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Review Article

ENGINEERING THE FUTURE OF FOOD: CELL SELECTION, CULTURE MEDIA, PRODUCTION, AND SUSTAINABILITY OF LAB-GROWN MEAT

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ABSTRACT

Cultured meat (CM), which was produced by growing animal cells in vitro instead of raising and killing whole animals, was considered a groundbreaking innovation in food production. Choosing the right cell types was a fundamental stage in the creation of CM, and the main sources included adult stem cells (such as FAPs, muscle satellite cells, and MSCs) and pluripotent stem cells (iPSCs and ESCs). Ethical considerations, differentiation possibilities, and scalability each had unique benefits. Cells were sourced, grown in bioreactors, introduced onto biocompatible scaffolds, and tissue was matured into edible products as part of the production process. Culture media and scaffold materials had a major impact on tissue formation and cell proliferation; research was still being conducted to find sustainable and serum-free substitutes. The ability of CM to reduce land and water use, and animal suffering while allowing safer, healthier, and more personalized meat products was what made it environmentally viable. Cost cutting, energy efficiency, mass media production, waste management, and societal acceptance were still issues, though. These challenges had to be removed in order to include CM in future sustainable diets, as defined by the FAO and WHO, while safeguarding cost and nutritional adequacy. According to modelling studies, under certain situations, CM might have provided a small contribution to low-impact diets. As the field developed, CM integration with multiple protein sources and sustainable food systems was seen as crucial to achieving environmental and global food security objectives.

Keywords: Adult stem cells, Pluripotent stem cells, Scaffolds, Sustainability.

INTRODUCTION

Urban expansion, demographic growth, and nutrition transition choices are all contributing to the abrupt rise in the order for meat across the world. In-spite of being entrenched in many cultures, livestock farming is coming under more scolding because of its considerable negative effects on the environment, moral dilemmas, and scaling issues. Cellular agriculture, which produces products of animal origin from cell cultures rather of entire animals, has become a viable replacement in feedback. The choice and developing of suitable animal cells that can copy the structure, texture, and nutritional content of traditional meat is the basis of the production of cultured or lab-grown meat. A primary procedure that influences the effectiveness, security, and caliber of cultured meat is cell selection. Definite animal cells, especially stem cells, can be segregated and grown to produce muscle fibers, fat, and connective tissues in vitro rather of hanging on all organisms. These cells can be produced from pluripotent stem cells like induced pluripotent stem cells (also known as or embryonic stem cells (ESCs), or they can be taken from living animals by tissue biopsy. Adult stem cells, such as myogenic satellite cells and mesenchymal stem cells (MSCs), are earlier on lineage-committed and thus more functional for producing particular meat tissues, in contrast pluripotent stem cells have the volume to transform into a wide range of tissues. Sourcing, cell multiplication in bioreactors, tissue regeneration with biological scaffolds, and eventual progression into a meat-like product are all

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aspect in the manufacturing of cultured meat. For the intend of aiding cell growth, simulating native tissue conditions, and guaranteeing sustainability, the choice of culture media and scaffold materials is necessary. Growth potential is quite impede by matters including the over prized of serum-containing media, the probability of uncontrollable modification in long-term cultures, and the ecological impact of particular scaffold materials. With prominence on pluripotent and adult stem cell sources, separation and extension methods, and their purposes in tissue maturation, this analysis strives to explore cell selection strategies for cultured meat. It also talks through ongoing progress in culture technologies, the justifiable suggestions of lab-grown meat, and how these improvements lineup with future sustainable diet substructures. By looking into the scientific, technical, and ethical dimensions of cell selection, this article provides all-encompassing outline of one of the most crucial factors in the cultured meat production system (Figure 1).

