The status of cultured meat and scientific challenges

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Abstract

Cellular agriculture is an emerging branch of biotechnology aiming to tackle the issues associated with conventional industrial animal farming for meat production, i.e. environmental impact, controversial animal welfare and unsustainability. Cultured meat can be produced by applying current cell culture practices and bio-manufacturing methods that are already being used for the production of biologics, utilizing mammalian cell lines and the production of cell and gene therapy products to produce tissue or nutritional proteins. However, to bring production at scale, significant improvements and modifications need to take place for such a process to be cost efficient and robust enough to serve for food supply. The present study looks at the different scientific aspects to be tackled in order to render cultured meat into a viable commercial option. It is an interdisciplinary endeavor covering aspects from cell selection and medium optimization to biomaterials and tissue engineering. The current status of each of these fields is documented together with expert insight of what are the remaining challenges to be addressed as well as their potential respective solutions.

Why culturing meat?

The promise of cultured meat rests largely upon resolution of the problems related to industrial livestock farming, thereby circumventing some of its undesirable consequences 1. The IPCC (2018) has stated that we need to substantially reduce our consumption of conventional animal products to avoid the worst effects of climate change, yet most consumers are not willing to do this [2]. Harnessing the potential of stem cells to multiply and form skeletal muscle and fat tissue allows a vast reduction in livestock necessary to produce meat. Advantages of cultured meat broadly fall into three categories: sustainability, animal welfare, and public health.

In terms of greenhouse gas emissions, water consumption, and land use, cultured meat is far more efficient than conventional meat [3-5]. Studies have, however, found that cultured meat might be more energy-intensive [3,5], and therefore some environmental benefits are dependent on a transition to clean energy sources 6.

Second, cultured meat presents incontestable advantages in terms of animal welfare [7]. The Sentience Institute (2019) estimates that 99% of animals used for food are factory farmed, and are therefore considered industrial products rather than sentient beings 8.

Third, there are substantial public health benefits from cultured meat production. Conventional meat is the most common food source of potentially fatal infections such as salmonella and listeria (Painter, 2013). The production process of cultured meat guarantees the absence of contaminants during cultivation and can also be realised without the use of antibiotics. Antibiotic abuse in agriculture is a large problem contributing to antimicrobial resistance in pathogens which affect humans 9,10.

Finally, the resource intensity of livestock meat production requires an estimated 70% of our arable land to be used for the purpose of feeding livestock [11]. With an anticipated 70% increase in global meat demand, in 2050 we will have insufficient planetary resources to provide meat to the world population.

What is cultured meat?

Cultured meat aims to copy conventionally produced meat, through (stem)cell and tissue culture. The idea to use cell and tissue culture to produce meat is old, with first references in utopian literature from the 19th century [12]. The discovery of stem cells made *in-vitro* cell production possible and opened the road to cultured meat. Stem cells can be isolated from

a simple biopsy from a living animal [13] and then expanded *in-vitro* to generate a large number of cells. Subsequently, the cells can be stimulated to differentiate into muscle or fat cells, depending on the isolated stem cell type. Tissue engineering techniques, typically involving a biomaterial scaffold that gives temporary or permanent support and 3D organization of the cells, lead to the assembly of a tissue that resembles meat in its sensory and nutritional qualities as closely as possible. In theory, one can approach mimicry of meat in different ways, ranging from single protein production of individual muscle proteins to fully fledged tissue engineering of a complex muscle tissue containing muscle, fat, blood vessels, nerves, fibrous tissue and perhaps resident immune cells, in a natural architecture (Figure 1). The generation and assembly of multicellular muscle fibers and fat organoids into a minced meat product lies in between these extremes. This review focuses mostly on tissue engineered meat as this method is most commonly employed by investigators and startup companies, because it is scientifically the most comprehensive and enables the production of a meat copy. It means that at the very least the final product contains mature muscle fibers.

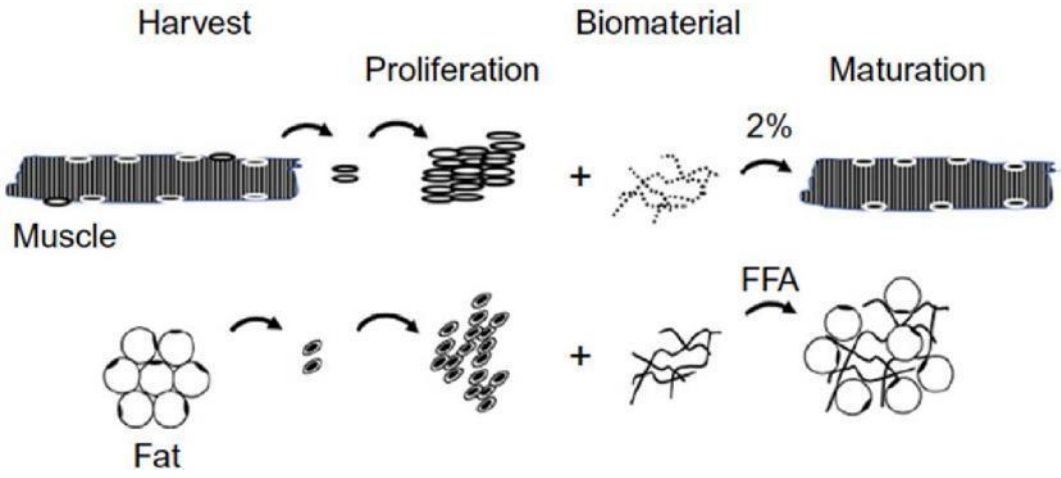


Fig 1. Principle of cultured meat. Stem cells are harvested from mature muscle tissue and expanded to large numbers. Using a gel biomaterial and a specific differentiation protocol

(2% growth factor medium for muscle and free fatty acids for adipose tissue derived stem cells), mature muscle fibers and pieces of adipose tissue are being formed.

