Chapter 8

In Vitro Cultured Meat

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ABSTRACT

The advent of in vitro cultured meat represents a groundbreaking advancement in food technology and sustainable agriculture. This chapter delves into the intricacies of lab-grown meat, exploring its potential to revolutionize the meat industry by offering a viable alternative to traditional livestock farming. In vitro cultured meat is produced by culturing animal cells in a controlled environment, allowing for the creation of muscle tissue that mirrors conventional meat without the need for animal slaughter. This method addresses a myriad of concerns related to environmental sustainability, animal welfare, and food security. In conclusion, in vitro cultured meat has the potential to transform the meat industry by offering a sustainable, ethical, and safe alternative to traditional meat. As research and technology continue to advance, cultured meat could play a pivotal role in addressing some of the most pressing issues facing global food systems today.

DOI: 10.4018/979-8-3693-4115-5.ch008

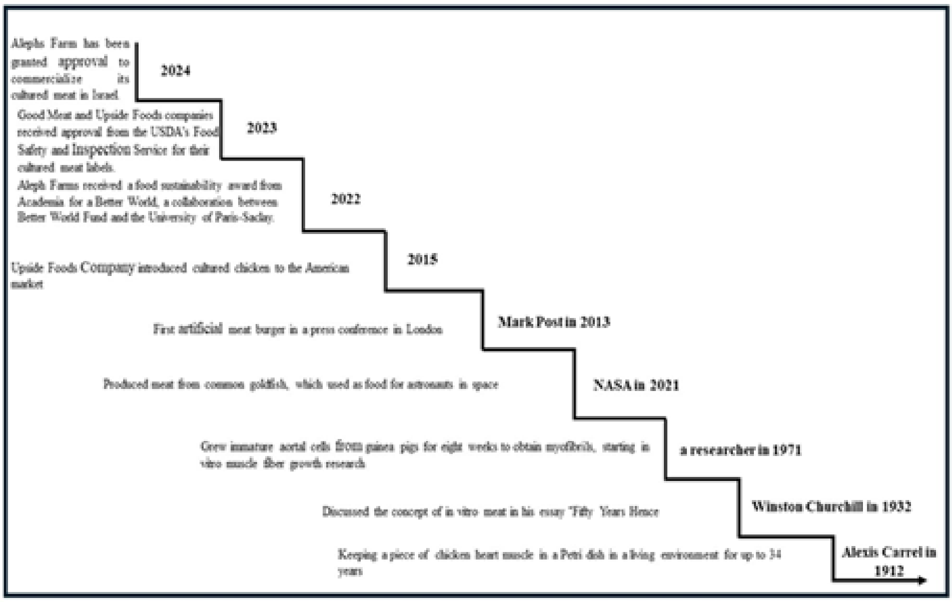
1. INTRODUCTION

In vitro cultured meat, also known as lab-grown meat, clean meat, or cultured meat, represents a novel approach to meat production by utilizing animal cells to grow meat in a controlled environment (Jin, 2024). This novel approach seeks to address a number of issues related to conventional meat production, including environmental effects and animal welfare concerns (Anomaly, 2023). In vitro meat production provides a sustainable and ethical substitute for traditional meat production by using cell cultures to produce meat (Padilha et al., 2021). The process entails separating live animal cells and promoting their development into connective tissue, muscular tissue, and fat (Jin, 2024). Through tissue engineering techniques, cultured meat can replicate the sensory and nutritional characteristics of traditional meat while minimizing the need for animal slaughter (Jin, 2024). In vitro meat production research seeks to control composition, lower production costs, and closely imitate traditional meat through the application of scientific discoveries and technical achievements. The primary objective of in vitro meat production research has been to sustain the viability and functionality of muscle stem cells by improving culture conditions (Choi et al., 2020). Other research has investigated the application of diverse culture media and methodologies to facilitate the expansion and differentiation of muscle cells for the purpose of producing cultured meat (Dutta et al., 2022). Decellularized tissues have also been studied as prospective scaffolds for cultured meat production, providing a conducive environment for cell proliferation and tissue growth (Singh, 2023). Additionally, decellularized plant-derived cell carriers have been suggested as a viable means of promoting cell proliferation in the manufacture of lab-grown meat (Thyden et al., 2022). Two benefits of producing meat in vitro are lowering greenhouse gas emissions and global warming associated with conventional meat production. The environmental impact of cultured meat has been a subject of study, with life cycle assessments comparing different meat substitutes, including lab-grown meat, insect-based alternatives, and plant-based substitutes (Smetana et al., 2015). These assessments have highlighted the potential of cultured meat to reduce the environmental footprint of meat production compared to traditional methods. In the context of reducing emissions and environmental consequences, microalgae were employed in the manufacture of lab-grown meat for a variety of advantageous and sustainable reasons. This included controlling composition, supplying the necessary nutrients, and bringing down the price of in vitro cell development (Rojas-Tavara, 2023). Regarding the challenges, consumer perceptions and acceptance of lab-grown meat play a crucial role in the adoption of this innovative technology. Studies have investigated the willingness of consumers to pay for in vitro meat and the factors influencing their food choices (Asioli et al., 2021). Factors such as ethical considerations, environmental sustainability, and health concerns have been identified as primary motivators for consumers interested in cultured meat (Rehman, 2024). Effective communication strategies and information nudges have been suggested to influence consumer preferences towards meat alternatives, including lab-grown meat (Segovia et al., 2022). In vitro cultured meat represents a revolutionary advancement in food technology with significant potential to address pressing global challenges. Therefore, this chapter has explored the scientific foundations, benefits, and challenges associated with cultured meat production.

2. HISTORY

The evolution of in vitro produced meat was evaluated using both theoretical and practical considerations. An early concept of growing a piece of chicken heart muscle in a Petri dish in a living environment for up to 34 years was pioneered by Alexis Carrel in 1912. In 1932, Winston Churchill wrote an essay titled “Fifty Years Hence,” which was later included in the book Thoughts and Adventures. In that essay, he addressed the idea of invitro meat. The improvement of meat production through tissue engineering techniques was proposed in 1953 by Willem Van Eelen, a Dutchman. In 1971, a researcher grew immature aortal cells from guinea pigs for eight weeks to obtain myofibrils, starting in vitro muscle fiber growth research (Bartholet,2011). In 1999, Symbiotic A, the world-renowned lab, produced modified in vitro cells by harvesting frog muscle biopsy (Catts and Zurr, 2002). In 2001, the National Aeronautics and Space Administration (NASA) undertook research on food production, which might potentially be used in space flight, and successfully manufactured meat from common goldfish (*Carassius auratus*). The muscle tissue was then provided to astronauts as food in space. Early in the new millennium, Jason Matheny promoted the idea of produced meat, co-authored a paper on cell-cultured meat, and established New Harvest, a company devoted to studying in vitro meat (Edelman et al.,2005). In August 2013, Mark Post from Maastrich University, Netherlands, launched a cell-cultured meat burger for sensory evaluation in a press conference in London after growing bovine skeletal muscle cells (Stephens et al.,2018). In 2014, the US-based nonprofit organization People for the Ethical Treatment of Animals (PETA) expressed their endorsement of CM by offering a reward of 1 million dollars to anybody capable of producing lab-grown meat using chicken cells (Kantono et al., 2022). In 2015, Upside Foods Company brought cultured chicken to the US market. On November 18, 2019, China's first product additionally had its public appearance. Zhou Guanghong, a professor at Nanjing Agricultural University, successfully cultured the sixth generation of pig muscle stem cells in a nutritional solution for 20 days, yielding a 5 g meat product. The emergence of new restaurants where it is possible to try cell meat products is mainly in Asian countries, and the USA. In December 2020, Singaporean regulators granted approval for the commercialization of lab-grown chicken nuggets in restaurants (Waltz, 2021). In late 2020, Eat Just, an American company, made its first commercial sale of CM at the “1880 restaurant” in Singapore. In 2020, the Singapore Food Agency authorized Eat Just's chicken bites for commercial sale, making them the first CM product to pass a food regulator's safety review (Carrington, 2020). In 2022, Aleph Farms received a food sustainability award from Academia for a Better World, a collaboration between Better World Fund and the University of Paris-Saclay. In 2023, two cultured meat enterprises (Good Meat and Upside Foods) have received approval from the USDA's Food Safety and Inspection Service for their cultured meat labels. Furthermore, Ivy Farm Technologies, a company backed by Oxford University, anticipates receiving authorization and commencing the commercialization of lab-grown pork in the United Kingdom by 2023. The company's objective is to achieve an annual production of 12,000 tonnes of pork, which is equivalent to the meat gained from the slaughter of 170,000 pigs (Mridul,2023). Moreover in 2024, in Alephs Farm has been granted approval to commercialize its cultured meat. Nevertheless, twelve European countries, including Italy, France, and Australia, as well as certain American states, including Alabama and Florida, have prohibited the consumption of cultured meat in 2024. Figure 1 presents a concise overview of the progression flow diagram for cultivated meat.

Figure 1. Historical aspect of cultured meat



3. FUNDAMENTAL

Myogenesis, the process by which muscle tissue is formed, is the first step in the production of tissue cultured meat (CM). Multipotent myoblasts from the mesoderm layer undergo a number of processes during embryonic development, including fusion, proliferation, and differentiation, to form myotubes, which eventually unite to become muscle fibers (Kantono et al., 2022). Making CM is therefore very similar to growing skeletal muscles.

