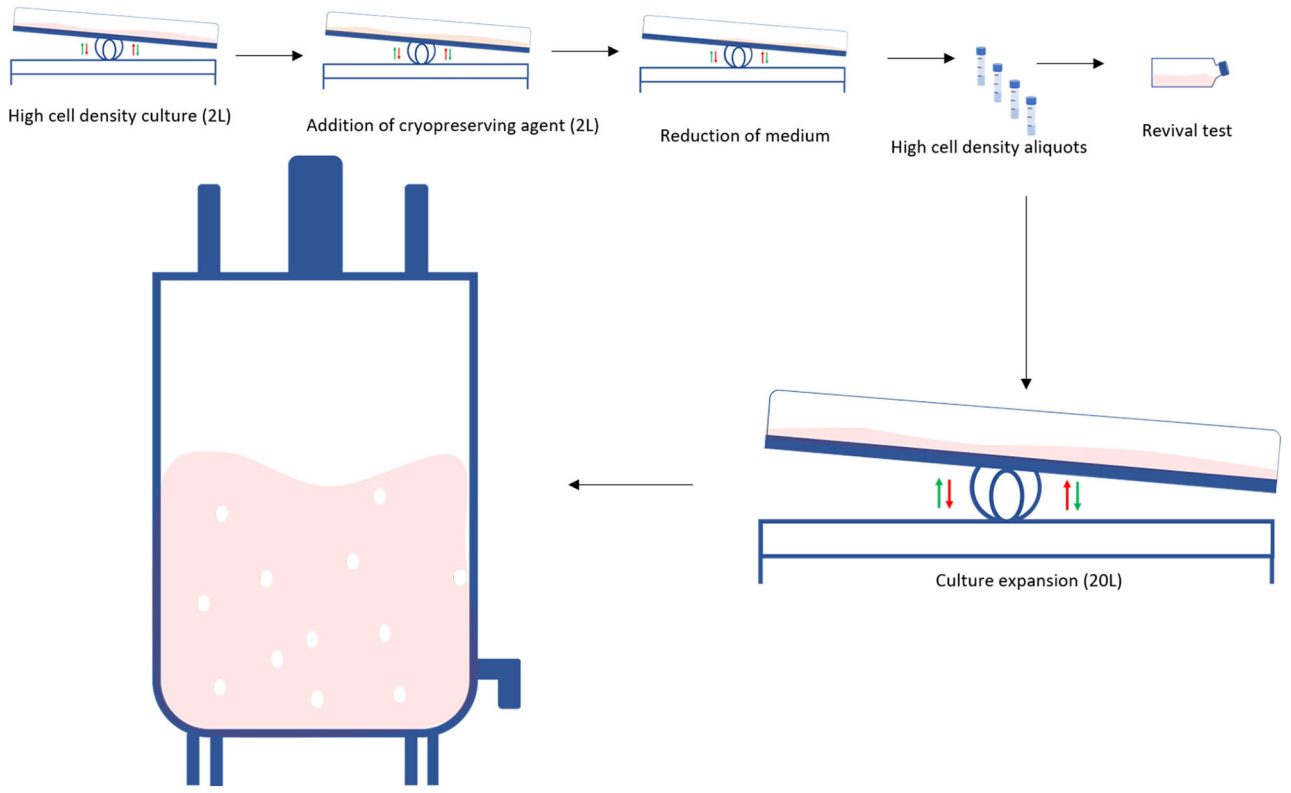

FIGURE 3 | Obtaining a high-density cell bank: High density cell banks are used to reduce the required steps in the traditional scale-up process to generate larger numbers of cells. Initially, cells are grown at a high cell density in a perfusion bioreactor, a cryopreservation is added to the culture and the volume is reduced. Cells are then banked as high-density aliquots in cryovials. When required, a test vial is revived. If the revival is successful, the high-density aliquots are seeded into a perfusion bioreactor which is subsequently seeded into a larger bioreactor.