Originally coined as 'in vitro meat' because the cells and tissue are cultured in vitro, the name of cultured meat is still a subject of debate. Currently, cultivated meat, cultured meat, cell-based meat and clean meat are the most prevalent names. Although some of the institutions represented by the authors favor a different name, for the purpose of this review we use the term cultured meat as a descriptor. Culturing meat is part of a proposed novel industry referred to as cellular agriculture, i.e. using cell-based biotechnology to replace traditional animal derived products such as meat, seafood, leather and milk. These endeavors have a common aim to reduce the negative societal impact of traditional livestock agriculture, while maintaining its widely acknowledged nutritional and cultural value.

Cell selection

A cultured meat bio-manufacturing process begins with one or more starting cell populations. The starting cell population may be homogeneous or exhibit various levels of heterogeneity. Although meat is a complex tissue, the current notion is that species specific skeletal muscle cells and adipocytes are the minimal necessary components of cultured meat. The suitability of the starting cells for manufacturing, is based upon their capacity for self-renewal and differentiation, in an environment where other animal components are minimized or eliminated.

Self-renewal is defined by a cell's continued ability to replicate and expand in numbers, while retaining its potential to differentiate in one or more tissue lineages. Stem cells such as embryonic stem cells (ESCs) can differentiate into any tissue and are known as pluripotent stem cells [14]. During embryonic development these ESCs give rise to progeny that becomes

more committed and loses pluripotency. For instance, so called mesenchymal stem cells (MSCs) have limited differentiation capacity but can still form bone, cartilage and adipose tissue. The progeny cells can remain quiescent in tissues as an adult stem cell, or can contribute to a developing or regenerating tissue as a transit amplifying cell [15], in a process called asymmetric division. Amplifying cells proliferate fast and extensively prior to differentiating terminally into post-mitotic cells that form most mature functional tissues. They present though a limited replicative capacity (i.e. amount of cell doublings they can undergo). Implementation of cells from various stages of stem cells has been proposed for cultured meat manufacturing [16,17.] Here, the suitability of a given stem-cell type for meat production, will be evaluated with respect to their capacity to expand and differentiate into skeletal muscle, the predominant constituent of most meats. Similar considerations however apply to the adipocyte lineage.

Adult stem cells of skeletal muscle, also known as satellite cells, constitute the most accessible myogenic progenitor in skeletal muscle tissues, and require little coaxing to differentiate into skeletal myotubes. It is these satellite cells, or rather their amplifying progeny called myoblasts, that were used to create the first cultured meat hamburger prototype [13]. Myoblasts propagate rapidly and egress from the cell-cycle as spindle-shaped myocytes that fuse with multinucleated myofibers during tissue repair and development [18.] Satellite cells and especially myoblasts require substantial optimization to increase their proliferative capacity for adaptation to industrial-scale cultured meat manufacturing applications [19.]

A primary consideration when isolating satellite cells for applications in cultured meat production is the muscle of origin. Skeletal muscle fiber types are broadly designated as oxidative slow-twitch, and glycolytic fast-twitch, respectively recognized as red and white meat. *In-vitro* porcine studies suggest that satellite cells retain the character from their originating tissue [20]. Additionally, purification of the starting satellite cell population from the

biopsy material can be performed relatively simply by differential adhesion protocols or by fluorescence activated cell sorting (FACS) based on biomarker characteristics [21-24].Industrial manufacturing of cultured meat at a scale sufficient to satisfy commercial demand heavily relies on cell propagation, starting in small planar culture system and gradually moving to large bioreactors [25,26.] As transient amplifying cells however, myoblasts can undergo a finite number of doublings and gradually lose their differentiation capacity. Therefore, efficient biomanufacturing could benefit from retaining satellite cells in their stem cell stage with presumably indefinite renewal capacity, while still being able to produce myoblasts. A recent study [21] demonstrated that this renewal can be extended *in-vitro* by inhibiting a cell signaling pathway known as p38-MAPK. Upon withdrawal of this inhibition, satellite cells retain their differentiation capacity. Similar interventions might lead to a more efficient use of satellite cells taken from a single biopsy.

Functional immortalization may provide another approach to extend the replicative capacity of skeletal muscle cells for industrial-scale expansion. For over four decades, differentiation competent, immortalized skeletal muscle cell lines have served as model systems in skeletal muscle biology research. Isolated from rat [27] or mouse [28] model organisms and spontaneously derived though consecutive passaging, these cell lines lack the species identity culturally acceptable for producing meat for human consumption [7]. Though a myogenic quail cell line has been described, the ability of this cell line to form mature myofibers is severely impaired [29]. Targeted genetic approaches developed for functional immortalization of human skeletal muscle cells [30] may provide alternative pluripotent cell lines from traditional livestock species for industrial biomanufacturing of cultured meat [31.] Unlike satellite cells, these so-called induced pluripotent stem cells (iPSCs) have an indefinite renewal capacity, because their early commitment to specific tissue lineages is inhibited. iPSCs are derived by reprogramming cells isolated from somatic tissues to the pluripotent state by directed expression of a combination of transcription factors often

including POU5F1, SOX2, KLF4 and MYC [32]. Human and mouse models have constituted most of the research and development reported on pluripotent stem cells to date, thus these findings still need to be translated to livestock species [7].