4. PROCESSING AND TECHNOLOGY

4.1 Cellular Tissue Origin (Cell Sourcing)

Cultured meat processes rely on the extensive proliferation of cells to generate sufficient biomass. Primary cell sources for CM biomanufacturing, including adult stem cells (ASCs), are obtained via biopsy or postmortem tissue from the designated location of the animal species of interest. The second alternative is to use pluripotent stem cells. ASCs have a limited replicative capacity of 50–60 divisions. The ASCs can be categorised into three types of satellite stem cells: myosatellite cells (MCs), adipose-derived stem cells (ADSCs), and mesenchymal stem cells (MSCs). Myosatellite cells have the capacity for self-renewal and proliferation, but their differentiation potential is limited to muscle cells. The second one is adipose tissue-derived stem cells, which develop from subcutaneous fat in adipose tissues and may develop into adipogenic, myogenic, chondrogenic, or osteogenic. The last one of ASCs, namely mesenchymal stem cells (MSC), are multipotent cells, which can be derived from non-muscle tissues such as bone marrow, adipose tissues, and placental tissues (Jervis et al., 2019), which can differentiate into different kinds of cells such as myocytes, adipocytes, and fibroblasts (Okamura et al., 2018). However, the regenerative properties of MSCs declined over time. Additionally, the myogenic differentiation of MSCs alone is inadequate. Therefore, MSCs are employed for co-cultivation with myoblasts, which secrete many growth factors that play a role in muscle regeneration and stimulate myoblast migration, proliferation, and differentiation. Pluripotent stem cells are categorised into embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). ESCs are obtained from the inner mass of blastocysts in the early stages of embryonic development. They possess pluripotent characteristics (Williams et al., 2012), meaning they can differentiate into the three primary germ layers: ectoderm, mesoderm, and endoderm. These ESCs are the best source since they can differentiate and proliferate into any cell type without restriction (Reiss et al., 2021). The challenges are somewhat determined by the cell type selected, including primary stem cells, which invariably lose proliferation and differentiation capacity during long-term culture (loss of stemness), whereas the stable maintenance of pluripotent cells can necessitate complex, expensive medium formulations (Bar-Nur et al., 2018). Invariably, primary cells undergo a senescent state during long-term culture, which is defined by permanent cell-cycle exit, widespread gene expression changes, and remarkable cellular flattening and enlargement (Di Micco et al., 2021). Decreases in proliferation are observed even before complete senescence. Proliferation rates in bovine SCs decrease to approximately 0.6–0.8 population doublings (PDs) per day until cells enter senescence at 20 to 30 PDs (Stout et al., 2022). To produce industrially cultured meat, the proliferative capacity must be significantly increased beyond these constraints (Melzener et al., 2021). Understanding and overcoming this obstacle require an understanding of the causal link between cellular senescence or death and decreased proliferation. The aging process involves a variety of cellular changes, including the accumulation of mutations at the genetic and epigenetic levels, changes in metabolism and morphology, and changed signaling system activity (Ogrodnik, 2021).

Table 1. Different cell types from different animal species for cultured meat production

1	**Types of cells**	**Characters of cells**	**Disadvantages**	**Site of cells**	**References**
Cattle	1. Adult stem cells (AS) (progenitor cells)	These cells may develop into adipogenic, myogenic, chondrogenic, or osteogenic.			**Okamura et al., 2018** **Witt et al.,2017**
	a. Adipose tissue-derived stem cells	Have ability to self-renew and proliferate.	they can only differentiate into muscle cells.	subcutaneous fat in adipose tissues	
	b. Satellite stem cells (Myosatellite cells, myoblast cells)	Multipotent cells, have the ability to differentiate into different kinds of cells such as myocytes, adipocytes, fibroblasts and chondrocytes.		Skeletal muscle cells	

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Table 1. Continued

1	Types of cells	Characters of cells	Disadvantages	Site of cells	References
	c. mesenchymal stem cells (MSCs)		The myogenic differentiation of MSCs alone is inadequate. Therefore, MSCs are employed for co-cultivation with myoblasts, which secrete many growth factors that play a role in muscle regeneration, stimulate myoblast migration, proliferation and differentiation.	Bone marrow, adipose tissue and placental tissues	
	2. Pluripotent stem cells	It is the best origin because they can differentiate and proliferate to all cell kinds without limits.			**Reiss et al., 2021**
	a. Embryonic stem cells (ES)			Inner cell mass of the blastocyst stage of embryo	
	b. Induced pluripotent stem cells (iPSCs)			iPSCs are obtained by inducing adult somatic cells using a set of identified pluripotency factors	
Chicken	Chicken muscle satellite cell				**Siddiqui et al., 2022**
	1-Satellite cells from the slow muscle	differentiate into slow and fast muscle fibers.			
	2--Satellite cells from the fast muscle	only differentiate into fast muscle fibers.			
Fish	1. Continuous fish cell lines (CAM) derived from *Carassius auratus* and *cromileptes altivelis*				**Li, Guo, & Guo, 2021;**

continued on following page

Table 1. Continued

1	Types of cells	Characters of cells	Disadvantages	Site of cells	References
	2. Fathead minnow (FHM) cell line from *pimephales promelaus*				**Chen et al., 2020**
	3. Cell lines developed from muscle cells of: *a) Danio rerio* *b) Paralichthys olivaceus* c) *Lates calcarifer*				**Vishnolia et al.,2020** **Peng et al., 2016** **Lai et al., 2008**

4.2 Seeding of Cells on Scaffold (Scaffolding of Cells, Cell Adsorption on Scaffold)

Stem cells (progenitor cells) are seeds on a scaffold; a biomaterial substrate used in the preparation of cultured meat. Scaffolding biomaterials, complex frameworks, are added to the culture media to provide structural support to the cells, enhance nutrient transfer, and facilitate cellular respiration (Schätzlein & Blaeser, 2022). Furthermore, scaffolding is required to replicate traditional meat tissue's 3D configuration by forming dense, integrated saturated meat with medium perfusion and a vascular system (Seah et al., 2022). Furthermore, scaffolding can promote cell cultivation without the use of serum, which can be achieved by incorporating bioactive chemicals into an edible scaffold rather than introducing them into the culture medium (Chen et al., 2024). Bioactive, flexible, having a large surface area, allowing growth media diffusion, and being edible, non-toxic, and allergen free are the fundamental features of scaffolding employed in cultured meat technology (Alam et al., 2024). The natural extracellular matrix (ECM) is composed of proteoglycans, collagen, and glycoproteins. Consequently, proteins and polysaccharides are expected to be the primary components of scaffold biomaterials (Bomkamp et al., 2022). For CM production, the ideal porosity range for scaffolds is 30% to 90%, with hole sizes ranging from 50 to 150 µm or larger. The scaffold thickness typically relies on processing methods (Bomkamp et al., 2022b). These scaffold biomaterials are animal- and plant-derived, as well as synthetic polymer biomaterials. ECM-enriched biomaterials such as elastin, gelatin, collagen, and fibronectin are all animal-derived biomaterials (Reddy et al., 2021), which are distinguished by their high extracellular matrix (ECM) content, ability to enhance cellular proliferation, and complete absorption by the human body. The second type of animal derived biomaterial, decellularized animal tissues, is mainly used in the biomedical field. Contrarily, plant-derived biomaterials are the best choice for developing meat biomaterials due to their nutritional content, low cost, good cellular compatibility, and perfect consumer acceptance (Ben-Arye, and Levenberg, 2019). For CM production, plant proteins, including soy, pea, zein, and glutenin, are abundant, competitive, and capable of being converted into scaffolding films with appropriate mechanical properties (Dong et al., 2004). Scaffold films, mainly produced from zein and glutenin, are used to stimulate the proliferation of stem cells and facilitate their differentiation into myotubes (Xiang et al., 2022). For the production of fibrous scaffolds, texture vegetable proteins (TVP) can be used (Bakhsh et al., 2022). Alginate, on the other hand, can be utilized to make self-assembling hydrogels with good mechanical characteristics, that disintegrate as cells move and secrete their own ECM (Sahoo and Biswal, 2021). For biomaterials that do not readily allow cell attachment, functionalization with short peptide sequences is a promising alternative. RGD-alginate is a well-studied tissue engineering system (Sandvig et al., 2015), and it could be a potential technique for cultured meat, however, additional peptide sequences that more closely approximate native ECM may be useful for attachment, migration, and maturation.

4.2.1 Scaffolding Types

1. Microcarriers

Microcarrier (MC) scaffolds are used for large-scale cell proliferation and are composed mainly of polystyrene, cross-linked dextran, cellulose, gelatin, or polygalacturonic acid (PGA) and coated with collagen, peptides containing adhesion motifs, or positive charges to promote cell adhesion. Their diameters are typically between 100 and 200 µm (Bodiou et al., 2020). According to Norris et al. (2022), larger MC enhances cell adhesion, while smaller MC results in higher growth rates because of increased shear stress. According to Bomkamp et al. (2022b), there are three possible scenarios for the use of microcarriers in cultured meat processing: first, cells are temporarily transported to MC to promote cell growth, and then they are withdrawn and processed. Second, a temporary carrier that dissolves or decomposes into free cells. Finally, the MC is an edible carrier that is incorporated into the finished product, which eliminates the costly cell harvesting steps and their corresponding yield losses, thereby reducing the cost of cultured meat. Hence, edible MC is a promising approach for cultured meat scaffolding that generates industry-scale cell mass while maintaining reduced costs (Levi et al., 2022). Edible biopolymers, including chitosan and alginate (Chui et al., 2019), starch (Zhang et al., 2017), zein (Li et al., 2016), and gelatin (Radaei et al., 2017), have been used to create MCs for various biomedical purposes. These MCs have the potential to be utilized in cultured meat production. For MC production, various technologies have been employed, primarily electrospray, spray drying, air jet milling, micro-grinding, dispersion polymerization, emulsion polymerization, photopolymerization, solvent evaporation, microfluidics, spherulitic crystallization, and air spraying (Morais et al., 2020). The production of microcarriers for cultured meat production is hampered by two challenges. The first is compliance with safety regulations regarding the use of food-grade and non-toxic crosslinkers, solvents, and surfactants in the formation of edible MC polymers. For instance, chitosan can be crosslinked using sodium tripolyphosphate (TPP) or genipin instead of toxic glutaraldehyde, and alginate can be crosslinked using CaCl2 instead of BaCl2. The final one is inadequate cell adhesion. To improve adherence, biopolymers can be modified with functional domains like RGD or integrin-recognized sequences (Yang et al., 2014), bioactive polymers (Chui et al., 2019), or crosslinked (Chui et al., 2019; Radaei et al., 2017; Zhang et al., 2017). Pig skeletal muscle, mouse muscle, and mouse adipose cells were grown on a 3D porous gelatin microcarrier (PoGelat-MC). Using 3D-printed molds and glutamine transaminase, minced pig muscle tissue was formed into centimetre-scale meatballs with excellent mechanical qualities and protein content (Liu et al., 2022). Additionally, MCs can be implemented in the production of cultivated flesh by facilitating cell proliferation or incorporating them into the final product. Matrix Meats (Brennan et al., 2021) and OMeat are two examples of commercial enterprises that have recently begun to develop edible MCs for use in cultivated meat.