Selection of cell

Selection of cells by growing animal cells instead than the entire creatures, cellular agriculture can reproduce the morphological and molecular traits belonging to the authentic cell structure of various type of organism cell (Ben Arye *et al*., 2020). The technical methods and material resources used to produce diverse textures, sensory allure and nutritional value scan have an effect on regarding the standard of in-vitro meat. Through mechanical or chemical incorporation (primary culture) or direct resection from intended organs or tissues, cellular origins may include can be acquired derived from living organisms (T.K. Hong *et al.,* 2020). Established cell cultures originating from a mixture of animal tissues and cells are capable of being cultured in a lab setting under thoroughly observed circumstances. Stem cells represent the primary origin for cell generation utilized as starting material as their capability to separate into a broad variety of cell types, including chondrocytes, adipocytes, endothelial cells, myofibers, fibroblasts, and more. Somatic stem cells along with embryonic-like stem cells constitute the main categories of stem cells that have the capability to distinguish and multiply, which are essential for generatingof manufactured meat. While adult stem cells/progenitors main target cell identity identified in the muscle tissue surroundings, like mesenchymal stem cells (MSCs), myogenic satellite cells, adipogenic/fibroblast precursor cells (FAPs) whereas true stem cells needed for meat production are induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). As a conclusion, the collection of cell lines is tailored and based on the needs of the preferred yields. Present methods of generating meat in a lab are usually correlated to the growing of skeletal muscle cells which regularly consists of fat and fibroblast tissues that are enhanced along with cartilage-forming cells for building connective tissues, cytoskeletal proteins, and endothelial cells that are grown together for blood supply intentions that upgrade the appearance of the meat. Yet, in spite of the skilled potential of viable cells, the use of specific adult stem cells in cultured meat generation has a notable concern in that the cells are subjected to cancerous transition in prolonged culture.

Pluripotent stem cells

PSCs with the capability to evolve into the mesoderm, endoderm, or ectoderm germ layers include iPSCs and ESCs. Since the cells can separate into all the cell types essential to produce cell-cultured meat (Post *et al.,* 2020). they turn into a feasible option for growing cell-cultured meat. Moreover, these cells conquer the challenges connected with functioning with multipotent or progenitor cells by being able to be developed for an prolonged period of time devoid of surrendering their pluripotency. In vitro myogenesis advancement has been achieved using Epiblast Stem Cells (EpiSCs), iPSCs or ESCs, in a variety of species, including humans, pigs, translational restorative medicine, and animal generation through cell-based meat growth. However, it is not possible to distinguish EpiSCs or ESCs from embryos, which develop ethical and faith-based dilemmas.

Adult stem cells

The more vastly used cell source for adult stem cells have primarily been used for producing culturing meat. These are unvaried progenitor cells discovered in distinct animal organs and tissues. Since, being multi-potent, can be differentiated into a limited variety of cell types most of which are definite to the tissue or organ in which they are detected. The production of cultured meat is best suitable for distinct types of adult stem cells. Mesenchymal stem/stromal cells (MSCs), fibro/adipogenic progenitors (FAPs) and Muscle satellite cells are the three important progenitor/stem cell types discovered in the vicinity of muscle tissue. Adipocytes, fibroblasts, Skeletal myocytes and chondrocytes are among the main mature cell types into which these progenitor cells can grow.

CELL CULTURE MEDIUM COMPONENTS

The following necessary elements are often found in cell culture media composition for muscle cell cultivation:

Nutrient solution at the base

Essential nutrients such as amino acids, vitamins, minerals and energy yielding compounds like glucose are one of the crucial elements required for cell growth that are provided by the onset nutrition solution. These elements are necessary to endure muscle cell cellular actions and biological processes (Z. Yang and H.-R. Xiong, 2012).

Both cytokines and growth factors

Growth factors take part in a pivotal role in intensifying muscle cell proliferation and separation, including fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF) (E.N. O'Neill *et al.,*2023), (S. Shaikh *et al.,* 2021). To change cellular reactions and signaling pathways, cytokines like interleukins and tumor necrosis factor (TNF) may also be added (K. Ahmad *et al.,* 2021).

Hormones

To manage biological processes and motivate muscle cell differentiation, hormones like insulin and dexamethasone are regularly add on to the cell culture media. Serum or Alternatives to Serum: Animal-derived serums, such as fetal bovine serum (FBS) (S.H. Yun *et al.,* 2023), (have prolong been make use of in cell culture formulations to offer a wide spectrum of proteins, growth factors, and hormones. Although, new serum-reduced or serum-free culture media engaging serum substitutes made from synthetic or plant-based sources are fetching more and more popular due to social and eco-friendly troubles.

Buffering agents

Bicarbonate and HEPES are two examples of pH buffering agents that are necessary to retain the cell culture medium at the ideal pH, which plays a crucial role in maintaining cell viability and experimental outcomes (S. Tyagi and S. Mani., 2023).

Bioreactor compatibility

The requirement of mass production bioreactor systems must be taken into account when putting together cell culture media. This covers elements like waste collection, nutrition delivery, and oxygenation in the bioreactor environment (J. Meneses *et al*., 2020). Regulatory Compliance: The capitalization of items made from cultured meat depends on the cell culture medium formulation meeting protection and regulations.