advantage of using HPLC is that components of the media will be separated by adsorption, liquid-liquid interaction, or affinity separation. The downside of using manual sampling methods is time consumption as well as not being able to test the samples in real time (32). Monitoring a bioreactor in real time is of major importance as it can lead to higher efficiency, productivity, product quality and overall cost reduction (33). For instance, cell density and viability are two of the most critical factors for a bioprocess and they should be measured in real time. Instruments facilitating these measurements are based on optical density, fluorescence or conductivity and are providing online measurements which subsequently will be verified using offline methods such as microscopy (34). At-line monitoring of substrate and reagents density can be performed using optical sensors, ultrasound sensors, UV-Vis, fluorescence and RAMAN spectroscopy (31). RAMAN and near infra-red (NIR) spectroscopic methods are popular in the pharmaceutical industry and are based on the interaction between light and matter. Both RAMAN and NIR are non-invasive methods that can provide useful information about cell culture bioprocesses albeit the interpretation of the spectra is complex and needs chemotactic and multivariate method (35). Near Infra-Red spectroscopy (NIR) is a popular method for in line bioprocesses measurements and combined with multivariate data analysis provides the opportunity to perform a real time measurement of a number of parameters (36). The information regarding the spectra is obtained using an FTIR spectroscope, acquiring the data with a probe that is inserted into the bioreactor system (37). NIR advantages include easy maintenance, being non-invasive and the identification of multiple analytes in the media. However, FTIR probes are expensive and the immersion of probes into the bioreactor broth requires thorough sterilization (31). Finally, it is worth mentioning *in situ* microscopy, this is capable of taking images of cells from inside a bioreactor without the need to take the sample out (38) since the field of view is fixed (34). Overall, despite the increasing scale of bioreactors, traditional monitoring methods are still in use, suggesting the need in implementing more reliable, automated and real-time systems (35).

SMALL SCALE TECHNOLOGIES–OR COMPACT TECHNOLOGIES

In this section we will describe four of the most commonly used devices to scale-up adherent cells at bench scale. An overview of the main technical characteristics and relative advantages/disadvantages is also presented in **Table 1**.

