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Popular Article

Cultured Meat

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Abstract

Cultured meat, also known as cultivated or *in-vitro* meat, is the meat produced in the laboratory by cultivating animal cells under controlled conditions. Key inputs in the process are stem cells, culture media, bioreactors, and scaffolds. Cultured meat production eliminates the need to raise and slaughter food animals and erases the issues of environmental pollution, food intoxication, and disease transmission. There is no issue associated with animal welfare. Although cultured meat has the potential to revolutionize the concept of human nutrition, some of the initial shortcomings like improvement in the colour and texture profiles, addressing the concerns of the consumers as it not being real meat, and development of a prototype for commercial production to effectively manage the demand and supply chain around the globe need to be dealt with. Appropriate cost-cutting strategies would give an edge to cultured meat over conventional meat.

Keywords: Cultured meat, *in-vitro* meat, benefits, limitations, consumer acceptance

Introduction

Cultured or cultivated meat is the meat produced from living animal cells and is identical to conventional meat at the cellular level. The method of cultured meat production of involves the use of the basic elements required to build muscle and fat enabling identical biological processes

happening inside the body of an animal. Conventional meat production entails major environmental pollution, consumption of fossil fuels and massive use of land, and water resources. Meat production accounts for 15 - 24% of the total greenhouse gas emissions worldwide and is the primary cause of deforestation in creating grazing land to raise the food animals (Steinfeld *et al*., 2006). Conventional meat production is extremely inefficient as according to the World Resources Institute, chicken which is considered to be the most efficient converter of crops into meat requires nine calories of feed to produce one calorie of flesh. Livestock provides merely 18% of calories consumed by humans in return for the use of 77% of the global farmland. Production of cultured meat would effectively address the concerns of animal welfare, safeguard mankind from the harmful residual antibiotics, growth promoters and other veterinary drug residues alleged to be transmitted from conventional meat to the consumers, and drastically mitigate zoonotic pandemics.

The first cultured meat was produced as a processed beef burger by Professor Mark Post and his co-workers at Maastricht University, Netherlands in August 2013. The Indian Institute of Technology, Guwahati developed lab-grown chicken meat in 2019. In the same year, the Center for Cellular and Molecular Biology, Hyderabad in collaboration with the National Research Centre on Meat, Hyderabad produced 'Clear Meat', a lab-grown chicken meat. The United States-based Company 'East Just' became the first company to receive regulatory approval for its *in-vitro* cultured chicken meat product. In December 2020, 'East Just' sold and served its first cultivated chicken meat nuggets at a restaurant named '1880' in Singapore making it the world's first commercial sale of laboratory-grown meat. Some other companies dealing with cultured meat of different species are 'Memphis Meats' and 'Because Animals' (United States); 'Aleph Farms' and 'Super Meat' (Israel); 'Mosa Meat' (Netherlands) etc.

Cultivated meat has a variety of benefits over conventional animal agriculture. Cultured meat production can be tailor-made to suit the specific requirements of processed meat products with predefined quality attributes. By manipulation of the growth medium, co-culture, or genetic engineering, it is theoretically possible to create products with desired textures, tastes, and nutrient profiles. The concentration of high saturated fatty acids allegedly contributing to cardiovascular disease can be effectively manipulated in cultured meat. Maintenance of aseptic conditions throughout the culturing process ensures contamination-free meat production. Since the production system of cultivated meat uses stem cells, cells from rare or endangered animals or even cells from

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samples of extinct animals could be used to produce exotic meats in bioreactors without any threat to the concerned species. Occurrence of the pale, soft, exudative pork and dark, firm, dry beef encountered primarily due to pre-slaughter mishandling of the animals could be completely avoided in cultured meat.

Method of Production of Cultured Meat

The process of cultured meat manufacturing commences with collecting and banking stem cells from an animal. Collected cells are then grown *in-vitro* inside a bioreactor to provide environmental conditions similar to that of an animal's body. Akin to *in-vivo* conditions that are vital for cell growth, cells are artificially provided with oxygen and basic nutrients such as amino acids, glucose, vitamins, and inorganic salts supplemented with proteins and other growth factors for their growth **(**Swartz and Bomkamp, 2021**).**

By changing the composition of the culture medium, the healthy immature cells are then activated to undergo differentiated into skeletal muscle, fat and connective tissue that make up the meat. The cells after proper differentiation are then collected, prepared, and packaged into final products. It is expected that the whole process will take about 2-8 weeks, depending on the kind of meat being cultivated. A similar theory is being pursued to produce milk and other dairy products.