Scaffolds

Support Structures For myoblasts to proliferate and differentiate, they must adhere to a scaffold or substratum. The scaffold and its by-products must be edible in the case of cultured meat and can be derived from non-animal sources (J.S.H. Seah *et al.,* 2022), (R. Dong *et al.,* 2020). Another obstacle is engineering a structure capable of mechanically lengthening attached cells to promote differentiation (M. d' Angelo *et al*., 2019). It is necessary to have a pliable substratum to avoid the disengagement of nascent myotubes, which usually experience spontaneous shortening (M.J. Post *et al.,* 2020). Three-dimensional scaffolds are necessary for the entire in vitro bioprocess of tissue formation (Y. Chen *et al*., 2024). A porous material that provides both mechanical stability and integrated network makes up these structures. They replicate the extracellular matrix (ECM), the natural habitat of living cells (K. Ahmad *et al*., 2021), to help adherent cells adhesion and differentiate into particular cell types. By promoting the development of blood vessel formation and different spatial organizations in the resulting product, scaffolding material can improve the look and structure of lab-grown meat to more closely resemble conventional meat. By allowing the culture media to flow continuously, the scaffold's porous shape promotes effective nutrition and oxygen delivery, waste product elimination, and the preservation of cellular metabolic processes while preventing death (S. Ng and M. Kurisawa, 2021).

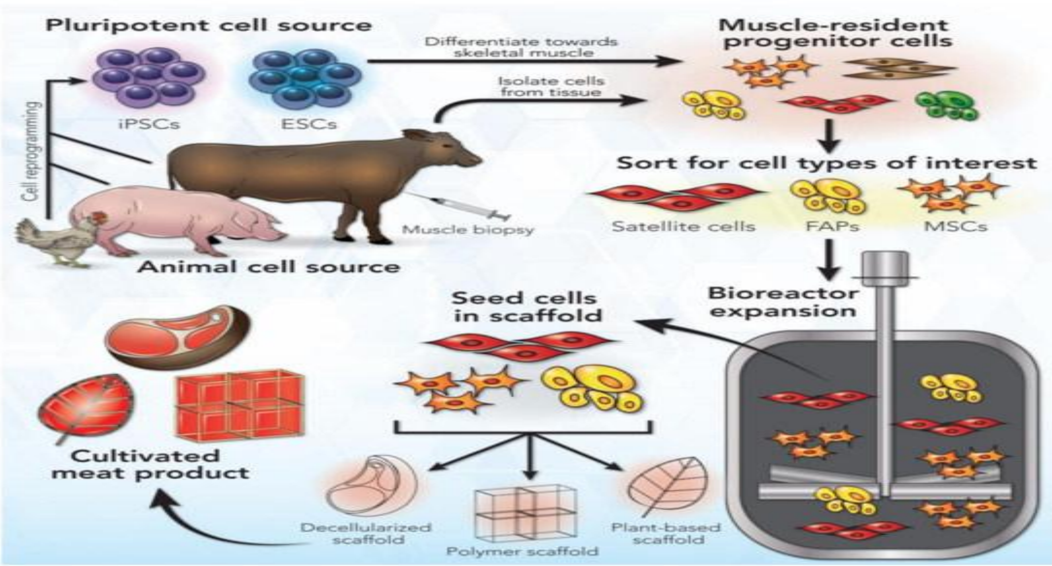

Figure 1. General workflow for cultivated meat production. Adapted from https://doi.org/10.3390/ijms22147513

It's critical to adhere to specific scaffolding guidelines while producing meat in a laboratory. Scaffolds must have the proper strength and structure, be safe for the body, and decompose over time. Additionally, they must be the proper size and texture. The substance used to bind the fat and muscles together throughout the meat-making process must have the proper stiffness for each. Compared to fat, muscles require a stiffer substance. The scaffolds must also have nutrition, be safe, not trigger allergies, and maintain the proper temperature. They should also improve the product's flavor and general appeal. It is crucial to maintain the conventional appearance and superior quality of ordinary beef in order to satisfy clients. Either natural edible polymers or synthetic polymers can be used to build scaffolds (N. Xiang *et al*., 2022). The latter can come from a variety of sources, including plants, animals, fungi, algae, or a mix of other polymers.

Zein scaffolds have been shown to be capable of efficiently binding, proliferating, and differentiating certain cells (T. Jiang *et al.,* 2015). This suggests that zein may be employed in the production of CM. Examples of plant-derived polysaccharides that may be utilized in the production of CM include starch, cellulose, and pectin. In addition to plant polysaccharides, algae-derived polysaccharides are important. Alginate is one example, which (M. Trujillo-Miranda *et al.,* 2023) used as a scaffold material for the 4D bio-fabrication of skeletal muscle spheroids. However, as alginate products can result in allergic reactions, it is important to be cautious (T. Mateti *et al.*, 2022).