2. Porous Scaffolds

Scaffolds with apertures that encompass a range of 10-100 µm (Zeltinger et al., 2001) are sponge-like structures that offer the mechanical stability necessary for seeded cells to establish tissues and deposit extracellular matrix. Moreover, porous scaffolds are particularly appealing due to their low cost and simple construction procedures. The scaffolds provide a three-dimensional platform for cell survival, proliferation, and maturation (Zhou et al., 2021). The size and distribution of scaffold pores play a vital role in cell culture during CM production. Larger pores are desirable for media perfusion as they facilitate the efficient transfer of nutrients and oxygen, mimicking the function of blood vessels (pseudo-vascularization) in CM (Singh et al., 2023). Additionally, a high surface-area-to-volume ratio and appropriate pore size contribute to achieving high cell density and help regulate cellular behavior (Carletti et al. 2011). However, the present porous scaffolds for cell-grown meat have challenges in matching food-grade material requirements with cell adhesion and proliferation capacities. Their cell differentiation efficiencies are also limited, which results in poor retention of typical meat properties like texture and nutrients (Chen et al., 2024). The selection of components and nutritional value is crucial for this scaffold, as it is designed to remain within the final product. Plant proteins, including soy (Ben-Arye et al., 2020), and plant polysaccharides, including cellulose (Abitbol et al., 2016), are frequently employed as scaffolding materials. The extrusion of soy protein powder results in the production of textured vegetable protein (TVP), a vegetable protein that is used as scaffolds for cultured bovine skeletal muscle. Coating the extruded TVP with fibrinogen enables effective adherence of bovine satellite cells, resulting in a cell seeding efficiency exceeding 80% (Ben-Arye et al., 2020). Moreover, the rapid freezing of a solution of hyaluronic acid and gelatin results in the formation of ice crystals concurrently with the formation of cross-links, which in turn leads to the formation of a porous scaffold that exhibited more than 90% porosity and was able to support the attachment, proliferation, and differentiation of porcine adipose-derived stem cells (Chang et al., 2013).

3. Hydrogel Scaffolds

Hydrogels are 3-D crosslinked hydrophilic polymer matrixes characterized by high water-absorption capacity. In this network, water is the dispersion phase and makes up at least 70% of the gel weight (Tan and Joyner, 2020). Hydrogels are created by the process of physically or chemically crosslinking synthetic, natural, or copolymers. Cell proliferation, motility, and differentiation are significantly influenced by hydrogel stiffness and diffusion kinetics. This is because an optimal rate of diffusion of micronutrients and signaling molecules is necessary to penetrate the hydrogel's thickness and reach the growing cells, which supports cell proliferation and growth. Conversely, high hydrogel stiffness restricts cell proliferation and migration (Freeman and Kelly, 2017). These hydrogels possess impressive mechanical capabilities, but they lack biocompatibility and adaptability. Additionally, they exhibit some level of cytotoxicity and may pose a risk to food safety (Ye et al., 2023). As a result, the focus of current research has shifted to natural polymer hydrogel. Natural polymer materials are typically derived from polysaccharides or proteins to form hydrogels (Ghanbari et al., 2021a). Proteins possess inherent advantages over polysaccharides in the development of hydrogels (Ghanbari et al., 2022). Proteins are composed of numerous amino acids, and numerous reactive groups can be employed as locations for chemical modification and crosslinking to generate polymer structures (Cuadri et al., 2016). Protein-based hydrogels have been widely developed and studied by researchers due to their excellent properties, which include high nutritional value, biocompatibility, biodegradability, adjustable mechanical properties, and low toxicity when compared to synthetic polymers (Farwa et al., 2022). Collagen, silk fibroin, and gelatin are among the most used protein hydrogel materials. However, most of these proteins are animal proteins with large application costs, and because of their complicated structure, structural alterations are frequently limited (Ghanbari et al., 2021b). Furthermore, plant-derived proteins may be safer than animal-derived proteins since they are less likely to transmit zoonotic infections (Surya et al., 2023). Soybean protein, being one of the most prevalent plant protein sources, is high in nutritional content, environmentally friendly, and available from a variety of sources, making it widely employed in the food business (Liu et al., 2023). Additionally, carrageenan, extracted from seaweed, can be utilized to produce food-grade hydrogels, which are extensively employed in meat processing (Yegappan et al. 2018).

4. Fibrous Scaffolds

Nanofibers are produced using spinning techniques such as electrospinning and have features that promote cell functions, including adhesion, penetrability, and a three-dimensional structure. Spinning processes can be utilized on various materials, such as soy (Phelan et al., 2020), gelatin (MacQueen et al., 2019), and polystyrene (Lerman et al., 2018). The fibres that are produced through spinning techniques are analogous to natural substances. The fibrous structure's advantage is its resemblance to the texture of a piece of meat, which can be advantageous for the 3D assembly of the meat and its flavor (Kolodkin-Gal et al., 2023).

4.2.2. Scaffolding Biomaterials

The structure and properties of scaffolds are influenced by the biomaterials used for scaffolding. Typically, scaffolding biomaterials have great porosity, biocompatibility, and ECM mimicking, as well as mechanical strength to direct cell adhesion, proliferation, and morphological changes (Sharma et al., 2015). Polysaccharides and proteins are two of the most frequent biopolymers with extracellular matrix-like properties. Edible biomaterials, derived from natural sources, have garnered considerable attention and are widely utilized in the field of cultured meat. This is mainly due to their abundant availability and the fact that they closely mimic real tissue in terms of chemical and biological properties (Su et al., 2021). Proteins and polysaccharides, which are polymers, are considered the fundamental components of scaffold biomaterials. These polymers may originate from natural or synthetic sources. Thus, polymer-based biomaterials are regarded as a promising option for scaffold fabrication (Khan and Tanaka, 2018). The FDA has certified many polymers for food use, confirming their safety. The edible polymers that have been approved by the FDA include pectin, chitosan (CS), gluten, gellan gum (GG), cellulose, gelatin (GL), collagen (COL), soy protein isolate (SPI), starch, glucomannan, and alginate (Alg) (Ali & Ahmed, 2018).

1. Natural Polymer (Biopolymer)

Natural polymers are materials derived from natural sources. They are divided into protein-based and polysaccharide-based biomaterials. Both types are naturally occurring polymers of animal and plant origin. Natural polymers are biocompatible, toxic-free, cell-adherent, and promote proliferation and differentiation. Despite this, their mechanical strength is low, and they are vulnerable to high temperatures (Del Bakhshayesh et al., 2018).

a. Protein-Based Biopolymer (Animal or Plant-Derived Protein Biopolymer)

Animal protein, plant protein, and fungal protein are the sources of protein-based scaffold biomaterials. Soy, pea, zein, and glutenin are plant proteins that are abundant, competitive, and capable of being converted into films with the necessary mechanical properties for the development of CMs (Dong et al., 2004). The growth of aligned cells and the subsequent development of aligned myotubes were effectively stimulated by the protein films composed of zein and glutenin (Xiang et al., 2022). So zein and glutenin may be promising candidates for future research in the production of CM. Textured vegetable proteins (TVP) are currently in high demand in a variety of culinary goods, including frozen dumplings, ham, sausages, and fish balls (Zhang et al. 2017). This suggests that global acceptance and satisfaction with TVP products are gradually increasing (Jones, 2016). Moreover, TVP may be implemented to generate fibrous scaffolds (Bakhsh et al., 2022). The absence of the arginine-glycine-aspartic acid (RGD) sequence in the plant protein scaffold hinders its ability to adhere to cells. To address this, Lee et al. (2022) applied a coating of fish gelatin/agar matrix onto textured vegetable protein, creating a more favorable environment for cell adhesion. Despite its high biodegradability and low cost, plant material has the potential to induce allergic reactions (Post, 2014). Hence, decellularized plants have lately been the focus of researchers interested in an alternative plant source (Thyden et al., 2022). Decellularizing tissues create an extracellular matrix with a vascular network that transports nutrients and oxygen (Contessi et al., 2020). Decellularized plant-based tissues show a natural fluidic transport system with plant arteries diverging from big, major veins into small capillaries. This system resembles mammalian tissue's branching vascular network (Harris et al., 2021). As a result, the unique structural properties of decellularized plant tissues were identified as promising scaffolding for cultured meat. To circumvent biopolymer-based edible scaffold problems, researchers have produced edible scaffolds from decellularized apple hypanthium and spinach leaves (Modulevsky et al., 2016; Jones et al., 2021). For cultured meat production, decellularized plant tissue scaffolds are a good choice because they do not contain any animal components, are cost-effective, ecologically friendly, easily scalable, and provide the necessary morphological and biochemical microenvironment for growing muscle cells (Jones et al., 2021). Broccoli, sweet pepper, spinach leaves, and green onion were among the plant tissues that were used for decellularization. Decellularized amenity grass was employed by Allan et al., 2021 for in vitro myoblast culture. The grass scaffold's striated topography facilitated the alignment and differentiation of myoblasts, which were preserved by their natural long, narrow structure and parallel vasculature system. Moreover, broccoli florets were chosen to serve as decellularized microcarriers in bioreactors to facilitate the scalability of cell proliferation (Thyden et al., 2022). Another protein biopolymer source is of animal origin. Animal-derived biomaterials, including elastin, gelatin, collagen, and fibronectin, have a high ECM content and promote cellular development. They are also fully absorbed by the human body. Nevertheless, scaffolds of a single material have inferior mechanical properties and certain collagen, produced from fish skin, and gelatin, derived from pigs and cowhide, components have challenges in terms of sustainability because of their high cost and vulnerability to ethical and environmental problems (Li et al., 2022b). A collagen gel-based meat model including smooth muscle cells (SMCs) was recently published by Zheng et al., 2021. In this model, SMCs reduced pressure loss, increased collagen, and made meat firmer, springier, and chewier than controls. These findings show that SMCs improve cultured meat texture by generating ECM proteins. Thus, another scaffolding biomaterial, such as a polysaccharide biomaterial, including alginate, is employed in conjunction with natural animal protein scaffolding, like Enrione et al., 2017 constructed an edible porosity scaffold utilizing freeze-drying technology that incorporated salmon gelatin, agar, and sodium alginate. This scaffold allowed muscle stem cells to adhere and grow, resulting in the necessary myogenic responses. Furthermore, by employing electrospinning, porcine gelatin, TG enzyme, and chemical crosslinking, the resulting microgelatin fibres facilitated the growth and alignment of muscle cells in a single orientation (Mendes et al., 2017). Moreover, Park et al. have recently devised a technique for producing enhanced cultured meat by utilizing fish gelatin's MAGIC powder and myoblast sheets. The powder, characterized by its edible gelatin microsphere (GMS) structure, displayed changes in shape and connection depending on the process of crosslinking. The researchers discovered that GMSs greatly improved the cultivation of myoblast sheets, resulting in more efficient cell sheets with meat-like properties compared to conventional methods. Due to the varied surface qualities resulting from crosslinking, the production of GMSs on a large scale was simply achieved. This research also determined that the quality of lab-grown meat, improved using GMS cell sheets, is similar in tissue characteristics to both soy-based meat and chicken breast (Park et al., 2021).

b. Polysaccharide-Based Scaffold Biomaterial (Animal or Plant-Derived Polysaccharide Biopolymer)

Certain plant polysaccharides, including alginate, pectin, konjac gum, and cellulose, possess the potential to serve as valuable biomaterials due to their physiological roles and excellent cellular adherence. In addition to plants, specific types of bacteria and algae also synthesize cellulose. Cass Materials, an Australian start-up company, is now investigating the application of fermented bacterial nanocellulose as scaffolding for CM. Initial studies have shown promising results, suggesting that muscle cells can attach to the very porous scaffolds and develop fibres (Le, 2020). Nevertheless, most of the cellulose-based scaffolds exhibited a porous structure, except for the green algae Cladophora, which was predominantly fibrous (Bar-Shai et al., 2021). Another potential scaffolding material for CM is alginate, a polymer made from brown algae. Pluripotent stem cells can be cultured in alginate-derived tubes, which are compatible with differentiation techniques and enable high cell densities and growth rates (Li et al., 2018). An edible three-dimensional scaffold (CS-SA-Col/Gel) composed of chitosan, sodium alginate, collagen, and gelatin were created by Li et al., 2022a. A robust cultured cell meat (CCM) model with strong adhesion sites was constructed utilizing a 3D 2-CS-SA-Col1-Gel scaffold that was created via freeze-drying and electrostatic interactions. This scaffold successfully promotes the growth of pig muscle cells. Not only that, but the look and texture qualities (such as chewiness and resilience) of this structured CCM model were quite like those of fresh pork.