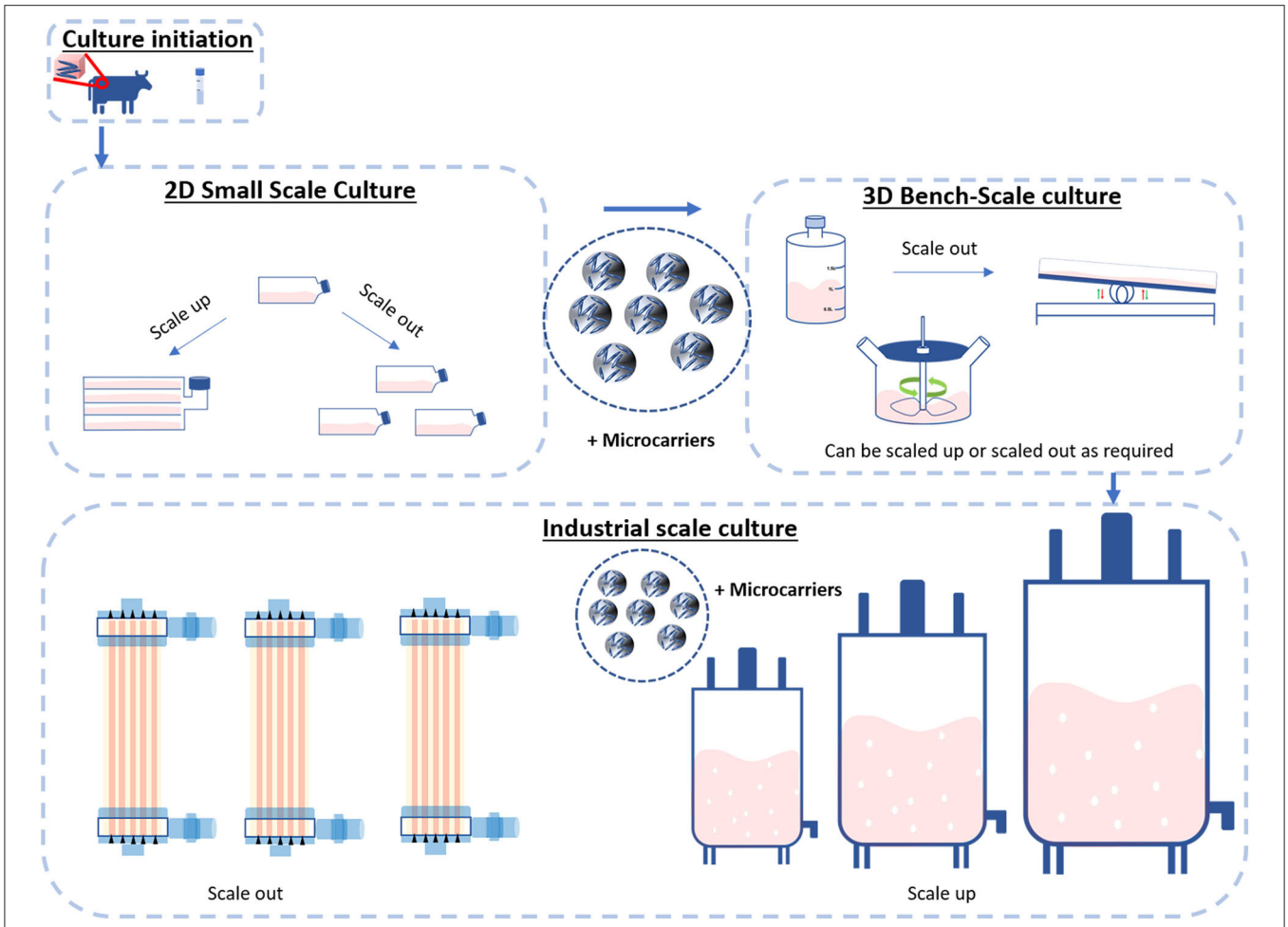

FIGURE 4 | Scale-up and Scale-out. Cultures are typically initiated from cryopreserved stocks or biopsies and cultivated in small-scale cultures such as flasks. These flasks can be scaled up with the use of microcarriers or scaled out with the use of hyper or multilayered flasks. The process then enters the bench scale stage. Here, the use of larger vessels such as roller bottles, perfusion bioreactors and spinner flasks can be deployed to both scale-up and scale-out the process. When required, the process then enters the industrial scale. This stage of bioprocessing enters two distinct streams: bioreactors scale-out (multiple bioreactors of the same size) or scale-up (further processing up to a single bioreactor).

T-Flasks

T-flasks are the most commonly used plastic consumables for early stage cell expansion, usually when growing cells starting from a cryovial (**Figure 5A**). T-flasks vary in size, ranging from a culture area of 12.5–225 cm^2 and are made of disposable plasma treated polystyrene, or tissue culture plastic (TCP) (39, 40). While conveniently economical, these flasks are labor-intensive and become cost-inefficient when expanding cells beyond bench scale, mainly because of their high footprint.

Multi-Layered Flasks

They are large T-flasks composed of stacked flat surfaces (**Figure 5B**). The aim is to increase the available surface by incorporating a multi-tray unit reaching a total area that depends on the number of layers, but generally reaching up to 2.5 m^2 (39, 41). This type of flask must be treated as an individual unit with the cells from each layer to be seeded, cultured and detached at the same time. Although being a useful device for scaling-up at bench scale, there are concerns regarding the cell quality and the associated labor intensity. For instance, there might be a heterogeneous availability and distribution of nutrients and gasses between the different layers of the flask (41). Moreover, simple operations like cell seeding, media change and cell detachment/harvest become challenging due to their size and weight. In this respect, system automation would greatly improve these day-to-day operations.

Roller Bottles

These bottles consist of cylindrical vessels to grow cells in a dynamic system (**Figure 5C**). They are usually placed in a heated environment on a rack that slowly revolves (ranging between 5 and 240 revolutions/hour). They are inexpensive and are a common method used for the initial scale-up of adherent cells (42). The cells attach and cover the inner surface of the bottle;

TABLE 1 | Table summarizing the advantages and limitations of the described small-scale systems.

Device	Surface area (cm^2)	Cell densities (ml^{-1})	Features	Disadvantages
T-Flask	25–225	1×10^5	• Low cost • Easy to use • Easy cell adaption • No special extra equipment required	• Scaling-out can be labor intensive • Inconsistency
Multi-layer flask	525–18,000	1×10^5	• Increased surface area compared to T-Flask • All layers are passaged at once	• Increasing in layers numbers impact in the difficult to handle it • Heterogeneity within different layers
Roller bottle	850–1,700	1×10^5	• Rotation provides better distribution of nutrients and oxygen • Require less media compared to planar flasks	• Scaling-out can be labor intensive • Require extra setup to roll bottles.
Spinner flasks associated with microcarriers	380/g	1.7×10^6	• Improved surface and cell yield • Homogeneous mass transfer • Relatively low-cost • Optimization prior to industrial scale-up in suspension	• Shear stress can be harmful to cells • Require optimization with cell line and microcarriers

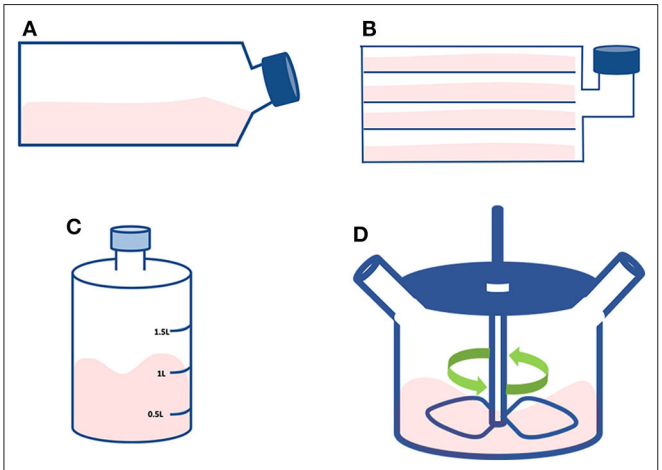

FIGURE 5 | Small scale technologies. Schematic representing the discussed compact technologies: **(A)** T-flask; **(B)** Multi-layered flask; **(C)** Roller bottle; and **(D)** Spinner flask.

hence the cells are cyclically bathing in culture medium and exposed to gases. In addition, the rotation provides a level of mixing, preventing gradients from forming within the medium that may affect cell growth. In this system, the cells are most of the time covered by a thin layer of medium, thus facilitating superior gas exchange (18). The surface available for cell expansion is between 500 and 1,700 cm^2, in a total volume ranging 1 to 1.5 L, suitable for culture volumes of 0.1–0.3 L (39). Like static flasks, rotating flasks are also labor intensive. For high cell numbers, a further constraint of a roller bottle process through scaling-out is the limitation in the control of O_2 and CO_2 in both the gas and the liquid phase of culture (39, 42).