Requirements for Production of Culture Meat

Cell Lines

Different types of cells can be used to cultivate meat. Cells that can self-renew and differentiate into the cell types which make up meat tissue (i.e. myofibers, adipocytes, fibroblasts, chondrocytes, endothelial cells, etc.) otherwise known as stem cells are mostly used in the process. Stem cells for meat cultivation can be obtained either from the embryonic stem cells or from the adult stem cells and the most common method of acquiring the stem cells is by using minimally invasive methods in live animals. In some cases, biopsy of a recently slaughtered animal where the tissue is still viable can be used to obtain these cells, which could be important for determining compliance to religious laws (e.g., Halal, Kosher). Stem cells of a particular organ can be obtained to produce other products such as cells from livers can be used to produce foie gras or that from the mammary glands can be used for milk production. The process of collecting cells from healthy animals possesses extensive documentation that ensures the quality and traceability of the cells. To prevent any risk of genetic

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drift of the cultivated cells, cryopreservation of the expanded cells is done as a master cell bank from which working cell banks can be serially subculture.

Cell Culture Media and Culture Conditions

To grow the cells outside the body, culture media should contain all the required nutrients and growth factors essential for the growth of the cells. Culture medium formulations are perhaps the most complicated task in designing an *in-vitro* meat production system. The medium should support and promote growth while being affordable containing edible components in large quantities.

The most important component of the medium is the serum. The serum used is mostly of animal origin like the foetal bovine serum. It has a large number of constituents in a highly variable composition (Shah, 1999). Nonetheless, the use of foetal bovine serum may raise ethical issues, so the use of commercially available serum replacements and serum-free culture media for culturing mammalian cells *in-vitro* becomes more important. Serum-free media also reduce operating costs and process variability (Froud, 1999). An example of a commercially available serum substitute specially designed to replace foetal bovine serum for growth of anchorage-dependent cells *in-vitro* is Ultroser G. It has a consistent composition containing growth factors, adhesin factors, binding proteins, hormones, vitamins, and trace minerals which are necessary for growth of the cell (Duque *et al*., 2003). Mushroom extracts are also used for the cultivation of fish explants (Benjaminson *et al.* 2002).

Growth factors are other important components of the culture media. Selection of the extrinsic regulatory factor must be specific to the type of the cell and also to the species since myosatellite cells of different species respond differently to the same regulatory factors (Burton *et al*., 2000). It is also likely that the formulation may be required to change throughout the culturing process. For example, the proliferation period may require one certain combination of growth factors and hormones while the differentiation and maturation phase may require a different set. These growth factors or hormones are supplemented into the media from external sources *viz*., transgenic bacterial, plant, or animal species which produce recombinant proteins (Houdebine, 2009). Alternatively, some synthetic paracrine signaling system can be set so that co-cultured cell types (a feeder layer) can secrete growth factors that can promote cell growth and proliferation in neighbouring cells. For example, co-cultured hepatocytes could provide insulin-like growth factors which stimulate myoblast proliferation and differentiation (Cen *et al*., 2008) as well as myosatellite cell proliferation

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in several meat-animal species *in-vitro* (Dodson *et al.,* 1996). Otherwise, autocrine growth factor signaling can play a role, as certain muscle cell-secreted growth factors such as insulin-like growth factor II stimulate myocyte maturation (Wilson *et al.,* 2003). Similarly, growth factor production can occur in genetically engineered muscle cells. Some of the other important components of the media required to support cell viability and vitality are glucose, water, amino acids, vitamins, salts, and other components as detailed in Table 2.

Regular contraction of the skeletal muscle cell is necessary for proper cell differentiation and to obtain a healthy myofiber morphology which in turn prevents atrophy (Datar and Betti, 2010). *In-vivo* muscles are supplied with nerves for regular and controlled contraction. Nevertheless, in the *in-vitro* system, the contraction of muscle cells must be stimulated by alternate means. Edelman *et al.* (2005) and Van Eelen *et al.* (1999) anticipated that mechanical stretching of scaffolds and expandable scaffold beads helped in providing contraction to the muscle cells. De Deyne (2000) said that the electrical stimulation was more effective than external mechanical contraction in promoting muscle development and feasible on large-scale cell production. Electrical stimulation induces contraction internally and helps in differentiation and sarcomere formation.