ESCs and iPSCs from agriculturally relevant ungulate species, such as pigs and cows, have only recently been derived successfully and characterized [33-36], while the derivation of bona fide ESCs or iPSCs from avian species, namely chicken, remains elusive. Established culture conditions have been unable to support stable, long-term culture of pluripotent cells derived from the avian blastoderm, and attempts of deriving iPSCs have resulted in partially reprogrammed cell lines [37].

Protocols established for differentiating pluripotent stem cells to skeletal muscle have taken alternate approaches with varied results. One approach relies upon culture regimens of growth factors and small molecule inhibitors to direct cells from the pluripotent state toward the myogenic lineage [38], whereas an alternate approach employs conditional activation of ectopically expressed transcription factors for programming cells to a myogenic lineage from a progenitor state. The later approach is reported to derive myogenic cells and direct their differentiation in a more efficient manner [39]. In fact, a variation of this programming approach was previously demonstrated in a porcine iPSC model resulting in contractile myotubes [40].

There is a strong precedent established for derivation and maintenance of pluripotent stem cells in serum-free [41,42] , and animal component free cell culture medium [43], as well as cultivation of these cells in a carrier-free suspension environment [44,45], features that would greatly facilitate industrial scale production.

Both the advancements in the maintenance of adult stem cells and the derivation of bona fide ungulate pluripotent stem cell lines opens up distinct yet promising avenues for manufacturing cultured meat. With technologies for production of cultured meat rapidly evolving, it is likely that multiple stem cell paradigms will find applications in industrial manufacturing based upon the advantages inherent to their respective biology.

Medium

Cells are cultured in a nutritious and oxygenated fluid which traditionally is referred to as 'medium'. As a result of the projected scale of cultured meat production, resource efficiency (feedstock, water and power usage), scalability of production and cost are critical boundary conditions. The cost of cell culture medium has been identified as one of the major cost drivers during upscaling of stem cell production 46.

The fundamentals of designing a good cell culture medium is qualitative and quantitative understanding of the physiology and metabolism of the target cell. Metabolism is regulated at cellular, molecular and genetic levels with different allosteric reaction rates. The availability and concentration of the substrates in cell culture media are obvious key parameters [47] in optimizing the overall yield of the metabolic reaction network towards a more efficient biomass production. Mammalian cells usually show inefficient consumption of carbon, nitrogen and energy sources and overproduction of metabolic byproducts such as lactate and ammonium [48]. There are two approaches to increase the metabolic efficiency. One is to use fed-batch or perfusion processes, which has been shown to increase cell density 3.4-fold [49] and result in a more effective metabolism, perhaps due to lower concentration fluctuations of substrate or metabolites. The other is metabolic engineering where the composition of the medium is optimized to drive metabolic pathways. The latter has been successful in optimizing medium for cell lines to produce pharmaceutical products 50-52 .

Besides productivity, the composition of cell culture media will also define the final characteristics of the cultured meat product. In the livestock industry factors such as climate, nutrition and stress define the meat product. For example, it has been suggested that acidosis caused by rapid glycolysis leads to degenerative changes in muscle fibers, which are solitary and rich in type II fibers [53-55.] Affected muscles show undesired

225 characteristics such as being pale, soft and exudative (PSE) [56]. In cell culture, an even more
226 direct influence on cellular metabolism and resulting cultured meat characteristics by the
227 medium is likely. In highly proliferating cells it has been shown that over 70% of the glucose
228 is metabolized to lactate with associated acidosis, leaving only 20 to 30 % of the glucose
229 entering the TCA cycle [57]. Nutritional deficiencies, such as lack of vitamins, cause
230 degenerative changes in muscle, as indicated in the case of vitamin D [58], vitamin E and
231 selenium 56.
232
233 Medium for proliferating cells need to be different than for differentiating cells as
234 metabolism changes from primarily energy and general nutrient usage to highly specialized
235 protein production. With more complex tissues that are composed of muscle and fat tissue
236 for instance, again different media compositions will be required.
237
238 Cell culture medium is not only important for productivity and quality, it also contributes to
239 the sustainability of the overall process. Animal derived components, including serum,
240 cannot be part of the medium, because they introduce contamination risks and undefined
241 substances and violate the ethical principle of using less animals, but most importantly, they
242 are unsustainable. Cell culture medium, where serum is replaced by proteins, growth
243 factors, sugars and fatty acids that take over the function of serum needs to be chemically
244 defined. Components that need to be present in high concentrations, such as glucose and
245 amino acids, will have a strong impact on the environmental footprint of the process. Today,
246 amino acids, the building blocks for protein synthesis and thus very important for meat
247 production, are most effectively produced through fermentation [59], using mainly glucose as
248 substrate. The industrial production of glucose is well established, with little waste
249 production and a high level of integration: 57% of the electricity and 59% of the heat input