Spinner Flasks

These devices are flat-bottom flasks commonly used at a bench-scale for stirred suspension cultures that can be used to initially validate microcarriers and media composition (43) (**Figure 5D**). The culture is maintained in suspension and the stirring is achieved by a magnetic stir bar, also called magnetic driven impeller (44). The media is inoculated with cells to fill the flask with a volume of 100–200 ml at a stirring speed of 50 rpm (45). Compared to the solutions mentioned earlier, spinner flasks can generate high cell numbers, provide a better aeration system, a more homogeneous nutrient supply, longer culture period and reduced costs. Microcarriers can be added to spinner flasks mainly to do preliminary tests before moving to larger bioreactors (7). Microcarriers are small spheres with a diameter ranging between 90 and 300 μm and available in different sizes, materials, coatings, and surface charges (46–48). Different sizes and materials impact on the microcarriers seeding density and cell harvesting methods (48). Cell adhesion treatments can enhance cell attachment and promote cell spreading (49). As the choice of the microcarrier depends on the cell type, product, and operational set-up, it is highly recommended to run preliminary tests with different microcarriers (48, 50, 51).

The critical following steps are: (i) cell dissociation from the carriers and (ii) harvesting cells from the media (7, 49). Many studies have reported challenges in efficiently detaching the cells from the carriers using classic enzymatic methods (7, 49, 52). To mitigate this problem, current solutions include coatings with thermo-responsive polymers (e.g., pNIPAAm) (53), degradable (e.g., made of PGA) (54) and edible (e.g., made of alginate or chitosan) microcarriers (7).

LARGE-SCALE BIOREACTORS–OR INDUSTRIAL SCALE

In this Chapter we will describe four of the most commonly used devices to scale-up adherent cells at industrial scale. An overview of the main technical characteristics and relative advantages/disadvantages is also presented in **Table 2**.

TABLE 2 | Table summarizing the advantages and limitations of the described large-scale systems.

Device	Max Capacity[a]	Cell density (ml^{-1})	Features	Disadvantages
Wave bioreactor (associated with microcarriers)	20 L/0.02 m^3	2×10^6	• Tool for intensified scaling-up • Low shear stress • Operation in different batch modes	• Scale-up to >100 L is challenging • Large space is needed
Stirred tank (associated with microcarriers)	2,000 L/2 m^3	2×10^6	• Easy of scaling up from benchtop to factory • Bioprocessing is well-understood • Flexible and automatic platform for very high-volume bioprocess	• Require optimization with cell line and microcarriers • Large volumes required • High shear stress
Packed bed	500 m^2/ 0.03m^3	3×10^6	• High density cell culture due to large surface available • Operation in different batch modes • Cell passage less frequent	• Packing material difficult cell harvest • Concentration of gradients
Hollow fiber bioreactor[b]	150 cm^2/ml^{-1} 0.00007 m^3	1×10^9	• Increased surface to volume ratio • *In vivo*-like tissue structure (blood vessels)	• Difficult to harvest cells • Concentration of gradients

[a] *Commercially available.*
[b] *Variable maximum capacity, as various cartridges can be connected in parallel.*

Rocking Motion Bioreactors

This type of reactor utilizes the wave motion of culture medium generated by a rocking platform to provide a cell-beads (microcarrier) suspension (**Figure 6A**). The beads are placed inside a disposable bag with ports allowing for air circulation and bag inflation (55). The disposable bag system has advantages for clinical applications in terms of safety providing the ultimate ease in operation and protection against cross-contaminations (55, 56). The chamber is placed on a special rocking platform causing low/negligible shear stress to the cells (55, 57). The agitation provides proper mixing and mass transfer while the circulating air provides the necessary oxygen exchange (57). Of note, new rocking motion bioreactors models have a higher mass transfer than the standard wave type bioreactors while inducting a relatively low shear stress. It is possible to connect culture medium bags for perfusion via additional ports and it can operate via batch, fed-batch, repeated fed-batch, and perfusion mode (12). This setting facilitates scale-up and automation, which has been demonstrated for culture volumes up to 500 L (58). Such a system is widely used for the expansion of mammalian cells, for example embryonic feline lung fibroblasts (59), neutrophils from HSCs (58), and T cells (60). Considering that these reactors allow high cell yields, they are the platform of choice when expanding High Density cell banks obtained from intensified processes (27).