2. Synthetic Polymers

Polyglycolic acid (PGA), polylactic acid (PLA), poly DL-lactic co-glycolic acid (PLGA), polycaprolactone (PCL), and polyethylene glycol (PEG) are synthetic polymers (Biswal, 2021). In contrast to natural polymers, synthetic polymers provide exceptional mechanical strength, which is essential for supporting tissue growth. However, these materials inhibit cell growth, generate hazardous chemicals after degradation, and have poor cell adherence (Tessmar and Gopferich, 2007). Furthermore, the hydrophobic nature and lack of cell recognition sites limit synthetic biopolymers, such as the arginyl glycyl aspartic acid (RGD) peptide motif (Tallawi et al., 2015), which is not yet approved for human consumption, limiting its use in in vitro meat production (Singh et al., 2023). Biomaterials in the food sector are limited because of their non-edible nature and susceptibility to deterioration, potentially toxic to tissue (Bomkamp et al., 2022b). The General Standard for Food Additives allows for a maximum amount of 1-70 g/kg of polyethylene glycol to be added (Codex Alimentarius Commission, 2021). Moreover, FDA-approved edible scaffolds include gelatin, chitosan, pectin, cellulose, starch, gluten, alginate, and glucomannan, all of which are natural biopolymers (Singh et al., 2023).

3. Self-Assembling Peptides (SAPs)

Self-assembling peptides (SAPs) have been investigated and utilized for tissue engineering scaffolds and 3D bioprinting materials due to their versatility and ECM-mimicking properties (Grey et al., 2022). SAPs are made up of monomers that can conform into structures according to the environmental features around them, allowing for use in a variety of functions (Lee et al., 2019). Self-assembly can be tailored for specific applications by changing the nature of peptide sequences, while more robust and complex materials with advanced design features are feasible by simple crosslinking with biological macromolecules (Hao et al., 2022). Amino acid side chains offer sites for chemical alterations, producing diverse supramolecular structures and adaptable hydrogels. These hydrogels can gain properties like shear-thinning, bioactivity, self-healing, and shape memory, expanding self-assembling peptide material applications. Supramolecular peptides can structurally assemble into nanofiber hydrogels based on distinctive building blocks. These hydrogels serve as nanomorphology-mimetic scaffolds for tissue engineering. Biochemically, peptide nanofiber hydrogels can have bioactive motifs and factors either covalently tethered or physically absorbed into them, providing various functions based on physiological and pharmacological needs (Hao et al., 2022). Self-assembling peptides known as CH-01 and CH-02 have been used to produce hydrogels that can act as scaffolds. The hydrogel was found to successfully mimic ECM and display a nanofibrous structure like that of collagen in natural meat. The hydrogels were able to support the adherence and proliferation of muscle myoblasts (Arab et al., 2018), suggesting a viable option in cultivated meat scaffolding. The utilization of SAPs in cultivated meat remains unexplored in the existing literature, despite their use in tissue engineering. This may be attributed to the high cost of conventional peptide synthesis, which could restrict the conduct of further research. Potential strategies to reduce the cost of SAP production for cultivated meat scaffolding include the optimization of current approaches by recombinant organisms. Additionally, cell-free systems (Zhao & Wang, 2022), which eliminate the necessity for microbial hosts, present another potential method for SAP production.

4.2.3. Scaffold Fabrication Methods

During the design and fabrication of the scaffolds, the practicality and requirements of the mechanical, biological, and physicochemical features are considered. Pore interconnectivity, form, pore size, porosity, strength, and degradation rate are critical factors that influence the manufacturing of scaffolds. The top-down and bottom-up approaches are the two main techniques employed in the production of scaffolds. The top-down technique involves the initial construction of scaffolds, followed by the subsequent embedding of cells within their microstructure. The top-down strategy for fabricating three-dimensional (3D) bio-scaffolds involves many processes, including electrospinning (Lannutti et al., 2007), phase separation (Liu and Ma, 2009), lyophilization (Eltomet et al., 2019), and self-assembly (Nie et al., 2017). Conversely, the bottom-up method emphasizes the creation of small-scale tissue components with precise micro-architecture, which are then combined to produce larger tissue structures (Lu et al., 2013). These building blocks can be created utilizing many methods, such as generating cell sheets (Lee et al., 2018), self-assembling cell aggregates (Napolitano et al., 2007), encapsulating cells in hydrogels (Wodarczyk-Biegun et al., 2016), and bio-printing cells (Park et al., 2017).

1. Solvent Casting

The simplest and most common method for scaffold synthesis is solvent casting, in which biopolymers are dissolved in an organic solvent, poured into a mould, and allowed to evaporate to form a thin, sheet-like scaffold (Deb et al. 2018). In the food sector, food-grade alcohol is the organic solvent of choice (Mancuso 2021).

2. Electrospinning

The electrospinning (ESP) technique creates a fibrous structure with fibre diameters ranging from 10 nm to microns, which could be exploited to build edible scaffolds for in vitro meat production (Seah et al., 2022). Nanofiber scaffolds in this approach are highly porous, have a large surface area, and mimic natural extracellular matrix properties. In addition to forming aligned fibres that may facilitate muscle fibre development, these nanofibers have the capacity to facilitate cell adhesion and oxygen and nutrient diffusion (via the spaces between fibers). Here, an electrically charged jet applies force to the tip of the needle, causing the polymeric droplet to form. The spinneret droplets burst and are stretched when they travel through the grounded collector from the spinneret tip when the charging solvent is subjected to high voltage, where an interplay between electrostatic repulsion and surface tension takes over. As the solvent began to evaporate, the jet finally hardened into nanofibers (Gañán-Calvo et al., 1997). A variety of scaffold biomaterials, such as polylactic acid (PLA), poly (lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), gelatin methacryloyl (GelMA), fibronectin, albumin, and gelatin, can be processed using spinning techniques (Bomkamp et al. 2022b). However, collagen, gelatin, whey protein, chitosan, cellulose, and starch are edible materials that can be considered in the food industry (Levi et al., 2022). Using immersion rotation jet spinning technology, MacQueen et al., 2019 cultured rabbit skeletal muscle cells and bovine aortic smooth muscle cells on a fibre scaffold composed of porcine gelatin, TG enzyme, and the chemical crosslinking agent EDC/NHS to produce meat-like products. The mature alignment of both types of muscle cells within anisotropic 3D muscle structure was confirmed by their adhesion to the gelatin fibers. This introduces a novel concept for the large-scale production of cultured meat.

3. Three-Dimensional Bioprinting (3D Bioprinting)

3D bioprinting is distinctive because it generates intricate and customizable structures in layers using 3D digital models created with computer-aided design (CAD) software. 3D bioprinting technology could be useful for both small-scale and large-scale production of customised cultured meat (Stephens et al., 2019). Additionally, light-assisted printing, inkjet printing, and extrusion printing are prevalent bioprinting techniques (Kacarevic et al., 2018). The majority of bioprinting processes use extrusion printing, which dispenses bioink as continuous filaments (fibres) instead of droplets using pneumatic pressure or a mechanical screw plunger. Bioink, a combination of various scaffold biomaterials, plays an essential role in 3D printing by creating the scaffolding necessary for the differentiation of stem cells into meat (Sun et al., 2018). Moreover, the printed scaffold offers a micro-milieu and a habitat for the developed muscle cells, which are usually cultivated in bioreactors that enable the transportation of nutrients on a large scale (Bishop et al., 2017). Vat photopolymerization-based, extrusion-based, and jetting-based bioprinting are the primary methods in 3D bioprinting. The bioink is deposited with high precision in extrusion-based bioprinting, resulting in customized 3D structures with excellent structural integrity. This is achieved through the continuous deposition of filaments. In extrusion-based bioprinting, the bioink is deposited with high precision, obtaining customized 3D structures with good structural integrity due to the continuous deposition of filaments. The entire process of bioprinting is carried out under the control of a computer (Ozbolat and Hospodiuk, 2016). Jetting-based bioprinting can produce ink droplets with a controllable size and low volume, depositing the ink in specific locations with high precision and without contact. Employing this technique, it is possible to use a variety of biomaterials as well as the incorporation of living cells (Li et al., 2020). Finally, Vat polymerization-based bioprinting is an emerging technology in the biofabrication of scaffolds applied in tissue engineering, used for its high resolution compared to other bioprinting technologies. Ink particles with a controlled size and low volume can be generated through jetting-based bioprinting, which deposits the ink in particular locations with high precision and without contact. This method enables the incorporation of living cells and a diverse array of biomaterials (Li et al., 2020). Lastly, vat polymerization-based bioprinting is a new technology that is being used in the biofabrication of scaffolds for tissue engineering. This technology is valued for its superior resolution in comparison to other bioprinting technologies. A cereal prolamin ink for 3D printing was generated using zein, a protein derived from barley and rye, and utilized to produce a fibrous framework that enables the attachment and growth of C2C12 and pig skeletal muscle satellite cells (Su et al., 2023). Moreover, Dutta et al. (2022 developed a bioink from alginate and gelatine-based hydrogel scaffolds with plant- or insect-derived hydrolysates for bovine myosatellite cells and produced cell-based pepperoni meat prototypes 20 × 20 × 5 mm in size. Also, Liu et al. (2021b) created a 15 × 15 mm 3D-printed structure for porcine skeletal muscle satellite cells using sodium alginate-gelatine and gelatine-methacrylate (GelMA)-silk fibroin. In a gelatine-based gel, Xu et al. (2023) produced a cell-based fish fillet measuring 20 × 12 × 4 mm utilizing piscine adipocytes and satellite cells. Prior studies highlight the benefits of integrating 3D bioprinting with biomimetic scaffolds derived from authentic tissue architectures to produce cell-based meat products. Recently, companies have used 3D bioprocessing technology for the biofabrication of cultured meat. 3D Bioprinting Solutions, in collaboration with KFC (Kentucky Fried Chicken), intends to manufacture and promote lab-grown nuggets. 3D Bio-Tissues Ltd. (3DBT) has formed partnerships with CPI (Independent Centre for Technological Innovation) and the United Kingdom government's High-Value Manufacturing Catapult to enhance cell culture media for the cultured meat business. Nissin Food Holdings, a Japanese company, is collaborating with the University of Tokyo to create printed meat cubes (State of the Industry Report, 2022). 3D printing technology has been used to successfully make several food varieties, such as chocolate, cakes, and breads, in addition to cultured meat (Li et al., 2022).