250 are produced by a combined heat and power (CHP) system [60]. It is based on hydrolysis of a
251 raw material such as starch which is naturally produced by plants through photosynthesis
252 and therefore requires the use of land and water.
253 To achieve media with the lowest footprint, ingredients need to be sourced and dosed
254 judiciously. As an example, from an environmental perspective, glutamine should be
255 avoided to be used as an energy source instead of glucose, . Also, alternative sources of
256 amino acids and peptides should be evaluated, such as biomass from algae and certain
257 bacterial cultures. These microorganisms not only provide cheap sources of enriched amino
258 acids, fats, vitamins and minerals, but also offer opportunities to couple the cultured meat
259 production with other sustainable processes like waste treatment or CO2 capture 5,61-65.
260 Furthermore, recycling of culture media has been increasingly investigated for cell culture
261 processes due to potential economic and ecological advantages. This strategy has been
262 successfully demonstrated in bacterial and algae cultures with promising results with
263 respect to cost reduction and extended batch duration [66-68.] In combination with perfusion,
264 this strategy could be particularly interesting for the cultured meat process, as it would
265 significantly minimize the use of sterile, purified water, which is energy intensive. However
266 medium recycling has not yet been applied to mammalian cell cultures.
267
268 Metabolic engineering will increasingly rely on constraint-based modeling and flux balance
269 analysis that have been widely applied to predict and quantify the metabolic state of cells
270 69,70. Multi-omic flux balance analysis can help to predict flux distributions in a more reliable
271 way based on limited experimental data due to comprehensive crosslink of multiple omics
272 [71]. Metabolic modeling will be a powerful tool to predict not only the functional state of
273 cells, but also optimal nutrient formulations for cell growth *in vitro*. In the future, more
274 efforts are necessary towards the study of interactions between genome and metabolites

using association mappings [72], in order to improve objective and comprehensive function (not only growth maximization) for modeling [73-77.] However, to effectively validate and employ these methods, quantitative information on metabolic pathways and deep knowledge of the effect of a huge number of medium components and of their synergies are required. To add complexity, this input will likely be species and cell type specific. In such a multi-variable field of research, it is to be expected that metabolic modelling fed and validated by large amounts of data is required to support the experimental work

Scaling up, bioreactors, automation

For cultured meat to become a viable alternative to traditional meat, production has to be scalable and economical, factors typically going hand in hand. The specifics of scaling depend on the final intended product and the number of doublings the stem cell can sustain. For a minced product, the scaling is different than for a full thickness meat product. This is primarily true for the final stage, i.e. the organoid or tissue production, but the cell production will likely be similar as long as the cell production and tissue production phase are separated.

The objective of cell production is through a seeding train and a set of bioreactors of increasing volume, to generate a maximum number of cells while minimizing the needed feedstock, materials and culture manipulations. The seeding train is used to expand from the initial harvest number, which is typically in the order of 10^3 cells to the desired batch amount, in the range of 10^{13} cells, to create 1 ton of cultured meat (muscle). Seeding train optimization is focused towards maintaining the cells at the phase of exponential growth, while preventing them from differentiating early, and is highly dependent on the cell type 78,79. Therefore, the initial culture is performed in regular culture dishes or flasks, and as cell number grows, the culture is gradually moved to bioreactors with controlled conditions such

as temperature, pH and dissolved oxygen and carbon dioxide. The industry standard for mammalian cell culture in bioreactors is a stirred tank bioreactor where cells are either in suspension or attached to microcarriers that are suspended in the agitated medium [80]. Cell suspension is beneficial because of higher achievable cell densities and ease of harvesting. However, most mammalian cells are anchorage dependent, meaning that they have to attach to a surface, hence the advent of microcarriers that, while suspended, provide surface for the cells to grow. Similar to mesenchymal stem cells, bovine myoblasts can be expanded on microcarriers in suspension 81.

Recent developments show some success in modifying induced pluripotent stem cells (iPSCs) so that they can grow in aggregates [44,82,] very similar to earlier achievements in embryonic stem cells (ESCs) from mice [83] and human [84,85.] More committed stem cells such as mesenchymal stem cells, can form aggregates and grow, but the aggregate size is hard to control [86], leading to unpredictable cell yield. No large-scale cell culture data using aggregates is available. Cells from the C2C12 myoblast line can also form aggregates, but here too, no data is available on suspension culture in aggregates. It is anticipated that aggregate culture of MSCs or myoblasts is more challenging because of their tendency to differentiate in a 3D environment.

Bioreactors

For the large-scale production of cells required to produce cultured meat, bioreactors are the preferred culture modality because of their scalability, controllability and higher achieved cell densities than planar systems can offer [87,88.] Experience with large scale cell culture of anchorage dependent mammalian cells is being developed mostly for the MSC cell therapy field 89.

The most commonly used bioreactors are stirred tanks and rocking bioreactors (also known as wave bioreactor). Alternate bioreactor configurations include perfused packed bed reactors, plug flow reactors such as hollow fibre, air-lift, vertical wheel and fluidized bed bioreactors but also novel modes of operation of the stirred tank and rocking bioreactor 25,90. In Figure 2, the schematics of these bioreactors are presented. The ultimate goal of these developments is to increase the medium conversion ratio, i.e. the percentage of nutrients in the medium that is converted to edible animal tissue, equivalent to the feed conversion in traditional livestock meat production. The most important levers to improve the medium conversion ratio are cell density (cell number/ml medium) and optimal use of medium through recycling techniques. A second, equally important, goal is to scale up cell production to achieve cost-effectiveness. In addition to the production of cells, tissues need to be formed by the cells. In the absence of a fully integrated system where cells can not only divide, but also mature as a tissue after (self) assembly, the tissue formation stage occurs in a different bioreactor that is optimally suited to condition the forming tissue. Here, the diversity in reactor designs will be even bigger depending on the type of tissue to be formed and its specific conditioning needs. The labor-intensive parts of the process will need to be automated to reduce cost, and perhaps even more important, the risk of microbial contamination.