Stirred Tank Bioreactors

Giving the existing broad knowledge on stirred systems, stirred tank reactors are possibly the most used system for large-scale culture of mammalian cells (15, 61) (**Figure 6B**). They apply the same operational principles as the spinner flask (agitation in a tank via an impeller), just in much larger volumes which can reach up to 2,000 L in a single vessel (62). The impeller keeps the solution in agitation to maintain the particles (i.e., organoids, suspension cells, or microcarriers) in suspension whilst homogenizing the distribution of oxygen, nutrients, and heat (63). The tank provides a closed and automated platform and can operate in different modes, such as batch, fed-batch, and perfusion (15). Considering that it is a suspension culture system, it offers the typical advantages of optimized footprint. When it is used to grow cells attached to microcarriers, it can provide *in situ* assistance in dissociating the cells from the carriers when cells reach confluency (18, 52). The strategy is based on coupling the addition of trypsin with intense agitation. The generated shear stress improves the cell detachment efficiency, thus increasing the final yield. In this particular case, fluid dynamics tells us that this brief and intense shear stress does not damage the cells because the detached cells are smaller than the Kolmogorov scale of turbulence (52). Industrial stirred tank bioreactors are available as single-use, however they are traditionally made of stainless steel (cGMP material) considering it is easy to clean, well-compatible with biologics and highly resistant to pressure and erosion (11).

Packed Bed Bioreactors

Also called fixed bed, they consist of a hollow tube packed at the bottom with immobilized surfaces such as scaffolds, microcarriers or porous fibers (**Figure 6C**). The cells are seeded on the fixed bed while fresh media is continuously circulating within the system transferring oxygen and supplying nutrients, whilst providing a large surface to volume ratio for cell attachment and expansion (18, 64). This impacts cell passaging: due to the high surface area, cells can be passaged less often, thereby there are savings on costs of culture media and operations (57). They are commercially available at cGMP bench scale (up to 4 m^2) and for industrial scale manufacturing (up to 500 m^2) (18, 57). High cell densities of 5.1×10^8 cells/mL have been reported with packed bed bioreactors (64). Very early progenitor cells (CFU-GEMM) were expanded up to 4.2-fold while later progenitor cells (CFU-GM and BFU-E) exhibited up