4. Freeze-Drying (Lyophilization)

Freeze drying is a method of drying polymeric solutions in which a substance is frozen to an extremely low temperature and then the surrounding pressure is lowered, allowing the frozen water to sublime (Deb et al., 2018). The process is distinguished by three distinct steps: the initial step involves the preparation of the polymer solution; the second step involves the casting or molding of the polymer solution; and the final step involves the freezing and drying of the polymer solution at low pressure. Sublimation and desorption are employed to extract the ice and unfrozen water, respectively, during the third phase. Freeze drying can generate scaffolds with pore sizes ranging from 20 to 200 μm and a porosity of approximately 90%. Temperature, polymer concentration, and freeze rate regulate the size of pores (Deb et al., 2018; Seah et al., 2022). So far, the freeze-drying technique has been used for preparing the scaffold using synthetic non-edible and edible polymers, which should be further investigated and replaced with plant- and biopolymer-derived edible scaffolds (Shit et al., 2014; Bomkamp et al., 2022).

4.3. Cells Scaling Up

Massive cellular proliferation is essential to producing cultured meat. There are two types of scaling up cells: the first is scaling up based on scaffolding material and the second is a scaffold-free approach.

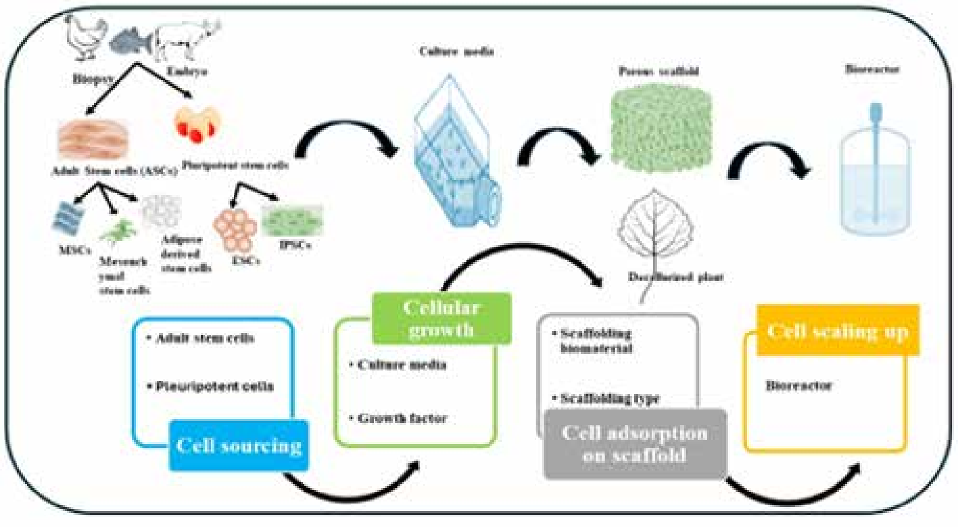
a. Cells Scaling Up Based on Scaffold Material

Bioreactors, a fundamental cell expansion technology, are responsible for the provision of the requisite stimuli and capacity to achieve the scale-up of cell sources for cultivated meat production. They are a controlled environment that contains a nutrient-dense medium containing essential amino acids, carbohydrates, and growth factors. It can also facilitate nutrient diffusion and cell development by stimulating or agitating the cells to promote their maturation and proliferation. In the initial phases of cultivated meat production, when cell proliferation is a top priority, a bioreactor is indispensable for the facilitation of large-scale cell culture as well as the simplification of medium recycling and replacement during the proliferation stage. Optimal culture conditions can be ensured by managing the biological conditions. To guarantee that the media is saturated with dissolved oxygen, oxygen can be introduced through sparges or upstream aeration. The pH is maintained at 7.2–7.4 by monitoring the carbon dioxide concentration as detected by sensors (Santos et al., 2023). Currently, the three primary bioreactor varieties are classified according to the method of medium introduction into the main vessel of the bioreactor: batch, fed batch, fed batch, and continuous (Spier et al., 2011). Also, the bioreactors can be classified according to how they mix their contents. Adding mixing to the bioreactor system promotes cell growth and development. Mechanical bioreactors mix with agitators or impellers. Currently, stirred tank bioreactors are the most common bioprocess scale-up bioreactors (Martin et al., 2004). Stirred tank systems are a viable bioreactor type for scaling up cultured meat production due to their proven reliability and scalability. The fundamental challenge in these societies is to ensure efficient delivery of nutrients and the elimination of waste at high cell concentrations. Bioreactor yield and operating cost are both affected by cell density, making it an important operational aim. Consequently, developing media formulations that sustain fast growth while reducing the formation of growth-inhibitory compounds such as ammonia is a significant biological problem (Kolkmann et al., 2022). Interventions to metabolically remodel cells for improved bioprocess adaptability can be better designed with an understanding of the mechanisms involved in nutrition and waste metabolite sensing (Shyh-Chang and Ng, 2017). While glucose is essential for anabolic activity, too much of it might inhibit cell growth (Furuichi et al., 2021). When glucose is present, it activates mTORC1, which in turn increases cellular anabolic responses (Leprivier and Rotblat, 2020). To keep growth rates constant, amino acid supplementation is essential, as it converges on mTOR signaling as well. Bioprocess optimization could be achieved by tinkering with the mTOR signaling network, which regulates cell size, proliferation rate, and glucose intake rate, among other cellular responses (Dreesen and ussenegger, 2011). Therapeutic options to target abnormally growing cells have emerged via inhibitor screening methods (Brüggenthies et al., 2022). Improving mTOR signalling in cultured meat through genetic or pharmacological means is an uncharted territory that could lead to fruitful discoveries in the long run, since cell proliferation is the end goal.

b. Scaffold-Free Approach (Cell Layering and Self-Assembly)

Layer-by-layer (LbL) assembly is a versatile and straightforward method for creating multilayer structures by self-assembly. It is feasible to create multilayer coatings with a precise structure and composition using a wide range of materials that are readily accessible. These coatings have various uses in the field of biomedicine (Zhang et al., 2018). This production process is rapid and easily expandable, and it has the capability to fabricate densely packed, multicellular, and textured tissues using standard culture plates without the need for a bioreactor. The three fundamental techniques for cell layering are stacking cell sheets, rolling a cohesive tissue sheet, and in situ deposition of cell-laden biomaterials. The initial method involves the utilization of a culture dish coated with a temperature-responsive polymer to create a tissue with many layers. The second approach involves enveloping an entire section of a slender tissue sheet around a cylindrical support and cultivating it until tissue fusion occurs. The third strategy involves the utilization of a handheld device to apply cell-laden biomaterials (Jo et al., 2021). Biomaterials are employed to enhance or inhibit cell adhesion, regulate cellular phenotypes, and offer three-dimensional structures for cell culture or co-culture. The biomaterials used for LbL assembly encompass a variety of substances, such as biomolecules, polyelectrolytes, particles, and colloids (Zhang et al., 2018). The successful co-culture of myoblasts and preadipocytes has already proven the possibility of using this method to construct meat-like tissues of varying dimensions and thicknesses. Scaffolds are unnecessary as the cells generate their own extracellular matrix (ECM), which remains intact and forms strong layers (Shahin-Shamsabadi and Selvaganapathy, 2022).

Figure 2. Steps of processing and technology of cultured meat



MSCs: myosatallite stem cells, ESCs: Embryonic stem cells, IPSCs: induced pluripotent stem cells.

5. ALTERNATIVES TO CULTURED MEAT

There are several alternatives to existing animal products in terms of food protein and energy sources:

5.1 Insects

In terms of converting biomass into protein or calories, edible insects can be produced more efficiently than conventional animals and potentially become a significant source of nourishment for humans (Tabassum-Abbasi et al., 2016). They include a lot of vitamins, protein, and fat (Nowak et al., 2016). Insects are more efficient at converting feed into consumable food than conventional meat, which only consumes 40% of the live animal weight. The fact that insects consume up to 100% of their feed partially explains this. As they are poikilothermic, insects consume less energy because they do not use their metabolism to heat or cool themselves. Compared with traditional cattle, they often have increased fertility, potentially yielding thousands of offspring (Premalatha et al., 2011). Rapid development rates and the fact that insects can achieve maturity in days, as opposed to months or years also contribute to efficiency (Alexander et al., 2017). A bone isotope study showed that insects have been a staple of human evolution (de Magistris et al., 2015), and a variety of species are currently consumed (> 2000 species) (Rumpold and Schlüter, 2013). However, the problem of limited consumer acceptance is widespread, especially in Western countries. The countries where a shift from eating animal products to eating insects would have the biggest effects are also the ones with high rates of animal product consumption per person. There are already indications that consumer perceptions may be beginning to shift in developed nations like the United States and the United Kingdom (Alexander et al., 2017). For instance, in the European Union, laws about novel foods and the permissible status of foods derived from insects dictate that insects cannot be processed and must instead be sold whole (de-Magistris et al., 2015). Insects are parasites or scavengers that primarily feed on grains. Their ability to scavenge indicates that they probably carry a variety of pathogens. Therefore, in addition to the standard food safety regulations for the development of insect-based foods, perceived pathogen risk overshadows the nutritional benefits of insects. The pathogens that could emerge in colonies that have been raised are not well understood. A recent study found that industrially raised mealworm and cricket samples, primarily composed of members of the Bacillus cereus group, contained a bacterial endospore fraction. Additionally, results indicated that norovirus genogroup II, hepatitis A virus, and hepatitis E virus were not found in the sample collection, suggesting a low risk of these viral pathogens affecting food safety (Vandeweyer et al., 2020). Inadequate handling, inappropriate culinary treatment, and eating insects at the wrong developmental stages are additional risks associated with eating edible insects (Kouřimská & Adámková, 2016). The EFSA states that the type of insect, the substrates utilised, the feed added to the rearing colonies, the production process, and the stage of harvesting of the insects all have a significant impact on the frequency and degree of contamination in insects and insect-based food products. Foods derived from insects and edible insects collected in the wild are also concerned about pesticide residues. Because the kinds of organic materials that wild insects eat are not regulated, they may eat pesticide-treated vegetation or crops, which could cause the pesticides to bioaccumulate in their tissues (FESA, 2015).

