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Prospects of Skeletal Muscle Culture for *In-Vitro* Cultured Meat Production

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Abstract

With the rising food demands and a proportionately growing population, the food business has developed various inventions, alterations, and ways to produce meat *in vitro*. This innovation has the potential to change the meat industry, with significant effects on the environment, human health, and animal welfare. As a result, animal cells, rather than actual meat from slaughtered animals, are employed to create cell-based meat, with cell division and proliferation taking place in the culture medium. Cultured meat is a novel alternative to traditional meat, which is more ethical, humane, and sustainable. This review analyses the obstacles related to technology, consumers, regulations, the environment, product costs, the economy, health and safety concerns, ethics, religion, and societal taboos.

Introduction

Recent data shows that the world population has reached 8 billion. In this scenario an imminent challenge humanity may face feeding 10 billion people by 2050 (UNPD, 2019) [1]. For most people, meat is an inevitable part of diet and nutrition. In 2014, more than 300 million tonnes of meat were consumed, and by 2050, consumption is expected to rise by 76% [2]. More than 70 billion animals are reared and slaughtered annually to supply the growing demand for meat [3]. Large-scale rearing and slaughter create serious ethical, health, and environmental issues. The main drawbacks of traditional meat production are the enteric emissions of greenhouse gases and large amounts of manure produced by livestock. In many regions of the world, slaughter is not done in a proper scientific and humane way; thus creating much pollution to the environment. There are increasing incidences of zoonotic diseases (viruses, bacteria, fungi, and other pathogens) transmission between humans and livestock. African swine fever, avian flu, and other animal disease outbreaks have made traditional livestock production a matter of risk [4].

Given these factors, a different method of producing extremely effective, environmentally responsible, and durable meat is essential. Cultured meat has been extolled as one of the “Top 10 Emerging Technologies of 2018” by the World Economic Forum and has been acknowledged as a potential solution to many ailments in animal farming [5]. Meat produced from cells is frequently referred to as *in vitro*, lab-grown, and clean. Cultured meat is edible muscle tissue created by culturing stem cells in controlled conditions and a physiological environment using synthetic tissue engineering and computational simulation technologies [6]. Cultured meat is prepared by culturing the animal cells *in vitro*, i.e., in a lab, by growing cells in a media containing a mixture of nutrients and growth factors. This would allow the proliferation, growth, and differentiation of the cells leading to the production of “animal-based meat”. In other words, cell-based meat is made outside the animal from which it originated by cultivating muscle stem cells [7].

Timeline of cultured meat production

In 1971 muscle fibers were cultured *in vitro* by Russel Ross. He was able to culture the guinea-pig aorta successfully [8].

In 1991, Jon F. Vein secured a patent to produce tissue-engineered meat for human consumption, where muscle and fat were integrated to create food products [9].

In 2001, Wiete Westerhof, van Eelen, and Willem van Kooten declared a worldwide patent on a process to produce cultured meat. The process employed a matrix of collagen seeded with muscle cells bathed in a nutrient media and was able to divide [10].

In 2001, the National Aeronautics and Space Administration of the USA began conducting cultured meat experiments, so astronauts could grow meat instead of transporting it. In partnership with Morris Benjaminson, they cultivated goldfish and turkey.

In 2003, Oron Catts and Ionat Zurr produced a few centimetres of “steak”, grown from frog stem cells, which they cooked and ate [11].

In 2004, Jason Matheny founded New Harvest, an organization promoting research on cultured meat [12].

The first cultured beef burger patty was created by Mark Post at Maastricht University in 2013. It was produced from over 20,000 thin strands of muscle tissue [13].

Industry-wise development

- Between 2011 and 2017, many cultured meat start-ups were launched.This includes Memphis Meats (now Upside Foods), an Israeli company named Super Meat, and Finless Foods - a San Francisco-based company working on cultured fish.
- Dutch start-up Meatable (consisting of Krijn de Nood, DaanLuining, Ruud Out, Roger Pederson, Mark Kotter, and Gordana Apic) reported in September 2018 that it had succeeded in growing meat using pluripotent stem cells from the umbilical cord. Meatable claimed to direct them to become muscle or fat cells as needed. The company claimed to avoid the usage of fetal bovine serum, thus eliminating any chance of killing any animals to produce meat [14].
- In 2019, Aleph Farms collaborated with 3D Bioprinting Solutions to culture meat on the International Space Station. This was done by ejecting meat cells onto a scaffold using a 3D printer [15].
- On April 27, 2022, the European Commission approved the request to gather signatures for the European Citizens' Initiative End the Slaughter Age to shift subsidies from animal husbandry to cellular agriculture.