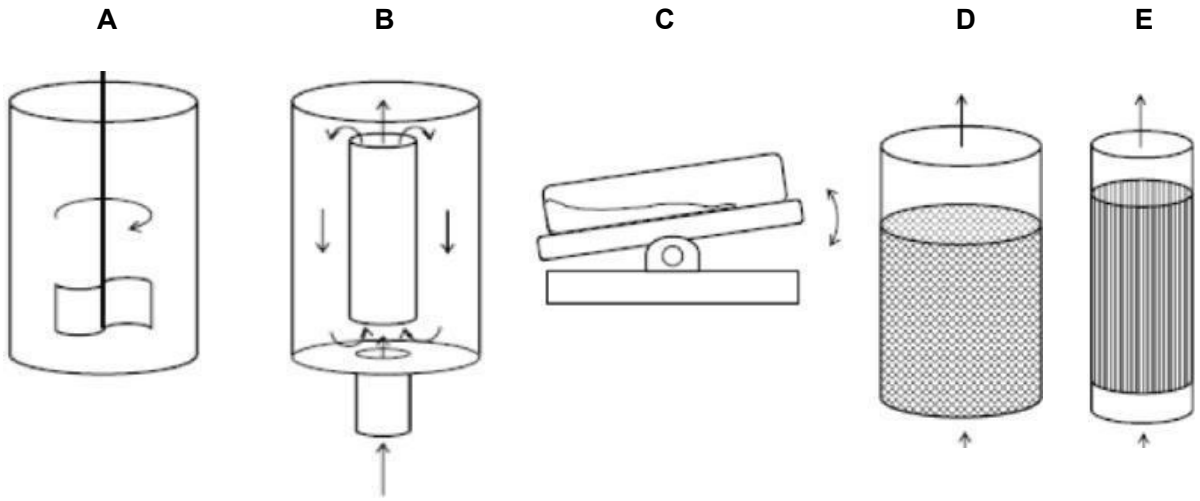


Figure 2: Most common bioreactor designs for mammalian cell culture. A: stirred tank; B: airlift; C: wave; D: flow through, E: hollow fibre.

Bioprocess development and optimization is also key to bring down production costs. *In silico* modelling of cell behavior will play a pivotal role in the next years, as to realize consistent production at scale, especially when the source material is primary cells, significant efforts need to be made to shift away from the current semi-scaled up systems and the “trial and error” upscaling approaches that currently dominate the field of cell & gene therapy 91,92.

Finally, the manufacturing process does not only include cell and tissue production, but also harvesting and purification of cells after production, cell storage, banking and transport, standardization and traceability of tissue harvest from animal donors, quality control of the produced tissues and regular food technology to process those into meat products.

Biomaterials

Biomaterial scaffolding is a key component to cellular agriculture, providing numerous functions to support cells towards tissue formation. Scaffold serves as an integrating support network onto and into which cells expand, differentiate and exploit their anchorage-dependent needs for survival and functions. Scaffolds also provide the porous network through which oxygen and nutrients flow and waste products are removed, thus maintaining cell metabolic functions and avoiding necrotic cores over time. To serve these purposes, a balance between morphology, structure and chemistry is required. Historically, scaffolding from biomaterials have focused on medically-relevant outcomes, for the fields of tissue engineering and regenerative medicine [93-97]. For such goals, the scaffold requirements are in part, different and more stringent than in the case of scaffolds utilized for cellular agriculture in foods (**Table 1**).

Scaffolding is usually degradable, but if it is not, it has to be edible in uncooked or cooked conditions. Edible and regulation-compatible scaffold material for food tissue engineering has to achieve physical goals such as texture, taste and thermal stability related to consumption, cooking and nutrition. It also has to be cheap and sourceable at large scale.

Biomaterial Scaffold Options – A variety of biomaterial scaffolds are being pursued for cellular agriculture, most of which are derived from biological sources but processed for desired structure and morphology, while retaining native chemistry (**Table 1**). To reduce cost, manipulation of the biologically sourced material should be kept at a minimum. Products derived from traditional livestock animals such as collagen are to be avoided since they are non-replicative and would therefore still require a substantial production of livestock for production. Thus, more promising materials are polysaccharides such as cellulose, starch (amylose/amylopectin), chitin/chitosan, pullulan, alginates, hyaluronic acid, and others [98]. Protein-based systems include fibrin, collagen/gelatin, keratin, or silk, where the materials are sourced through recombinant technology. Other materials of interest include the family of polyesters, polyhydroxyalkanoates, expressed in bacteria and other systems [99]. Finally, complex composite matrices generated from plants and microorganisms are also actively

pursued, including lignins, plant matrices (e.g., decellularized leaves), fungal mycelia and others [100]. Aside from biopolymers, there are a number of synthetic polymers that can be considered, including a range of polyesters. Generally, these systems are safe in the human body and can have a tailored degradation rate via chemical hydrolysis [REF]. Benefits of synthetic polymer systems are consistent quality and supply, but cost and requirement for surface functionalization may be limitating.