5.2 Microalgae

With an estimated 200,000 to 800,000 species, microalgae are a varied group that may grow quickly in a variety of settings when photoautotrophic conditions are met (Koyande et al., 2019) and can be cultivated in both warm and cold areas because of their remarkable resilience to harsh climatic conditions. Microalgae have demonstrated superior yields compared to those of conventional crops. By consuming nutrients found in wastewater, microalgae lessen their reliance on chemicals and freshwater (Brennan & Owende, 2010). Another significant feature that improves the use of microalgae as a continuous source of protein is their broad resistance to high pH and salt concentrations. Moreover, microalgae exhibit a remarkable capacity to concentrate vital nutrients and useful chemicals necessary for human well-being (Wells et al., 2017). These qualities have identified microalgae as one of the most reliable sources of protein. These were the subjects of studies conducted in the latter part of the 20th century that dealt with alternative agriculture and food. Up to 50–70% of the dry weight of microalgae is protein; this contrasts with 17.4% of beef, 19.2–20.6% of fish, 19–24% of chicken, 27% of wheat germ, 36% of soybean flour, and 47% of eggs (Koyande et al., 2019). The protein content of some high-protein microalgae (as % dry mass) has been reported as 60–71% (*Spirulina maxima*); 63% (*Synechoccus sp.*): 42–63% (*Spirulina platensis*), 53% (*Chlorella pyrenoidosa*), 57% (*Dunaliella salina*), 49% (*Dunaliella bioculata*), 52% (*Tetraselmis maculata*), 50–56% (*Scenedesmus obliquus*), 43–56% (Anabaena cylindrica), and 48% (*Chlamydomonas rheinhardii*) (Wang et al. 2021). Challenges: Lack of knowledge about the health benefits of microalgae and limited incentives for producers are major barriers to the successful use of this alternative protein source. Microalgae production has a minimal carbon footprint; if these challenges are successfully overcome, the use of microalgae in the food and nutraceutical industries could help meet the dietary protein needs of the growing world population and address climate change (Koyande et al., 2019). The unappealing taste, smell, and colour of microalgae alter the organoleptic qualities of processed foods, which presents a challenge for food scientists attempting to use them as food or as a source of ingredients (Verni et al., 2023). Another problem that needs to be solved to maximize the nutrient content of algae is the extraction of nutrients from microalgae. Bioactivity, bioavailability, and bioaccessibility are crucial factors to consider. The extracellular matrix (ECM), which is made up of pectin, cellulose, alginic, fibrillary peptidoglycan layers, and some polysaccharides (i.e., cellulose, hemicelluloses such as xyloglucan, mannans, glucuronan, beta-glucan, and lignin), is a multilayered, highly complex cell wall found in microalgae (Quesada-Salas et al., 2021). Sorting out the species that can react to cell wall disruption techniques is important because different microalgal species have varying degrees of cell wall complexity. Consequently, when selecting the microalgae species to be used as protein extraction subjects, it is essential to delve into the parameters that represent ease of cell disruption and protein recovery (Fatima et al., 2023).

5.3 Imitation Meat

Without utilizing meat products, imitation meat or meat analogues aim to replicate particular types of meat, including nutrients and aesthetic characteristics (such as texture, flavour, and appearance). The most popular fake meats, such as tempeh or tofu, are probably those made from soy (Malav et al., 2015), which has been producing and consuming tofu or soybean curd from coagulated soy milk for centuries. It can be further cooked to more closely resemble meat products in terms of flavor and texture. For example, flavoring can be added to make it taste like gammon, sausage, chicken, beef, or lamb (Malav et al., 2015). Soy and tofu contain high levels of protein while being low in fat (Sahirman & Ardiansyah, 2014). The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) of soy and beef is similar, suggesting that their protein values in human nutrition are comparable (Reeds et al., 2000).

5.4 Aquaculture

Salmon and other carnivorous fish can eat up to five times as much fish as they eventually produce as feed (Ma et al., 2009), which poses a challenge to the growth of farmed carnivorous fish (Diana, 2009), thereby lowering the likelihood of significant replacement with current animal products. Because herbivorous and omnivorous species have much lower "fish-to-fish" conversion ratios—carp, for example, currently has a ratio of 0.1, and further reductions are predicted, this problem is less severe for these species (Tacon & Metian, 2008) because consuming feed derived from fish is not necessary for their nutrition—two-thirds of aquaculture's total output comes from freshwater systems, which dominate production. The primary species are either herbivorous or omnivorous, with carp producing the most, though more recently, tilapia and catfish have also become more popular (Bostock et al., 2010).

6. OPPORTUNITIES AND CHALLENGES

6.1. Opportunities for Cultured Meat

With the introduction of enriched and functional foods, consumers are more open to trying goods modified to have specific nutritional qualities (Burdock et al., 2006). There are numerous methods available for producing designer meat in vitro. It is possible to modify the culture medium's composition to affect the flavor of cultured meat and fatty acid makeup (Bhat et al., 2013), and the health benefits of meat can be increased by incorporating elements such as specific vitamins into the culture medium, which may have a positive impact on health, and co-culturing with different cell types may improve the quality of the meat. Supplementing with fats after production allows for better control over the ratio of saturated to polyunsaturated fatty acids and overall fat content (Bhat et al., 2013), and in vitro production systems have significant environmental potential. Because the circumstances in an in vitro meat production system are controlled and manipulable, it will not only significantly lessen ecological hazards but also ensure sustainable production of designer, chemical-safe, and disease-free meat (Bhat & Fayaz, 2011). In vitro, meat has the potential to significantly lessen animal suffering and eliminate the need for animal consumption (Hopkins & Dacey, 2008). Since a product from a bioreactor is not subject to the same environmental fluctuations as animal products and is not location- or soil-specific, it presents opportunities for new production locations or alternate land uses. This makes it a more dependable alternative. Moreover, animals are notoriously unreliable as a raw material to produce meat from a commercial standpoint due to illness, stress, and uneven growth (Bhat & Bhat, 2011). In the current meat production systems, it takes several weeks rather than months (for chickens) or years (for pigs and cows) before the meat can be harvested. The growth of meat in in vitro systems requires much less time than traditional meat production methods. This implies that less time will be needed to maintain the tissue, which will result in less feed and labor being needed per kilogramme of in vitro cultured meat (Bhat et al., 2013).

6.2. Challenges of Cultured Meat

While many people support in vitro meat because of its potential benefits to the environment and climate, as well as because animal activists support it, there are also concerns and criticisms surrounding it (Welin, 2013).

1. Sensorial Characteristics

The color and appearance of in vitro meat might be challenging to compete with those of conventional meat. In 2013, a sensory panel at London's Riverside Studios reported that the cultured meat produced and tasted was colorless. A small amount of red beetroot juice and saffron were added to the meat to improve its color (Bhat et al., 2015). Therefore, it is necessary to develop new meat processing technologies to improve the flavor and appearance of in vitro meat products. Initially, scaffold-derived tissue monolayers and yolk-like blobs of self-assembling muscle fibres were used to produce in vitro meat, which was subsequently used to prepare communal meat products. Nonetheless, numerous attempts have been made to employ tissue engineering techniques to create more enticing meat products by seeding scaffolds with muscle cells to produce the final product. Additionally, there have been attempts and proposals to develop scaffolds that enable 3-D tissue culture and complex meat structuring using edible and natural biomaterials like collagen (Hopkins & Dacey, 2008).

2. Alienation to Nature

The fact that the in vitro meat production system might make us more aloof from animals and the natural world and contribute to our urbanization is another issue. Cultured meat is compatible with our growing reliance on technology, which raises concerns about our growing alienation from nature (Welin, 2013). Less land will be impacted by human activity if livestock farming is abandoned, which is beneficial for the environment but may also drive humans away from it (Bhat et al., 2015).

3. Cost of Production and Economic Disturbances

The primary potential barrier is the extraordinarily high cost of cultured meat, although market penetration and large-scale production are typically linked to sharp price reductions. Industrial-scale in vitro meat production is only possible if a reasonably affordable method for producing a product that is qualitatively comparable to already available meat products is developed and given government support similar to that given to other agribusinesses (Bhat & Fayaz, 2011), and the economies of these countries that depend on the export of meat to other countries and engage in large-scale conventional meat production will undoubtedly be impacted by the production of meat in vitro. Employment in the agricultural sector will be affected by this technology in nations where cultured meat production has been widely adopted. These production centers will lessen environmental pollution because they are close to cities, which will save transportation costs, but perhaps this will not be so good for the countryside (Bhat et al., 2015).

4. Social Acceptance

One of the biggest obstacles to the public's acceptance of cultured meat is its unnaturalness (Welin, 2013). In vitro meat's unnatural nature worries some potential customers, but as Hopkins and Dacey point out, just because something is natural does not mean it is good for you (Hopkins & Dacey, 2008). Furthermore, consumers may view in vitro meat as fake meat rather than the real thing, which would make them devalue it similarly to how they would artificially flowers or synthetic diamonds (Hopkins & Dacey, 2008). Many opponents of the idea of producing meat in vitro are concerned that because this technology can culture human muscle tissue, it may lead to cannibalism with fewer victims (Hopkins & Dacey, 2008). Another argument is that original cells obtained from an animal in a morally dubious manner must be used to create in vitro meat and that doing so will morally contaminate all subsequent generations of tissue (Hopkins & Dacey, 2008).