Cultured meat

Cultured meat or *in vitro* meat production is a new concept that could have an enormous impact on our meat production systems and the overall impact of meat production on the climate. Since the first public revealed of a cultivated piece of meat by Marc Post in 2013, the arena of cultured meat has expanded considerably. Several small-scale industrial start-up companies, attracting increasing investments, are now focused on delivering the first genuine cultured meat product to consumers, based on bovine, porcine, avian, and Pesci cells [16]. Stem cells have been isolated from diverse sources from different animal species, like adipose tissue, skeletal and cardiac muscles, bone marrow, dental pulp, heart liver, and fetal adnexa (amniotic membrane, cord blood, and Wharton's jelly) [17].

Satellite cells are skeletal muscle stem cells [18]. They can be used for cultured meat production. Stem cells are a type of progenitor cell that may multiply and differentiate between developing specific functions [19]. Cultured meat can be created by cultivating animal cells that show an extreme capacity to proliferate and can differentiate into mature muscle fibers. Several stem cell types can theoretically be utilized for this purpose, e.g., embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs), however the most reliable and well-studied cell source at this time are the satellite cells (SCs) [20].

Satellite cells used for lab-grown meat

Satellite cells (SCs) are the principal stem cells of the musculoskeletal system. Satellite cells show the capacity to proliferate and undergo myogenic differentiation. It can be isolated after extended tissue storage for increasing the practicality of cultured meat production [21].

Beginning with mononucleated myoblasts with a restricted ability for proliferation, muscle tissue creation occurs during embryological development [22]. Multinucleated myotubes are created when myoblasts merge, eventually developing into non-proliferative myofiber [23]. Except in situations needing repair or regeneration, the quantity of myofibers seldom increases postnatally. Satellite cells produce fresh myofibers or add more myonuclei to existing ones [24].

For the isolation of the muscle stem cells, whole muscles are minced and subjected to an enzymatic treatment, and then the satellite cells are separated using differential centrifugation, pre-plating, percoll gradients, or a combination of these techniques. Besides these, other methods may also be employed [25].These myoblasts fuse to form myofibers when growth stimulants are removed from the culture media, and following fusion, they begin to contract randomly [26].

The scientific field of producing cultured meat is very much in its infancy, and the knowledge regarding the complex biology and intricate biotechnical techniques needed for such a production system is scarce. The interplay between many research fields, such as stem cell isolation and characterization, bioreactor design and cell culture scale-up, growth media optimization, three-dimensional scaffolds, and sensory and nutritional evaluations, complicate cultured meat research. However, this interdisciplinary approach is necessary to develop a sustainable, nutritional, and tasty *in vitro* meat product [27].

The animal stem cells can be obtained from a muscle biopsy from live animals or sampled at slaughter. Theoretically, a large cultured meat production can be sustained without animal slaughter leading to a significant reduction inthe number of animals required for global meat production [28].

Process of *in vitro* meat production:

1. Muscle samples were taken from a suitable animal.
2. Collection of appropriate cells with the ability to create muscle.
3. Separation of stem cells from all other muscle components.
4. Induce cells (myoblasts) to grow and proliferate.
5. Dramatically increase the quantities of these cells in a bioreactor.
6. Provide some framework/scaffold to facilitate forming.
7. Provide nutrients and oxygen close to the developing cells or fibers; muscles need something like a circulatory system to remove metabolic waste.
8. The resulting product into a product that mimics meat.