Testing and Methodology Considerations – Well-established methods to study morphology, structure and chemistry can be pursued for the characterization of these materials including SEM, AFM, TEM, X-ray, FTIR, elemental analysis and rheological assessments(Table 2). However, the additional unique features for scaffolds related to cellular agriculture, include aspects of texture, digestion, cook-loss, water-binding capacity and taste that are less commonly considered in medically-related scaffold designs. Each of these features must be assessed with appropriate methods to ensure compatibility for human consumption as part of foods. For example, nutritional analyses, including extraction and chromatographic quantitation of key nutrients, mechanical testing to assess texture (e.g., Warner-Bratzler shear force, water-holding capacity and cook-loss from the meat industry), and nutritional safety need to be considered.

Additional Considerations - Additional factors to be considered for cultured meat applications include degradation lifetime *in vitro* and during digestion. This is preferably established with *in vitro* screening simulating the gastrointestinal conditions (pH, mechanics and digestive enzymes). Such screens would be performed on both pre- and post-thermally modified ‘cooked’ versions of the scaffolds to compare outcomes, similar to testing of other novel food ingredients [101.]

Ultimately, cost of scaffolds used in culturing meat is an overriding issue to consider where scaffolds should be a small component of the total costs. Key to minimize cost of goods and to guarantee consistency is production of those materials at large scale. Many of the polymers on Table 1 are already being produced at scale.

Table 1. Some polymer options for scaffolds for cellular agriculture via non-animal sourcing. There is insufficient data to date to ascribe these scaffold polymers to specific food tissue engineering outcomes.

Biopolymer Class	Specific Type	Source, features
polysaccharides	Cellulose and its derivatives (CMC, HPMC, MC)	plants, bacteria
	starch (amylose, amylopectin)	plants
	chitin/chitosan	crustaceans, insects, fungi, yeast
	hyaluronic acid, methacrylate derivatives	heterologous expression
	alginate	plants
	agarose	plants
proteins	collagen/gelatin, zein, methacrylate derivatives	heterologous expression
	silk	silkworms, spiders, heterologous expression
	elastin	heterologous expression
	keratin	heterologous expression
	Laminin	Heterologous expression
polyesters (PHAs)	polyhydroxyalkanoates (and variants of homopolymers, copolymers)	heterologous expression
synthetics	polylactic/polyglycol acids	chemical synthesis

	polycaprolactone	chemical synthesis
	polyethylene glycol	chemical synthesis
	polyvinylalcohol	chemical synthesis
complex natural composites	mycelia	fungi
	lignin	plants
	decellularized tissues	plants

419

420 **Table 2**. Scaffold design features (physical, chemical, biological considerations) for biomaterials to be

421 considered in cellular agriculture applications.

Property	**Features to consider**	**Analyses**
Physical		
Processability, structure, thermal stability (cooking)	Rheology, flow behavior, thermal stability, changes in structure with temperature	Viscometer, rheometry, dynamic mechanical analysis, differential calorimetry, thermal gravimetric analysis
Architecture, Texture	Crystallinity, porosity, content	Instron compression testing, XRD, FTIR, Warner-Bratzler Shear Force
Surface Features	Chemistry, functionalization	Immunohistochemistry, NMR
Morphology	Fiber size, surface topography, porosity, alignment, manufacturing approaches	SEM, mercury porosimetry, histology; fibers (extrusion, electrospinning), films (casting, rolling), sponges (porogens, gas evolution , freeze fronts for alignment), hydrogels (self-assembly, covalent crosslinks, selective chemistry)

		3D printing
Chemical		
Edible/digestibility/stability	Polymer chemistry, enzymes, chemical hydrolysis	In vitro mimetic solutions (enzymes – proteases, oxidases, hydrolases; chemical composition, gut/saliva simulants, pH, bile, etc.), macrophage screens, LPS assays, endotoxin screens, chemical screens for residuals (e.g., antibiotics, endocrine mimics, etc.)
Biological		
Safe for human consumption	GRAS, nontoxic	Various assays - bacterial toxicology assays, 3D tissues in vitro screening
Source/Sourcing	Consistent source, scalable	Composition analysis
		Viscometer, rheometry, dynamic mechanical analysis, differential calorimetry, thermal gravimetric analysis
Taste	Palatability, flavor- & aromatic- compounds (or as byproducts of cooking), Maillard reaction products (for sugar-based scaffolds), oxidation, stability	Tasting-panels, chromatography, GC/MS, TBARS assay
Nutrition	Metabolites, metals, sugars, amino acids, vitamins	Digestion, analysis via HPLC/MS, metal analysis
Cell and tissue compatibility	Surface chemistry, metabolites, physical structure, morphology	FTIR, NMR, SIMs Tissue mimics in vitro (oral cavity, stomach, intestine)
Environmental		
Sustainability	Water, land, energy footprint, greenhouse gas emissions related	Life-cycle assessment

	to production, synthesis, processing	

Consumer Acceptance

One of the major questions about cultured meat is whether consumers will buy it. Indeed, consumer acceptance is a necessary component for cultured meat's commercial success in the short term, and for its ability to bring about societal benefits in the long term.

Survey data on this question is rather inconsistent, and is dependent on a number of factors including the phrasing of the question and the nationality of the sample [102,103.] Table 3 shows a summary of the results of nationally representative survey questions about cultured meat to date.

Table 3: A summary of nationally representative survey questions on cultured meat.