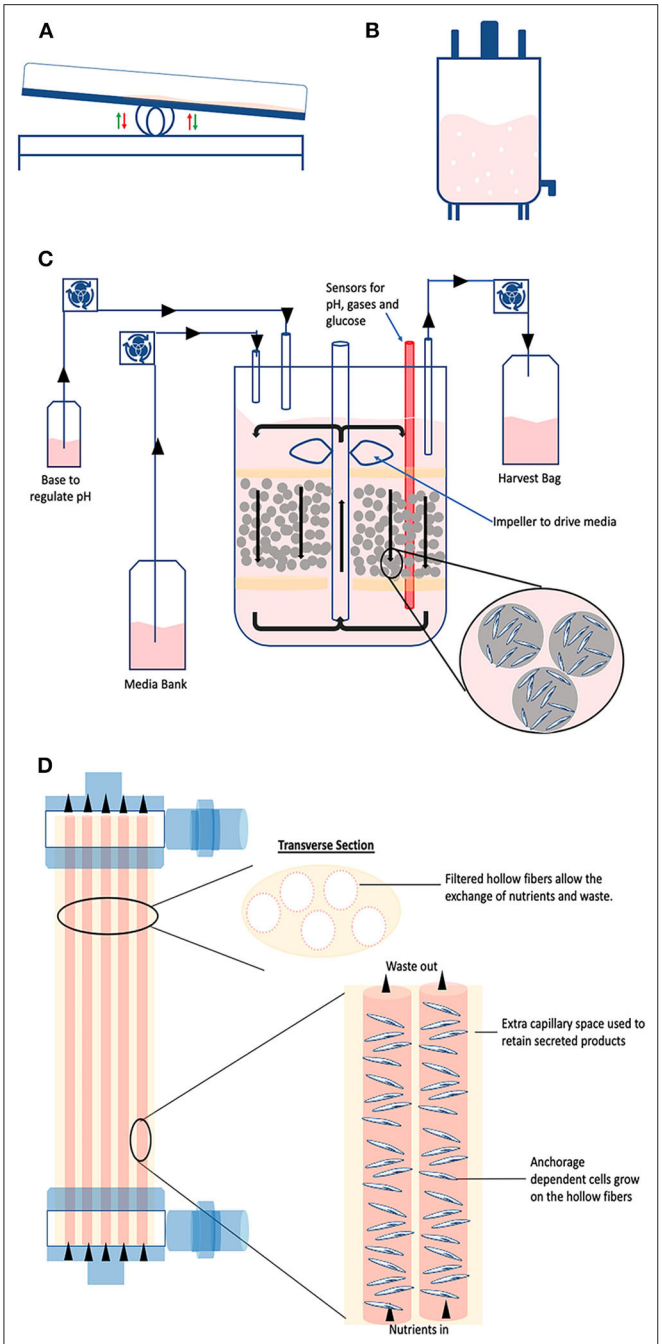

FIGURE 6 | Large scale technologies. Schematic representing the discussed industrial technologies: **(A)** Wave; **(B)** Stirred tank; **(C)** Packed bed; and **(D)** Hollow fiber bioreactors.

to seven-fold and 1.8-fold expansion, respectively (65). Moreover, an average seven-fold expansion of MSC was reported with a starting cell density of 6.0×10^7 cells, after 7 days of culture (66). Additionally, the perfusion operation offers the monitoring and control of the process conditions (18). It has to be noted that the structure of the reactor does introduce a risk of formation of an axial and radial concentration of gradients, especially at a large-scale (18). Cell harvesting can also be problematic due to the presence of high cell densities and the difficulty of effectively introducing the detachment supplements into the culture (18).

Hollow Fiber Bioreactors

They consist of a cylindrical chamber stacked with semi-permeable hollow fibers (**Figure 6D**). Cells can be inoculated both within the fibers and on the extracapillary surfaces, permitting high cell densities in the order 1.0×10^9 cells/mL (67, 68). The fibers mimic blood vessels in coordinating nutrient supply and removal of waste while oxygen exchange is managed by diffusion between intra-capillary and extra-capillary spaces (57, 67). The culture medium can flow through the fiber or chamber or both using proper channels and ports. Depending on the inoculation method, pore size for the semi-permeable membrane can be chosen to determine which particles shall pass through or retained by the membrane. For instance, if the cells are inoculated in the intracapillary surface, then the media is perfused from outside or extra-capillary space. This flow operation is known as intra-capillary inoculation with extra-capillary perfusion (69). Due to its perfusion nature, it allows automated monitoring and control of metabolites concentration which is important in maintaining process consistency (18). However, there is the potential dissociation of longitudinal concentration gradients as culture medium or dissociation reagent flows down the fibers, meaning the nutrients distribution can be inconsistent along the hollow fibers (67). Strategies to overcome these limitations include the use of oxygen carriers that increase the flow rates and/or rotate the hollow-fiber bioreactor in timed cycles to reduce oxygen gradients (70). Hollow fiber bioreactors have been employed to expand MSCs and human umbilical cord derived HSCs. The culture was carried out with seeding densities of 800,000 cells/ml to demonstrate a semi-continuous production model with up to 14,288-fold expansion while maintaining pluripotency markers (69, 71). In order to increase cell production, it is also possible to connect various units in parallel (scale-out) (72).

CONTINUOUS CELL CULTURE AS AN ALTERNATIVE APPROACH

More than 30 years ago, the concept of continuous bioprocessing was introduced as an alternative to batch production; in general terms it means creating a continuous process of turning flowing raw material into intermediate or final products (73). A broad range of industries have adopted continuous processes, spanning chemical and oil refineries to food and life sciences (74–76). The reason for this implementation relates to the proven benefits continuous processing brings to reducing process cycle times, materials and energy used as well as waste production (77). Recently, the biopharmaceutical industry, with the rise of cell based therapies, had to become more competitive in terms of bioprocessing to reduce the costs of manufacturing (78). For this reason, great efforts have been made in implementing continuous platforms to achieve better efficiency and become more cost