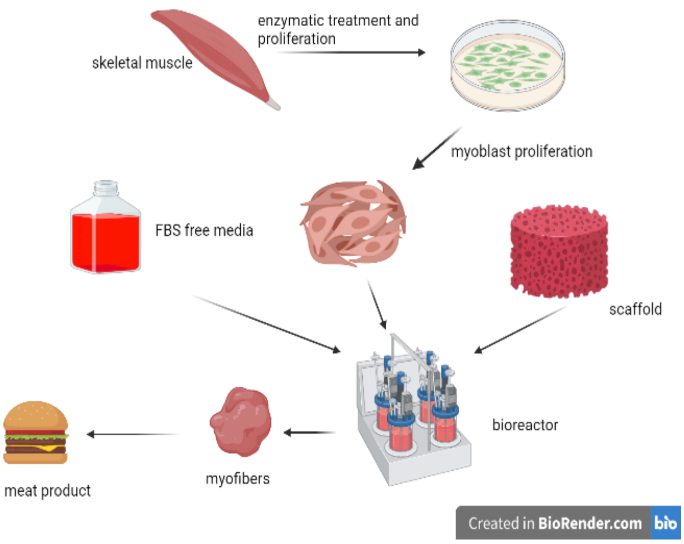

Figure 1: The process involved in the cultured meat production.

Techniques used for culture meat production

Scaffolding methods

In scaffold-based methods, proliferating embryonic myoblasts or adult muscle satellite cells are linked to a carrier or scaffold, such as a collagen meshwork or micro-carrier beads. It is then perfused with a culture medium in a stationary or revolving bioreactor. These cells can fuse into myotubes, which can later develop into myofibers, by introducing several environmental signals. The resulting myofibers can then be taken out, prepared, and eaten like meat [29].

Explant culture

Explants made using tissue culture techniques have the benefit of containing all the tissues that make up the flesh in the proper quantities and closely resembling an in vivo environment. Using foetal bovine serum 15% as the nutritional medium. However, the absence of blood circulation in these explants prevents considerable development since cells turn necrotic when isolated from a source of nutrients for more than 0.5 mm [30]. According to Vladimir Mironov, a branching network of the edible porous polymer through which nutrients are perfused and myoblasts and other cell types can adhere, can be utilized to generate very artificial muscle using tissue-engineering techniques. The use of artificial capillaries in such a system for tissue engineering has been suggested [31].

Technical obstacles

It is feasible to generatevery small pieces of muscle that acquire an adequatenutrient and oxygen supply through diffusion, but cultured muscles with blood vessels within to exchange gases and nutrients have yet to be developed. However, current progress in biomaterial technologies is looking forward to making this possible [32].

1. Cultured muscle tissue should be able to get physically stretched to seem similar to natural meat [33]. Fibroblast stem cells can organize collagen or collagen/matrigel into tight fibers between the anchors to develop tension within the developing muscle fibers [34].
2. Imposition of the cyclic stretching protocol used in a study failed to improve protein synthesis, but some researchers observed the positive effects of cyclic stretches on muscle maturation [35].
3. Growth in vitro of a single layer of myocytes and muscle fibers on a base of collagen fibers has been achieved, but the formation of steak-like three-dimensional (3D) structures will require a 3D framework or scaffold and a means of ensuring that every cell/fiber has a continuous and adequate supply of nutrients and oxygen, as well as a means of removing waste products such as CO_2.
4. Contractile proteins and other types of proteins in muscle are important for the texture, colour, and taste of cultured meat. For example, myoglobin, the haeme-carrying protein of muscle, is responsible for the colour of meat and is an important source of iron in meat [36]. Contractile activity of the muscle will stimulate myoglobinsynthesis, which may further enhance the colour of cultured meat.

Table 1: Article related to *in vitro* meat production.

Subject	Title	Comments	References
Clean meat	Opportunities for applying biomedical production and manufacturing methods for the development of clean meat industry Consumer acceptance of cultured meat: A systematic review	Production and manufacturing Consumer acceptance	Specht et al. [37] Bryantand Barnett [38]
Meat	How muscle structure and composition influence meat and flesh quality. Flavour of meat and meat products	How muscle structure and composition influence meat and flesh quality Flavour (book)	Listrat et al. [39] Shahidi [40]
Developmental	Fetal programming in meat production	Muscle development in farm animals	Du et al. [41]