Survey	Sample	Question	Would eat	Don't know	Would not eat
YouGov (2013)	1,729 UK adults (18+)	"Imagine artificial meat was available commercially, do you think you would eat it?"	19%	19%	62%
Pew Research (2014)	1,001 USA adults (18+)	"Would you... Eat meat that was grown in a lab?"	20%	2%	78%
Flycatcher (2013)	1,296 Netherlands adults (18+)	"Suppose that cultured meat is available at the supermarket. Would you buy cultured meat in order to try it?"	52%	23%	25%

The Grocer (2017)	2,082 UK adults (16+)	“Would you ever buy ‘cultured meat’ grown in a laboratory?”	16%	33%	50%
Wilks and Phillips (2017)	673 USA adults (18+)	“Would you be willing to try *in vitro* meat?”	65%	12%	21%
Surveygoo (2018)	1,000 UK and USA adults (18+)	“Would you be willing to eat cultured meat?”	29%	38%	33%
Bryant et al. (2019)	3,030 USA, India, and China adults (18+)	“How likely are you to try clean meat?”	52%	34%	13%

Although samples and question wording surely affect survey responses, the main differences here appear to be based on the amount of information given to participants. The three most optimistic survey results come from longer, cultured meat focused, surveys that gave participants plenty of positive information [103-105.] The most negative results, meanwhile, come from surveys where participants are given very little information about cultured meat, often as part of a longer omnibus survey (Pew Research, 2014; The Grocer, 2017; YouGov, 2013). Indeed, this explanation fits with the finding that positive (and negative) information about cultured meat influences attitudes in the direction of the information [106.]

Various experimental studies have demonstrated a number of ways in which acceptance of cultured meat can be increased. When cultured meat is primarily framed as a high-tech science innovation, it is significantly less appealing than when the focus is on its societal benefits or its similarity to conventional meat [103]. Similarly, overly technical descriptions are less appealing than more straightforward descriptions [107], and names like ‘lab grown meat’ which invoke science and unnaturalness are significantly less appealing than names like ‘clean meat’ which highlight the benefits relative to conventional meat [102]. Consumers are

also more likely to choose cultured meat when the price is lower, and when the perceived popularity amongst others is higher [108].

Data shows that most Americans (57.3%) are 'not at all familiar' with cultured meat [109]. Familiarity with the technology is a major predictor of acceptance whilst food neophobia is a major predictor of rejection (Bryant et al., 2019; [110]. Furthermore, focus groups on the topic have charted the course of initially negative attitudes towards cultured meat, which often become less negative after further consideration of the concept [111,112]. Therefore, despite a lack of meaningful longitudinal data, it is likely that attitudes and intentions towards cultured meat will become more positive as more people become aware of it and more familiar with the concept.

Various studies have found higher acceptance of cultured meat amongst men compared to women, amongst younger people compared to older people, and amongst omnivores compared to vegetarians [104,105,108,109,111,113]. Bryant and Barnett (2018) have argued that the gender disparity may relate to women having more cautious stances towards foods in general [114], whilst the age trend is likely due to higher openness to new experience amongst younger people [115].

The findings regarding vegetarianism are interesting - since cultured meat circumvents the primary ethical and environmental motivations for vegetarianism [116], one might think that vegetarians should be no more likely to reject cultured meat than omnivores. However, it is common for vegetarians to acquire an emotional disgust reaction to meat in general, which may supersede rational reasons for avoiding meat [117,118]. The relative lack of appeal of cultured meat to vegetarians should not be a major concern for producers or advocates: those who avoid meat are a small fraction of the market, and are, in any case, not contributing to the problems of conventional meat production. Moreover, it is important for the long-term ability of cultured meat to displace demand for conventional meat that it does

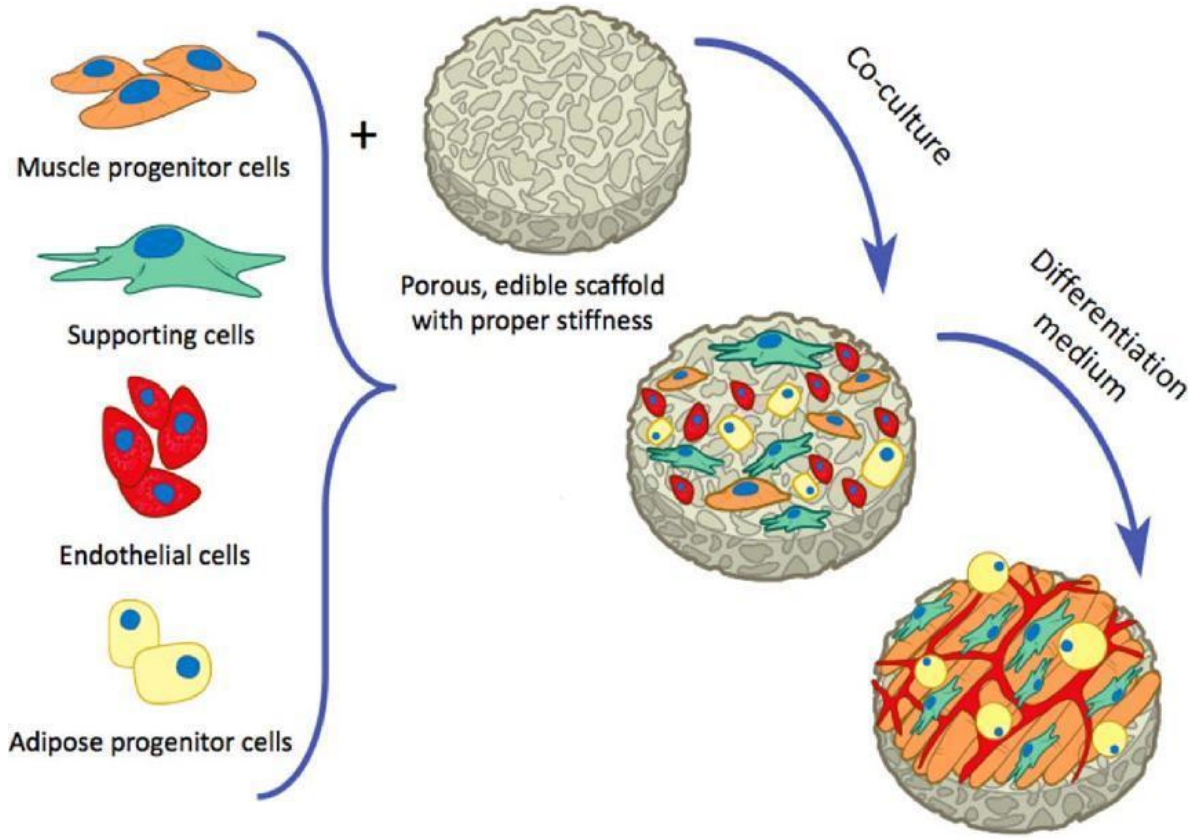
not come to be seen as a product for vegetarians.