MATERIALS AND METHODS

Method of preparation

The traditional process to manufacture farmed meat still remains in its initial phases of evolution. Consequently, a usual flow chart may be developed using the knowledge which every stage of the process can be altered for particular uses. In addition, there is still room for invention and cost and scale improvements in the production process. Cell sourcing, derived muscle-specific cell variants from the main cellular origin, cell separation to detach the targeted muscle-resident progenitor cells, cell inoculation in a biological tissue engineering scaffold, massive growth of the targeted cells of interest in a growth chamber, and the production of a cultivated meat product after the seeded cells have matured are the step by step in this process (Figure 1).This procedure starts with sourcing cells, which can be done in two alternative methods. The first and most popular method is to use primary cell sources, which are post-mortem tissues or tissue biopsies taken from the preferred origin of the targeted livestock species. Using a pluripotent cell source, such as early stage stem cells (ESCs) or programmed pluripotent stem cells (iPSCs), is the alternative. Muscle-specific stem-like cells can be procured from animal striated muscle tissues when initial cells are used. The progenitor cells of interest can terminally convert into differentiated cells and body structures, like cultivated meat, once they have proliferated to adequate number in the bioreactor. The progenitor biological units are regularly strew into a biological tissue scaffold to facilitate this discrimination process. This entitles the cells to stick to the framework and grown-up into consume meat. In order to best recreate the flavoring and steadiness of meat, the scaffold may also determine the final product's form and cellular structure.

Cell culture sustainability

With the aim of enhancing cell growth at affordable cost, publicize, and strengthen in-vitro meat production, the correct culture media and scaffolds are necessary. The media ingredients produce from horse serum, bacteria, and plants, or fetal calf serum (which is predominantly used for culturing cells for therapeutic purposes), should meet the needs of proliferating cells. The specific component that causes growth in serum is still mysterious (Mannello. F and Tonti GA., 2007). but media should contain vitamins, metabolic regulators, antibiotics, amino acids, nutrients like fatty acids, minerals, chicken embryo extract, metabolic regulators, hormones, different growth factors, and inhibitors in a certain proportion (Aswad H *et al.,* 2016). The confirmation of produced meat may be impeded by the use of serum in culture medium; therefore, serum-free media for myoblast augmentation must be confirmed. Because the scaffold imitates their natural environment, myoblast cells have been shown to distinct better in materials acquired from animals, primarily collagen, which causes the cells to align, reside, compact, and produce muscle fiber. When it came to tissue creation and cell differentiation, scaffolds made from artificial materials from ergonomics could not produce positive outcome (Bian W and Bursac N., 2009), (Snyman C *et al.,* 2013). Collagen is not considered as a viable supply and introduces dissimilarity when it is integrated during scaffold creation. In order to create compact biocompatible, nontoxic, tissue, and structurally well-built scaffold materials that make it simple for proliferating cells to stick and interconnect must be found. Particular substances, like polymeric sugar chains, provide all the required properties for the creation of scaffolds, but they lack myoblast cell binding sites (Bhat ZF *et al*., 2019).

Based on reports plant cellulose, alginates and chitins supplemented with short peptides to enhance binding sites are relevant materials for the fabrication of scaffolds on a large scale (Sandvig I *et al*., 2015), (Canavan *et al.,* 2005) scrutinized the use of polyglycolic acid, polyurethanes, and polylactic acid for scaffold creation and noted the issue with scaffold removal since take away the scaffold with mechanical force or enzymatic action may distress the scaffold and cells. Moreover, these sheets can attach to different substrates and stack to create three-dimensional products. Without a support base, removing cell sheets from a scaffold may comprise cells to aggregate due to contractile force, forming multicellular detachable spheroids. For this reason, a contact surface such as a hydrophilic gel or membrane is required (Lam MT *et al.,* 2009).