Myogenic stem cells	Satellite cells and the muscle stem cell niche	SC-extended review	Yin et al.[42]
Intramuscular fat (IMF)	development, genetic and nutritional control, and identification of putative markers Adipogenesis from Bovine Precursors	In farm animals,intramuscular fat content in meat-producing animals: Adipogenesis protocols for clean meat	Hocquette et al. [43] Mehta et al. [44]
Extracellular matrix	Muscle derived fibroblasts	Skeletal muscle fibroblasts in health and disease	Chapman et al. [45]
Gene Expression	Transcriptomics of meat quality	Transcriptomics and meat quality	Guo and Dalrymple [46]
Biomaterials	Biomaterials based strategies for skeletal muscle tissue engineering: Existing technologies and future trends	Scaffolding	Qazi et al. [47]

Challenges involved in the production of cultured meat

Culture media

The media used for usual cell or tissue culture often contains foetal bovine serum. Foetal bovine serum (FBS) is not only expensive, but the usage of FBS has ethical issues, especially when it comes to the production of cultured meat. Currently, the culture media uses pharmaceutical-grade amino acids and other nutrients, which are costly. If food-grade ingredients can be used for the mass production of the CM, the cost incurred for its production can be reduced.

Scaffolds for the meat production

Suitable scaffolds of appropriate thickness and mechanical properties must be used. This would allow cell attachment as well as the proliferation of cells. Further, such scaffolds must be biodegradable and edible [48].

Regulatory challenges

As the production of CM involves harvesting stem cells from livestock animals and growing these cells for food purposes, strict regulatory supervision is required. The current position is that no laws or regulatory bodies would look over this matter. In the United States of America, in 2018, the US Department of Agriculture's Food Safety and Inspection Service (FSIS) and the FDA declared that they would collectively control cultured meat production.

Under the Food Standards and Safety Act, 2006 of India, the Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purposes, Functional Food, and Novel Food) Regulations, 2016 (Novel Food Regulations) have been issued to regulate a wide variety of innovative food items that includes cultured meat.

The extract below from the Novel Food Regulations paraphrases the definition of Novel food:*"(Novel Food Regulations) defines novel foods as foods that (a) may not have a history of human consumption; (b) may not have an ingredient in it that has a history of human consumption; (c) are obtained through new technology which give rise to a significant change in the composition, structure or size of the food which may alter the nutritional value, metabolism or the level of undesirable substances".*

The manufacturers of 'cultured' meat must comply with the Food Safety and Standards (Packaging) Regulations, 2018 (Packaging Regulations), and strictly adhere to Food Safety and Standards (Labelling and Display) Regulations, 2020 as well.

Consumer perception

In a study by Flycatcher (2013) conducted in the Netherlands with n =1300, more than half of the participants (52%) were willing to try cultured meat, while almost one quarter was doubtful (23%), and another quarter reported they would never try it. Therefore, consumers should be able to accept cultured meat as a part of their diet and should feel the same juiciness, taste, and palatability they feel with the traditional source of meat [49]. The scientific community should transparently communicate the science of cultured meat to the public so that public acceptance will increase eventually [50].

Conclusion and future prospects

Cell-based meat production for human consumption is a growing industry, having developed from a straightforward notion. A major change in animal farming is inevitable as this concept is still in the works. Furthermore, because cell-based meat is safe and does not involve animal killing or suffering, many people who do not eat meat might start doing so because of the adoption of this technology. Cell-based meat will appeal to those who, for ethical reasons, favour vegetarianism. It is possible to alter meat quality to make "designer meat" over the long term because in vitro meat production is a managed and manipulable technology. Cultured meat/ *in vitro* meat is thought to have several benefits over conventional meat, including better animal welfare, more efficient resource use (land, energy, and water use), lower greenhouse gas production, and the ability to control the nutrient composition of the product.

However, because of notions of "unnaturalness" and "artificialness," consumers may be hesitant to adopt such products. In addition to having enough of the required nutrients and oxygen, the culture media surrounding the developing cells and fibers in culture must also include the growth factors and bioactive substances necessary for healthy muscle development.Successful production of cultured meat in the future would ensure meeting the predicted global demands for meat. This would play a complementary role alongside conventional meat products for meeting the needs of the hungry. The extent to which they constitute competitors of conventional meat remains to be seen. It is too soon to accurately assess the readiness withwhich consumers will accept cultured meat products. Thereal test will be when such products are on the market.

Winston Churchill says in his work Thoughts and Adventures (1931): "We shall escape the absurdity of growing a whole chicken to eat the breast or wing by growing these parts separately under a suitable medium."

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