Experts anticipated that cultured meat would be more appealing to consumers in America and Asia compared to Europeans [119] and now data appears to bear this out. Whilst the British were amongst the most accepting of cultured meat in Europe in a 2005 survey [120], they are substantially less accepting than their American cousins [121]. Americans, in turn, are less willing to eat cultured meat than those in China and India [109]. Such differences may be related to the different roles animal agriculture plays in these societies and cultures.

Of course, a major limitation of all the research on consumer acceptance of cultured meat is its hypothetical nature. Since there are no cultured meat products currently available commercially, researchers have been unable to observe the nature of consumer preferences in practice or explore specific aspects of the product which are appealing. However, as Bryant and Barnett (2018) observe, consumer perceptions of cultured meat are similar to perceptions of genetically modified food in terms of demographic trends [102]. Some consumers view these technologies as conceptually similar [122], and attitudes are often underpinned by similar sets of concerns.

Complex tissues

Currently, most cultured meat tissues consist of muscle tissue only[16], and minced meat products from muscle and fat are made by separately growing muscle fibres and adipose organoids to later be combined in the final meat product formulation. Meat is not only muscle, but a tissue composed of muscle, fat and connective tissue [123], ideally mimicked by culturing a tissue with similar composition. To grow these different cells together in a single tissue, a more advanced tissue engineering approach is therefore needed(Ben-Arye, unpublished), [124-126] (Fig 3).



509

510 Figure 3: Production of complex meat products from muscle, fat, connective tissue and
511 vascular cells, using a scaffold method.

512

513 The advantage of culturing complex tissue is not only that the composition of the produced
514 tissue will better approximate regular meat, but also that mutual beneficial interactions
515 between different cell types can be leveraged. The minimal requirement for such a complex
516 tissue seems to be the presence of muscle fibers, adipose tissue, fibrous and vascular cells,
517 by combining their respective progenitor cells and differentiating them to their final,
518 functional, phenotype.
519 Evidence of beneficial interactions between vascular cells (endothelial cells: ECs) and
520 skeletal muscle and adipose tissue derived stem cells shows that ECs secrete growth
521 factors and cytokines promoting proliferation and differentiation of muscle progenitors into
522 fibres [127], as well as promoting adipogenesis[128]. In addition, extracellular matrix components
523 (ECM) secreted by microvascular endothelial cells and fibroblasts stimulate preadipocyte

differentiation and muscle maturation, while providing texture to meat [129-132]. The most challenging component of this complex tissue is the adipose tissue, with little scientific evidence of applicability in cultured meat[133]. Currently adopted protocols to stimulate adipogenesis in human and murine cells are not suitable for generating edible tissue [123]. Thus, food compatible adipose tissue differentiation from common livestock animals should be established first, before addressing the challenge of combining fat cells with muscle cells. Co-culturing of different cells typically requires an elaborate optimization of growth medium and differentiation protocols [134]. The formation of a complex muscle tissue is also dictated by the properties of the scaffold biomaterial which, to be suitable for muscle and adipose tissue formation, it should be formulated to yield appropriate stiffness[135], for both tissues 136,137. However, adipose tissue requires low stiffness, whereas muscle tissue requires a higher one, a suitable combination might therefore be challenging.

Formation of cultured muscle fibres and muscle contractility can be further promoted via mechanical and/or electric stimulation applied on the complex tissue construct 138,139. Achieving muscle contractility presents an added value for cultured meat, as it stimulates muscle cell production of proteins such as myoglobin, which is responsible for the red color of meat and is an important source of iron [140].

Finally, thickness is another aspect of engineered complex tissues. To create attractive meat analogues, instance thickness of 1 cm or more is needed. This scale is far beyond the diffusion limits of oxygen and nutrients. To prevent tissue from dying, a channelling and perfusion system that allows even and sufficient delivery of oxygen and nutrients and adequate effusion of metabolic waste, is required [141,142]. The channelling system could come from spontaneously assembling ECs into a network of blood vessels or from a printed hierarchical vascular tree as has been recently demonstrated at small scale [143]. The functionality of the artificial blood vessels may affect muscle maturation through paracrine interaction or may just be a conduit system. The channels or blood vessels likely do not contribute appreciably to the taste and texture of the cultured meat product. Cost effective

scaling up of whole thickness perfused engineered tissue is obviously a massive engineering challenge.

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