Bioreactors

Even if cultivated meat products for sensory evaluation have been produced in the laboratory using standard cell culture dishes and stacked flasks, commercial production of cultivated meat will require the use of bioreactors. The bioreactor provides controlled conditions for cultivating the cells *in-vitro*. It controls the optimum temperature, oxygen levels with the proper delivery of cell culture media. Bioreactor enables monitoring of other important parameters such as metabolite levels, pH, and biomass accumulation. Different types of bioreactors are designed based on how the medium is mixed and whether the cells are grown in suspension or adhered to a solid surface. Continuous stirred tank bioreactor is most commonly used (Meyer *et al*., 2017). In general, continuous stirred tank reactors permit the growth of cells in suspension *via* mechanical stirring while maintaining high mass transfer of oxygen. Suspension growth can also occur with attachment-dependent cells through the use of microcarriers (Swartz and Bomkamp, 2021). Direct perfusion bioreactors are more suitable for scaffold based myocyte cultivation (Datar and Betti, 2010). Some general optimizations important for cultivating muscle cells in a bioreactor are discussed in Table 3.

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A variety of methods can be selected for up scaling cultivated meat production into commercial scales. Broadly speaking, these methods can be broken up categorically into batch, fed-batch, continuous, and perfusion types (Meyer *et al*., 2017). In batch culture, a vessel is filled with a fixed volume of media and cells are grown to their maximum density before being harvested or transferred to a larger vessel. In fed-batch culture, cells grown in a vessel are fed fresh medium from an in-line, independent feed vessel at variable rates to maximize properties such as exponential cell growth or cell densities. In continuous culture, cells are grown in a vessel and a fresh medium is added *via* an in-line feed vessel at an optimized flow rate, while product, cells, or medium are simultaneously collected in an independent collection vessel at the same or alternative rate. Perfusion culture is a subset of continuous culture wherein the cells are retained *via* a substrate or collection method, permitting medium recycling integration and high cell densities in a smaller space.

Scaffolding

Myoblasts cells are anchorage-dependent and can contract spontaneously. Scaffolding provides structural support to cells to adhere, differentiate, and mature to produce structured and thick meat products. An ideal scaffold should have a large surface area for growth and attachment, be flexible to allow for contraction, provide access to oxygen and medium diffusion which is analogous to the vascularization of real tissue and should be easily dissociated from the meat culture. Scaffolds, together with the cell culture medium, regulate the growth of cell populations and cell differentiation. In some instances, scaffolds are used as microcarriers to aid in the proliferation of anchorage-dependent cells in suspension bioreactors. Biomaterials used in making scaffold can be categorized broadly as natural (cellulose, fibrin, laminin, hyaluronic acid, gelatin, alginate, chitosan); synthetic (pluronic, poly (ethylene glycol) (PEG), polyglycolic acid (PGA), poly 2-hydroxy ethyl methacrylate (PHEMA), and poly acrylamide or composite materials consisting of both natural and synthetic biomaterials.

Conclusion

The concept of cultured meat is to develop meat in the laboratory by using the ideas and technologies that were initially developed and used in stem-cell research, regenerative medicine, and biomaterial engineering. Although the laboratory scale study on the development of cultured meat has attained considerable success, the major hurdle is its large scale production. Till now, the

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application of cultured meat is limited to the ground meat used in hamburgers, hotdogs as the main component or as a substitute. Production of three-dimensional cultured meat resembling traditional cuts with proper textural characteristics would help in the ready acceptance of the products by the consumers. Strict adherence to the rules and regulations of good cell culture practice (GCCP) and good manufacturing practice (cGMP) of food and drug production is indispensable in the development of meat in the laboratory (Coecke *et al.*, 2005). To popularize cultured meat amongst the consumers, a vigorous campaign on mission mode on the health and environmental benefits of cultured meat may be the desired step. A comprehensive study on large scale production, cost-cutting on production, and establishment of a global market chain would go a long way in establishing cultured meat as an alternative to conventional meat.

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