7. LEGISLATIVE CONSIDERATION

7.1. Regulations Consideration

In numerous countries, the production and sale of cultured meat (CM) lacks clearly defined rules. So, food regulatory agencies should develop rules to promote the acceptance and commercialization of cell-based meat. In November 2018, the FDA, USDA, and Good Food Institute released a joint statement on CM rules (Bryant, 2020), indicating that a collaborative agreement might alleviate consumer trust concerns and promote future confidence. In 2019, the USDA and FDA announced a formal agreement to regulate cell-cultured meat products from livestock and poultry cell lines, adding clarity to the US regulatory approach. According to the agreement, the FDA will have regulatory authority over components of the manufacturing process that occur prior to cell harvest and product development. These stages include cell line isolation, selection, and banking, as well as cell proliferation and differentiation into specific tissue types. The USDA will oversee processing items downstream from harvest, including product testing, inspections, labelling, and safety evaluations (Fish et al., 2020). Regulatory approval has been granted to new products, including poultry breasts, one year later (Kantono et al., 2022). However, CM-producing countries have implemented thorough legislative measures to ensure consumer safety. The Singapore Food Agency (SFA) has released guidance on its safety assessment requirements for novel foods, outlining specific information submission requirements for the approval of cultured meat products. The sale of cultured chicken meat from Eat Just Inc. was authorized by the SFA on December 1, 2020, marking the first-ever approval of cultivated meat worldwide (Barbosa et al., 2023). In Europe, the EU Novel Food Regulation (EUNFR) specifically covers food products that are created using tissue culture techniques. Consequently, CM must obtain formal clearance from the European Food Safety Authority (EFSA) before it can be available for sale. Nevertheless, the precise details regarding the required tests and certifications for this product, such as food safety, disclosed components, and nutritional value, are still uncertain (Chodkowska et al., 2022). Moreover, Regulation (EC) No. 178/2002, General Food Law (GFL), outlines the process for awarding food permits in the European Union. The Novel Food Regulation (Regulation (EU) No. 2015/2283) governs the pre-marketing authorizations for foods derived from animal cells or tissue culture. Genetic engineering in the production of cultured beef may trigger the application of the Regulation on Genetically Modified Food and Feed (Regulation (EC) No. 1829/2003). Nevertheless, the United Kingdom (UK) has ceased to participate in the EU's common food authorization procedures. To sell its products in the United Kingdom, any cultured meat company must apply for authorization from the UK Food Standards Agency (FSA) starting in May 2021

(Barbosa et al., 2023). Food Standards Australia New Zealand (FSANZ), a legislative authority responsible for developing food standards in Australia and New Zealand, has recently suggested the inclusion of cell-based meat in their current Food Standards Code. Nevertheless, it is imperative to obtain specialized premarket certification (Tingwei et al., 2019). To sell their products in any country, cultured meat manufacturers must submit for inclusion in the approved new foods list, as per FSANZ regulations regarding novel foods. This necessitates an evaluation of the safety of the production process by FSANZ, which is anticipated to last for a minimum of 14 months. The purpose of the safety assessment is to verify and establish that the product does not pose a health risk (Barbosa et al., 2023). In Canada, the submission of comprehensive information in a pre-market approval application is required for cultured meats, which are classified as novel foods. This information must include molecular characterization, nutritional composition, toxicology, allergenicity, and the types and levels of chemical contaminants, all of which serve as evidence that the food is safe for consumption. However, in Japan, cultured meat has an existing regulatory structure and may not require pre-market approval, depending on the production procedure. The Japanese government is building a regulatory framework to ensure food safety and customer acceptance.

7.2. Religious Consideration

Religion may influence how people perceive CM. In Judaism, some rabbis would consider CM Kosher if the cells came from a Kosher slaughtered animal (Kenigsberg and Zivotofsky, 2020). According to Islamic views, the use of CM is permitted (halal) if the cells are obtained from an animal slaughtered in accordance with Islamic dietary laws, and the growth medium used to produce the cells is also halal (Verbeke et al., 2015). Contemporary jurists believe that cultured meat is Halal only if the stem cells are sourced from a Halal animal and no blood or serum is used (da Silva & Conte-Junior, 2024). However, Jews, Muslims, and Buddhists showed less preference for CM compared to traditional meat. Moreover, Hindus are likely to consider cultured meat as a way of avoiding harming animals, and some may decide it is permissible to consume as long as it is not beef, as cows are considered sacred animals (Kenigsberg and Zivotofsky, 2020). According to religious standards, Christian people can consume any type of meat because it is considered clean. Consequently, cultured meat may be consumed if it is available on the market (Jagadeesan & Salem, 2020).

8. CULTURED MEAT MARKET

8.1. Customer Preference

The major obstacle to the development of CM is the lack of customer preference. Furthermore, consumer demographics, including gender, age, country of origin, eating patterns, and other social parameters, also influence meat consumption (Liu et al., 2023a). Cultural attitudes towards CM will exhibit substantial variation, as indicated by certain studies. Nevertheless, CM effectively reduces greenhouse gas emissions (GHG), ensuring that consumers recognize CM as an environmentally sustainable option (Bryant et al. 2019). Moreover, as the occurrence of zoonotic infections such as salmonella and *E. coli*, which are linked to conventionally produced meat (Bryant et al., 2019), has decreased in the CM, certain customers consider the CM a safe choice. Furthermore, with a lower fat level in CM (Bouvard et al., 2015) than traditionally farmed meat, approximately 28.6% of consumers in Europe judged CM to be healthy (Kantono et al., 2022). Also, consumer attitudes towards the prospect of cell-cultured meat differ greatly among regions. In Europe, more attention is being paid to meat production's environmental, sustainability, and animal welfare issues, but it is uncertain how much of this attention will change consumer behaviours or receptivity to meat alternatives (Santeramo et al., 2018). In 2013, a questionnaire poll was conducted on the first cultured beef burger manufactured in the United Kingdom. About two-thirds of consumers expressed an interest in consuming it (Guardian, 2013). Surveys done in the United States and Italy revealed that two-thirds and 54% of respondents, respectively, were willing to try (WTT) cultured meat (Mancini and Antonioli, 2019). In a study conducted to assess consumer attitudes towards cultured beef in Germany (n = 1000) and France (n = 1000), most consumers expressed a willingness to purchase cultured meat as an alternative to traditional meat when it became available (Bryant & Barnett, 2020). A global sample of customers (n = 3,091) from numerous countries, including China, the United States, the United Kingdom, France, Spain, the Netherlands, New Zealand, Brazil, and the Dominican Republic, were surveyed to determine their tendency to try and purchase cultured meat. Food curiosity, the importance of meat in a diet, and consumers' realistic perception of cultured meat as a viable option all had a positive impact on their willingness to test, buy, and pay more for it (Rombach et al., 2022). In South Africa, Tsvakirai and Nalley (2023) examined how psychological motivators and deterrents affect customers' desire to sample cultured meat. The study found that implicit views influence neophobic and neophilic attitudes, while concerns about social, cultural, and economic disturbances may prevent adoption. Additionally, several customers studied expressed reluctance to support sustainable lifestyles due to associated costs. However, they argue that the government should coordinate these efforts. Despite this, Morais-da-Silva et al. (2022) found that 58.8% of respondents preferred plant-based protein over cultured meat. Furthermore, based on their perception of cultured meat as being unnatural compared to traditional meat, 204 customers showed limited acceptance of it (Siegrist et al., 2018). In general, the willingness to try, eat, or pay is regulated by the respondents' age, gender, degree of education, and countries of origin. However, we found several interactions between these factors. African respondents from the richest and most educated countries were more WTT cultured meat (Kombolo Ngah et al., 2023). In terms of health, taste, and naturalness, according to Francekovi'c et al. (2021), the term cultured meat elicits curiosity but can also cause emotional resistance, particularly in Croatia, Greece, and Spain, where the proportion is lower. Surveys conducted in the United States and Italy showed that 2/3rds and 54% of respondents, respectively, were willing to try cultured meat. Additionally, vegetarians believe that cultured meat is morally justifiable and could serve as a healthy substitute for meat. Vegetarians feel cultured meat is morally acceptable and a healthy meat substitute. In addition, research by Chuah et al. (2024) suggests that customers' preferences and willingness to pay (WTP) can be greatly influenced by education on the benefits of cell-based seafood. This, in turn, could increase the marketability of these products. Several factors influence customer preferences, including the following:

1. Gender

In comparison to women, men exhibit a higher level of interest, a willingness to try (WTT) (Bryant et al., 2020), and a WTP (Kantor & Kantor, 2021) for cultured meat. Nevertheless, in Germany and, particularly, in France, women exhibit a lower level of willingness to consume cultured meat than males (Bryant et al., 2020). On the other hand, gender did not influence the willingness to eat and willingness to try (WTE and WTT) of cultured meat among African respondents (Kombolo Ngah et al., 2023).

2. Country of Origin

The likelihood of purchasing cultured meat is higher among Chinese citizens (59%), followed by Indians (56%), than among Americans (30%), which implies that government incentives for investment are in place (Bryant et al., 2019). Most French consumers believed that cultured meat lacked health benefits, taste, and natural features (Hocquette et al., 2022). Cattle are considered sacred animals in India, where cultured meat is widely consumed. While countries with large Muslim populations, including Malaysia, Qatar, and Indonesia, consider cultured beef to be Halal (Hocquette et al., 2024). Moreover, approximately 54% of respondents expressed an interest in trying cultured meat in a study conducted for Italian consumers (n = 525) (Mancini and Antonioli, 2019).

3. Age

Younger respondents exhibit higher WTT, WTE, and WTP than older respondents (>31 years of age) in Brazil (Chriki et al., 2021). However, in Europe (Grasso et al., 2019) as well as France and Germany (Bryant et al., 2020), elder respondents (65 years of age or older) show a low acceptability of cultured meat.

4. Income

In Brazil, respondents with the lowest monthly income (<3000 BRL) showed higher acceptability (WTT, WTE, and WTP) of cultured meat compared to those with the highest income (>15,000 BRL) (Chriki et al., 2021). Moreover, the initial cost of "artificial meat" will be higher, making it unaffordable for many consumers, particularly those in Africa (McKinsey, 2022).

5. Education

Cultured meat, a novel technique, will necessitate more trained and competent personnel. Education will thus play a critical role in ensuring that cultured meat is produced globally. Additionally, education had an interaction effect with income on WTP and WTE. This is since education is more readily available to individuals with higher incomes, particularly in certain developing countries (Kombolo Ngah et al., 2023). Food neophobia is more prevalent among respondents with lower levels of education (van den Heuvel et al., 2019).