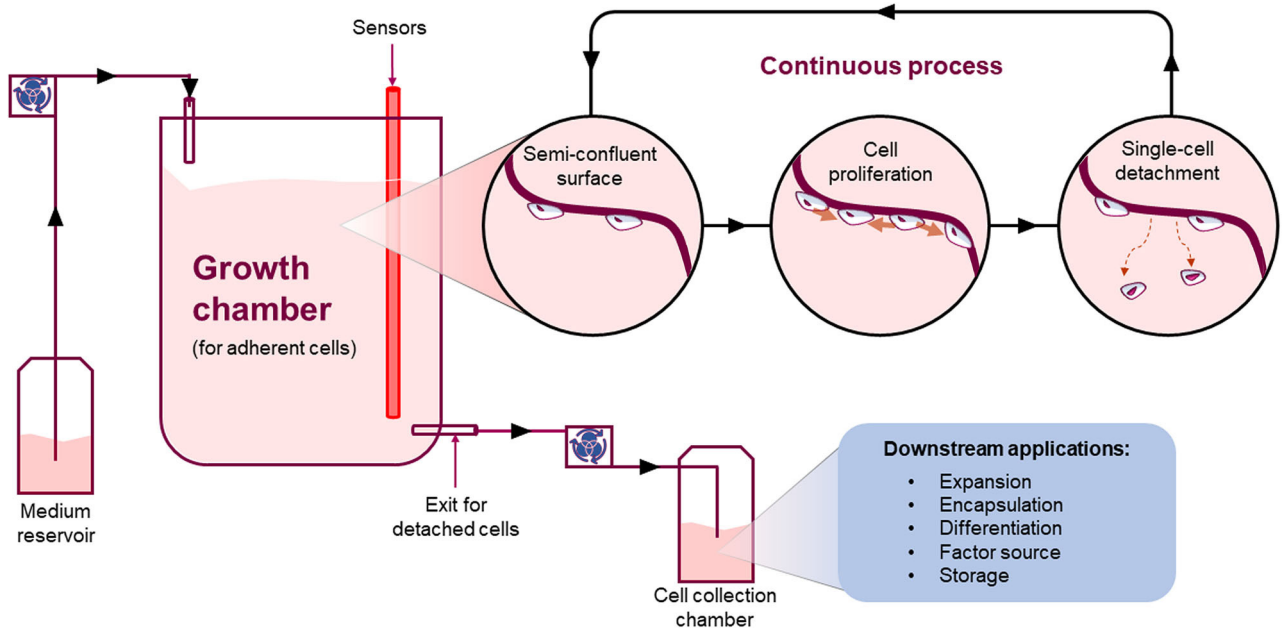

FIGURE 7 | *Continuous process.* Schematic showing an example of how a continuous process for the manufacture of adherent cells could work. Such system will allow adherent cells to grow onto a surface and detach continuously as single-cells at a steady-state. The detaching cells will leave an empty space for neighboring cells to grow into maintaining a stable number of cells in culture whilst continuously harvesting single cells from the system.

effective (79). Focusing on this sector, a more efficient *in vitro* cells expansion at large scale is still demanded for adherent cells.

The way of culturing adherent cells has not changed in the last 50 years. The only approach taken relies on incrementally increasing the surface available for the cells to grow in the lowest volume of culture media. In other words, to accommodate as many cells as possible using the smallest volume of media. Thus, when scaling-up the manufacture of adherent cells at industrial scale, the bioreactors footprint still represents a hurdle due to the difficulties in operating and monitoring high-volumes tanks. Such approaches led to the use of cells-bead surfaces that are stirred in suspension (as we mentioned above via using microcarriers and packed bed reactors). However, the challenges associated with cell-detachment and the subsequent harvest, are still not resolved.

Moreover, forecasts on the future demand of adherent cells to be manufactured seem to go far beyond the current capabilities of established platforms. In particular, the demand for adherent cells for industries such as allogeneic cell therapies and cultured meat will increase exponentially over a relatively short period of time; meaning there is a pressing need for new and innovative enabling technologies.

An alternative approach is to develop a new bioreactor that allows the continuous manufacture of adherent cells, based on the well-known benefits of continuous bioprocessing compared to batches (**Figure 7**) (77). In order to do that, the critical steps are: (i) how to detach single-cells, (ii) how to maintain the system in equilibrium between detachment and proliferation (steady-state), and (iii) how to collect cells continuously.

Importantly, a continuous system has the potential to provide several advantages compared to current batch systems such as: reduction of footprint and resources, overall lower production costs, increase product quality, reproducibility and yield over time implementing a closed and automated system. For instance, a recent paper showed proof-of-concept data suggesting that an area of just 155 cm^2 (like a medium size tissue culture flask) can generate over 1 million of cells every 24 h (76). Thus, such a small area could generate ∼100 million cells when working continuously for 3 months. Additionally, a continuous system applied to adherent cell manufacture could facilitate true single-cell real time QA. In such a system, single cells are continuously detaching and moving under-flow from the area where they were growing to the next downstream process. A checkpoint could be inserted allowing a decision to progress the cell to the next downstream process or be discarded accordingly. This could be applied to each and every cell manufactured. In turn, this new way of manufacturing cells can either change the following downstream processes or drive the development of a collection system can fit with current downstream processes. Either way, it will be a critical step that has to be thoroughly considered and planned before being implemented.

In general, the benefits of continuous systems over batches are well-known and proven by different industries, and there is no reason, at this time, why they could not be applied for this specific application. However, like any new technology or process, there will be challenges and learnings but there is always the opportunity to advance and improve.