In-vitro meat sustainability

Meat produced in vitro is thought to be more viable and sizeable. The construct of cultured meat is still in its infancy, and the literature now approachable makes it challenging to given an idea of its sustainability. In order to encounter the rising population's command for meat, innovative, effective and relevant management strategies in animal husbandry, agriculture and in-vitro meat production must be followed. We still know very little about many outlooks of industrial-scale in-vitro meat production, including growth inhibitors, the production of growth factors and antibiotics used in wide-scale media preparation, waste production, energy requirements, process efficiency and the cost of properly persuading of it, greenhouse gas emissions, etc. Some nations oversupply meat and then export the excess to raise finances for the importation of crucial products. The atomization of in-vitro meat production will result in self-sustenance in the developed world under such circumstances. This will have an effect on nations that mostly transport live animals or meat. In order to save money on transportation, make raw materials more easily accessible, and improve architecture, the in-vitro meat production will be discovered close to cities or urban areas. Sophisticated machinery, robotic processes, and differentially trained/skilled labor are all necessary for this manufacturing system, which also runs the hazard of not using the less skilled labor used in conventional meat production. Animals are also raised for uses other than meat, such as hormones, natural casings, leather, wool, etc. After their fertile life are over, animals in a number of developing nations are still sent to be exterminated. Instead of depending only on one food source, we should modify our food sources and take a comprehensive approach to promising food security. (Fairlie S. 2010) suggested using conventional farming, cutting back on the amount of meat ingested overall, producing meat in vitro, and utilizing cutting-edge technologies like nanotechnology, stem cell engineering, and synthetic biology to make sure sustainability and improve human-nature relationships. Countless micro-sized beads with positively charged exterior surfaces made of dextran, cellulose, gelatin, or plastic were created for large-scale cell culture.

Sustainable diet through cultured meat

The FAO and WHO Citation 2019 defines eco-friendly diets as "dietary intake that advance all proportions of individuals' well-being and health, have less ecological pressure and effect, are inexpensive, equitable, guarded, and accessible and are indigenously acceptable." Therefore, for CM to be incorporated into future diets, it needs to be reasonably priced, palatable, nutrient-dense, and environmentally benign. Using a linear programming perspective (Mazac. R *et al.,* 2022), carried out a dietary optimization study to see how newly developed foods, including CM, fit into the future sustainable diets. In many dietary optimization inspect, experimenters employ the mathematical technique known as linear programming to establish the optimal meal combination to meet one or more intention, such as decreasing environmental impact or achieving dietary nutrient adequate (Wilson. N *et al.,* 2013) Nine new food products—mycoprotein, insect meal, microbial protein, kelp, cloudberry culture, ovalbumin, cultured milk, microalgae and CM—were investigated in this study to see if they could be included in diets that were upgraded for water, land, and global warming potential (GWP) while still meeting nutrient adequacy standards. Their discovery demonstrated that, among the nine novel food products evaluated, insect meals and cultured milk were the most frequently selected foods in upgraded diets when animal-based foods were inaccessible. Even when optimizing for water use, the greatest contribution of CM was noted, although it was chosen in very modest amounts (0.10 g/day). CM was chosen at 29 g/day under a water use depletion scenario after eliminating insect meal, cultured milk, and all meals derived from animals.

Pros and cons

Pros of lab grown meat

1) Advantage of lab-grown meat is that it uses minimal water and land than present meat manufacturing techniques, which decreases environmental pollution (Mottet A *et al.,* 2017).

2) Reduces the use, distress, and death of animals substantially.

3) Assure the long-lasting generation of meat products that are free of chemicals and illnesses.

4) Engineer meat by modifying its constitution, for as by managing fat, to make it more nutritious and more beneficial.

5) Decreased food related and zoonotic sickness rates.

6) Fast generation.

7) Wild animals and preservation.

8) Approach to unique meat.

9) Vegan meat.

10) Alternative reference of protein.

Cons of lab grown meat

1) Product characteristics, such as color and look, in contrast to standard beef.

2) The increased cost of processing.

3) Financial disturbances: jobs, trades of meat, etc.

4) Moral concerns and public approval.

CONCLUSION

In summary, by utilizing specific animal derived cells, cellular agriculture and cultivated meat mimic the characteristics of conventional meat, providing a promising alternative for environmentally viable food production. Despite ethical dilemmas and the possibility of tumorgenesis transformation in adult stem cells, pluripotent stem cells offer substantial benefits for differentiation into different cell types. Advancement in cell source and scaffold design could enhance sustainability by reducing the environmental effects of conventional livestock production. Lab-grown meat processing strategies are also changing. Although lab-grown meat offers advantages like less animal exploitation and healthier product options, there are limitations as well, including issues with customer acceptance, production costs, and the impact on the traditional meat industry's bottom line. In the end, the future of lab-grown meat hinges on further research and cooperation amongst stakeholders to tackle ethical, financial, and ecological challenges, establishing it as a key element of a more sustainable food network for a growing population that is expanding.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICS APPROVAL

Not applicable.

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request.

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