8.2. Market Capacity

The cultured meat market is gaining traction as a sustainable, long-term alternative to food production. The expansion of this sector is indicative of the pursuit of more ethical solutions as well as the potential economic impact on countries that integrate this technology into their production models in the short and long term (da Silva & Conte-Junior, 2024). Animal cell-based meat, poultry, and seafood products that are analogous to traditional products are ready to be introduced into the market (Dolgin, 2020). Currently, over 150 organizations globally are engaged in the development of technology, either by contributing resources or manufacturing final products. The total amount of committed capital in this endeavor is projected to reach $2.8 billion by 2022 (Good Food institute, 2022). The initial cultured chicken nugget product has obtained regulatory permission for commercialization in Singapore (Singapore Food Agency, 2020), and the development of regulatory procedures for these goods is underway in numerous other regions. The United States Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) Food Safety and Inspection Service (USDA-FSIS) have officially agreed to collaborate in the regulation of cell-cultured beef and poultry products. The FDA will have exclusive jurisdiction over the regulation of seafood products (Post et al., 2020). Sustainable alternatives to traditional beef meet the increasing demand for ethical and environmentally sustainable diets. To date, there are 32 emerging start-up companies in the cultured meat production sector. Out of these, 25% are dedicated to producing cultured cattle, 22% to cultured chicken, 19% to cultured pigs and shellfish, and 15% to cultured exotic meat. North America accounts for 40% of the companies, followed by Asia (31%), and Europe 25% (Kumar et al., 2021). Startup companies and research groups worldwide are exploring cultured meat technologies to make them more accessible to customers, with the majority of groups based in the United States and Europe (Rubio et al., 2020). Mumbai, the first city in the world, hosted the first laboratory-grown meat research Centre, the Centre for Excellence in Cellular Agriculture, which cultured animal cells extracted painlessly. Their research focuses on developing and optimizing the most important cell lines to improve this sector (Porto and Berti, 2022). Australia, on the other hand, is the most recent nation to have entered the emerging laboratory-cultured meat industry, with two producers. Vow Food, a biotechnology start-up headquartered in Sydney, is one such producer that has secured approximately USD 20 million in venture funding. Vow Food's objective is to replicate meats that are currently produced and marketed on a large scale in Australia, with an emphasis on premium quality (Young et al., 2022). Glen Neal, the general manager of risk management and intelligence at FSANZ (Food Standards Australia and New Zealand), has indicated that cultured meat may be available for purchase in 2023 (Bowling, 2022). Singapore stands out in the global cultural meat landscape due to its limited production and export of traditional meat. Moreover, in 2023, cultured meat will become an essential part of the U.S. food chain. Brazil, a major meat producer and exporter, is currently engaged in innovative research on the production of cultured poultry meat, with sensory and nutritional analyses anticipated to be fulfilled by 2024 (EMBRAPA, 2023a). UPSIDE Foods, founded in 2015, was the inaugural CM startup. Since that time, there has been a rapid and significant expansion, resulting in the establishment of numerous enterprises in over 20 countries. In 2021, at least 21 new companies debuted, representing tremendous growth, as there had previously only been 86 CM enterprises (Santos et al., 2023). In Europe, the Netherlands, the origin of CM, committed EUR 60 million in financing in April 2022 to promote the construction of a national cellular agriculture ecosystem through the National Growth Fund. Spain made a large investment of EUR 5.2 million in a CM project managed by BioTech Foods in 2021 (Vegaconomist,2022). The Good Food Institute cites an increase in investment in cultured meat enterprises, demonstrating the production sector's confidence in this emerging market. In 2022, governments around the world offered significant funding and grants to cultured meat businesses, primarily for research and development initiatives. Global investments in cultured beef totaled $896 million. Several countries spearheaded the investment push, including Australia, China, the European Union, India, Japan, New Zealand, Qatar, Singapore, South Korea, Spain, the United Kingdom, and the United States (Bomkamp et al., 2022a). In 2021, invested capital increased by approximately 336% from 2020, reaching USD 410 million (GFI, 2021). The global cultured meat industry is anticipated to grow to $0.20 and $0.39 billion by 2023 and 2027, respectively, up from $0.16 billion in 2022 (da Silva and Conte-Junior, 2024). Moreover, the United States has made substantial investments in the production of cultured meat, with 43 companies currently engaged in the research of this technology, as indicated by data from the Good Food Institute (GFI) (Bomkamp et al., 2022a). Scaling remains a prominent obstacle in the industry, as it is crucial for lowering prices in the commercialization of cultured meat. In 2022, multiple collaborations were established with the aim of enhancing cultured meat production by facilitating the exchange of technologies, infrastructure, and resources among enterprises. Industries assure that they will be able to provide a restricted level of demand soon once cultured meat is globally regulated (Bomkamp et al., 2022a). Nevertheless, with the continuous growth of the cultured meat industry, there is an anticipated rise in the global production and distribution of cultured meat alongside traditional meat. Future Meat recently inaugurated its inaugural factory, which is dedicated to the production of meat that is derived from poultry, pork, and lamb cells (Porto and Berti, 2022). This achievement is a significant milestone in the technological advancement of the cultivated meat market because it serves as a catalyst for product industrialization. “MeaTech 3D Ltd.” has successfully printed a 104 g cultured steak using its proprietary 3D bioprinting technology. They derived the steak from adipose and muscle cells. It is reputed to be one of the largest cultivated steaks to have been produced in recent years (Dadhania, 2022). Since 2015, various private cultured meat enterprises have formed in many countries, including the United States (Memphis Meats, now known as Upside Foods) and the Netherlands (Mosa Meat) (Chriki et al., 2020), encouraging cultured meat production within the next five years (Zhang et al., 2021). In 2016, the Good Food Institute, a nonprofit organization, was founded to promote new meat alternatives, such as cultured meat. As of early 2022, 60 of the 112 global enterprises were working in cultured meat processing. In October 2023, Cell MEAT submitted a request for certification from the Ministry of Food and Drug Safety (MFDS) of the Republic of Korea for the provisional use of Dokdo prawns

(*Lebbeus groenlandicus*) cell culture as a food ingredient (Cell MEAT, 2023). New amendments to an application received from Vow seeking approval of cultured quail were announced by Food Standards Australia New Zealand (FSANZ) in December 2023 (FSANZ, 2023). Cell-based chicken, beef, and seafood mixed products like burgers and nuggets cost $66.4/kg to $2200.5/kg (Guan et al., 2021). These costs are significantly higher than the retail price of conventional seafood products, such as salmon, tuna, and shrimp, which are priced at approximately $10.17/lb, $10.29/lb, and $9.05/lb, respectively (USDA, 2022).

Poultry Meat Products

Memphis Meats, a food technology company, effectively manufactured and launched cultured meat products in 2016 (Newman, 2020). In 2019, a cell-cultured poultry nugget manufactured by JUST was priced at $50 USD (Van Loo et al., 2020). In late 2020, the Singapore Food Agency (SFA) approved Eat Just Inc.'s sale of cultured chicken meat, making it the first government to approve the commercialization of cultured meat. A restaurant sold the product for approximately US$23. In 2013, Mosa Meat, a Dutch startup company, produced its first beef burger, which cost roughly $330,000 (Luiz Morais-da-Silva et al., 2022). Eat Just's cultivated chicken (GOOD MeatTM) was authorised for sale by the Singapore Food Agency (SFA) in December 2020. Moreover, Memphis Meats, a California-based startup, created cultured duck meat *(Hocquette et al., 2022).* Gourmey, a French startup company, used duck egg cells and adjusted nutrients to produce artificial foie gras (ethical foie gras) (Guan et al., 2021). In 2020, JUST used cultured duck cells to produce duck pate and chorizo (Profeta et al., 2021).

Beef Meat Products

Mosa Meat, a Dutch startup company, generated cultured beef from cow stem cells in a medium without bovine serum, resulting in cost-effective CM (Bryant and Barnett, 2020). In 2016, Memphis Meats, a California-based startup, created the first cultured meatballs with cell-cultured beef (Stephens, 2022).

Pork Meat Products

In 2018, Meatable, a Dutch startup company, utilized stem cell technology to readily extract particular cells and create cell-cultured pork meat. New Age Meats, a San Francisco startup company, has produced prototype pork sausages using muscle and fat cells from live pigs (Profeta et al., 2021).

Table 2. Start-up companies for cultured meat production (beef products, pork products, poultry products and fish and shellfish products)

Species	Name of Company	Country	Name of Product
Cattle	Mosa meat	Netherland	Beef burger
	Modern meadow	USA	Meat steak
	Memphis meat	USA	Meat ball
	Aleph farms	Israel	Cultured steaks using proprietary 3-D technology
Pork	New age meat	USA	Pork sausage
	Higher steaks	UK	Pork belly and bacon
Poultry	Memphis meat	USA	Chicken tender
	JUST	USA	Chicken nuggets
	SuperMeat	Israel	chicken
	Memphis meats	USA	Duck meat nuggets
	Peace of meat	Belgium	Chicken meat
	JUST	USA	Duck pâté & chorizo
Fish and shellfish	Finless Food	USA	Bluefin tuna
	Bluefin Foods	USA	Bluefin tuna
	Blue Nalu	USA	Tuna, mahi mahi, red snapper
	Memphis Meats	USA	Coho salmon
	Wildtype	USA	Salmon
	Cultured Decadence	USA	Lobster
	Sound Eats	USA	Whitefish, zebrafish
	Shiok Meats	Singapore	Crab, lobster, shrimp
	Umami Meats	Singapore	Japanese eel, red snapper, grouper, yellowfin tuna
	Magic Caviar	Netherland	Caviar
	Bluu Biosciences	Germany	Salmon, trout, carp
	Cell Ag Tech	Canada	Whitefish
	Another Fish	Canda	Whitefish
	Avant Meats	China	Fish maw, sea cucumber, whitefish

9. CONCLUSION

In conclusion, in vitro-cultured meat represents a promising solution to the challenges faced by traditional meat production systems. Through advancements in tissue engineering, cell culture techniques, and sustainable practices, lab-grown meat offers a sustainable, ethical, and environmentally friendly alternative to conventional meat. The promise of cultured meat lies in its ability to provide a sustainable and ethical alternative to conventional animal agriculture. It offers solutions to critical issues such as environmental degradation, animal welfare concerns, and the increasing demand for protein from a growing global population. Cultured meat production requires significantly less land, water, and energy, and it has the potential to dramatically reduce greenhouse gas emissions, making it a key player in the fight against climate change. Moreover, in vitro meat can enhance food security by reducing dependency on traditional livestock and mitigating the risks of foodborne pathogens. The controlled environment in which cultured meat is produced minimizes contamination risks, ensuring a safer and more consistent product. However, the path to widespread adoption of cultured meat is not without obstacles. Technical challenges remain, including the need to improve the efficiency and scalability of production processes and to develop cost-effective culture media. Additionally, consumer acceptance and regulatory frameworks will play crucial roles in the successful commercialization of cultured meat. Public perception and education are vital to overcoming skepticism and fostering acceptance. Efforts to communicate the benefits and address misconceptions about cultured meat will be essential. Moreover, regulatory bodies must establish clear guidelines to ensure the safety, labelling, and marketing of cultured meat products. While challenges remain, continued research, innovation, and collaboration among scientists, policymakers, and industry stakeholders will be key to realizing its full potential. As we move forward, the development and adoption of cultured meat could lead to a more sustainable, ethical, and resilient food system for future generations.

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