COMPUTATIONAL FLUID DYNAMICS MODELING FOR SCALING-UP CELL PRODUCTION

Computer models have been employed to run algorithms and equations to predict the behavior of, or the outcome of many natural systems. Numerical simulations have become a resourceful tool not only for predictions, but also to accelerate the development of systems and devices in both natural and human systems (80). In particular, computational fluid dynamics (CFD) is a branch of fluid mechanics that uses numerical analysis and data structures to analyze and solve problems that involve fluid flows. Computers are used to perform the calculations required to simulate the free-stream flow of the fluid, and the interaction of the fluid (liquids and gases) with surfaces defined by boundary conditions.

Since design, construction, and evaluation of bioreactors for large-scale production is costly and time consuming, computational methods may give some insights into the fluid mechanics within bioreactors. Thus, critical limiting factors, such as insufficient mixing as well as inhomogeneous nutrient and oxygen mass transfer, may be identified early in the process design (81). CFD analysis can provide details of fluid velocities, pressures, solute or particle concentrations, temperatures, stresses, and heat/mass fluxes throughout the flow domain (67, 82). These are all important parameters to design bioreactors and scaling-up strategies. Specifically for bioreactor design, CFD is a resourceful tool to address important questions and investigate optimal parameters such as reactor type and dimensions, gas spargers design, foaming/foam control, hydrodynamic stability, mass transfer capacity, mixing, dissolved oxygen concentration/distribution as well as controversial topics such as bubble-induced cell damage (15, 67). In addition, process critical fluid flow parameters, which are hard or even impossible to measure, can be predicted by CFD (83). For instance, in the case of shear sensitive cells, the power input has to be found optimal to generate sufficient mass transfer without causing critical shear stress levels that can ultimately damage the cells (81).

CFD simulations of bubble columns, air-lift reactors or stirred tanks have been part of the work routine among chemical engineers (67). CFD analysis has been used to predict the flow behavior inside capillaries in ultra-filtration devices (84), which can be applicable for cell separation and/or cell concentration purposes (67). CFD has been also used as tool to help to understand bubbles coalescence (bubble burst), caused by gases mass transfer in the bioreactor environment, which has been a controversial subject in bioprocessing for decades (81, 83).

CFD has been widely performed for stirred-tank bioreactors at various volume scales (85, 86). Besides the classical bioreactors made of glass or stainless steel, the fluid flows in small (87), bench top (88), and pilot scale (89), helping to identify, for example, death zones or stagnant zones, where fluid flows very slowly or does not flow at all (90), impacting in the mass transfer and in the final product viability. Li et al. explored the CFD model to estimate the mass transfer and mixing performance of a reactor to scale-up cell production for cultured meat applications (91). The same approach is already widely used for the design and manufacture of several medical devices and is well-suited for conducting optimization studies to evaluate far more design alternatives than the build and test method, impacting in the reduction of design cycle time (15, 67).

We could say that CFD is a "weather forecast" for bioprocessing engineers assisting them to predict *a priori* the behavior of adherent cells growing within bioreactors. Successful scale-up of bioprocesses requires that laboratory-scale performance is equally achieved during large-scale production to meet economic constraints (92). Most importantly, CFD can reduce time- and cost-intensive trial-and-error experiments, which is especially important if the availability of the biological material is limited (i.e., primary tissues or stem cells). When the main engineering parameters, such as power input, mixing time, and (oxygen) mass transfer coefficient, are simulated and predicted, it is possible to optimize cell growth and productivity, whilst maintaining high product quality (81).

CONCLUDING REMARKS

There are a considerable number of technologies available to scale-up cell manufacture. These have been developed mainly to be used by the biotech and pharma industries. At the moment, there are no commercially available bioreactors that are designed *ad hoc* for cultured meat applications. Thus, it is likely that two different strategies are adopted by groups working in the cultured meat field: (1) try to adapt their cell manufacture process around existing batch technologies; (2) develop manufacturing platforms in house, that are very specific for their needs. It has to be considered that this field will have to generate cell numbers that are possibly the highest among all existing industries (1). However, the question remains as to whether current technologies will be capable of meeting such considerable cell demand. We believe, based upon current commercially available technologies, that batch processes will not be capable of generating the required number of adherent cells in an efficient way. Moreover, we believe that a drastic change in the way we have been growing and manufacturing these types of cells must happen, developing new systems bringing, for instance, the well-known advantages of continuous bioprocessing into play.

Great ideas and honorable goals in this field need to be coupled with new and highly innovative enabling technologies to support them. The great challenge of efficiently producing cultured meat products at scale, gives the possibility to develop new concepts and bioprocesses that did not exist before, driving innovation across multiple disciplines along with it. We believe that continuous cell manufacture could be one of these new concepts helping cultured meat companies achieving their goal. But surely, more are yet to come to drive innovation even further.

AUTHOR CONTRIBUTIONS

CB, JA, and LD wrote the manuscript. MM and LG edited and provided intellectual contribution to the manuscript. CC edited, provided intellectual contribution, and approved for publication. All authors contributed to the article and approved the submitted version.

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