

## Review Article

# Biodegradable and Nutritionally Fortified Scaffolds for 3D Culture and Printing of Clean Meat

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**Received:** January 08, 2025; **Accepted:** January 30, 2025; **Published:** February 04, 2025

## Abstract

Taste, safety, and nutrition are critical drivers in meat selection for most consumers. Cultured meat production faces the challenge of replicating natural taste while requiring innovative approaches to enhance its nutritional profile without compromising health benefits. With advances in the techniques to create complex and layered tissues, introducing and engineering nutritional elements as desired, 3D Bio-printing offers highly effective alternatives to animal-derived meat in terms of its overall dietary quality. It is now possible to create scaffolds that provide tissue architecture, recreating natural scaffolds such as collagen or bone to approximate the anatomy of animal-derived meat. This paper will examine the overall trends, developments, possibilities, and potential challenges in maintaining and improving the nutritional value of clean meat by fortifying the 3D scaffolds with multiple nutrients to create a healthier and more viable alternative to natural meat. To produce edible tissue such as clean meat, various cell types involved need to be cultured on a scaffold that mimics their natural environment, have components safe for human consumption, and are cost-effective. The scaffold can be composed of either a 3D scaffold or hydrogel that provides cells with compatible 3D microenvironment. Ideally, the scaffolds should mimic the different layers of the skeletal muscle connective tissue. This review will discuss the scaffolds and hydrogels potentially used to produce clean meat; and specifically examine the current research trends in upgrading scaffolds from the perspective of their ability to be biodegradable and add nutritional value to the end product.

**Keywords:** Scaffold; Cultured meat; Food Technology; Nutrition; Food Safety

## Introduction

The global human population is estimated to reach 9 billion by 2050. Consequently, the pressure of food requirements for such a large and growing population has become a big concern. The current production of crops is sufficient to provide enough food for the projected global population of 9.7 billion in 2050. However, very significant changes to the socio-economic conditions of many (ensuring access to worldwide food supply) and to the dietary choices of most, such as replacing most meat and dairy with plant-based alternatives and greater acceptance of human-edible crops currently fed to animals, especially maize, as directly-consumed human food, would be required.

5935 kcal/p/d of crops directly edible by humans are grown alongside 3812 kcal/p/d of vegetable matter eaten by other animals but not directly digestible by humans (i.e., GP&S). This total of 9747 kcal/p/d is more than four times the average dietary energy requirement for a healthy life (ADER) of 2353 kcal/p/d [1]. Farmed animals consume the equivalent of 5550 kcal/p/d in total. However, they return just 594 kcal/p/d to the human food chain in the form of meat (including 54 kcal/p/d of farmed and wild fish) and dairy products, while 4956 kcal/p/d is lost from the human food chain. This is in line with the estimates of the conversion rate of plant material to meat and dairy products ranging from 7-12%.

However, given cultural and taste preferences, it is unlikely that meat consumption will be reduced because of environmental or societal concerns. On the other hand, high pressure on land will only continue; and while relatively more efficient methods of farming or measures to convert plant material to meat, such as factory farming may be pursued, this is not likely to be a sustainable solution. Given this demand for meat, livestock systems will contribute to addressing the global food and nutrition security issue. Animal farming must produce larger quantities of high-quality and affordable meat, milk, and eggs through production systems that are environmentally sound, socially responsible, and economically viable. Despite the wide range of economic, environmental, cultural, and social services at local, regional, and global levels provided by livestock farming, a significant proportion of livestock is raised nowadays within the factory farming model. Similarly, with a lower contribution to greenhouse gases (GHG) and water usage than extensive agriculture, factory farming is mainly focused on efficiency (i.e., the quantity of milk or meat produced) rather than on other services and impacts such as interaction with the environment, climate change, reduced use of antibiotics, animal welfare, or sustainability.

Therefore, developing efficient protein production techniques to sustain growing global population while complying with challenges

such as environmental and animal welfare issues is the need of the hour [2]. One such solution is cultured meat, a sustainable animal meat alternative for consumers seeking responsible choices without dietary changes [3,4,5,6]. Since the first publication about cultured meat in 2008, the number has increased considerably after 2013, and in 2013 the first hamburger with cultured meat was prepared and tasted on a televised program [7].

## Environmental Impact of Clean Meat

Cultured meat could deliver reduced water use, greenhouse gas emissions, eutrophication potential, and land use compared to conventional livestock meat production. As per some methods [8] compared to conventionally produced beef, sheep, pork, and poultry, cultured meat involves approximately 78–96% less greenhouse gas emissions, 99% less land use, 82–96% less water use, and 7–45% less energy use. The overall picture is that cultured meat could have less environmental impact than beef and possibly pork but more than chicken and plant-based proteins. However, all three Life Cycle Assessments note that cultured meat technology has significant scope for innovation that could reduce the energy requirements below those used in these assessments and subsequently deliver better environmental outcomes than these models predict.

Another potential benefit is that cultured meat could be less prone to biological risk and disease through standardized production methods, and tailored production could contribute to improved nutrition, health, and well-being [6].

## Safety and Nutritional Benefits of Clean Meat

One of the major safety challenges faced by livestock is antibiotic resistance [9]. Cultured meat is produced and stored in a controlled environment compared to livestock, and close monitoring prevents any sign of infection. In addition, the nutritional content of cultured meat can be controlled by adjusting fat composites in production medium. A 2020 review on cultured meat gives an example of such controlled nutritional enhancement. For example, saturated fats can be replaced by other fats, such as omega-3 while controlling the risk of higher rancidity. Furthermore, the positive effect of any (micro) nutrient in cultured meat can be enhanced by introducing it in an appropriate matrix [2].

## Economics and Market of Clean Meat

The future of clean meat is promising, with the cultured meat sector expected to grow at a compound annual growth rate (CAGR) exceeding 15%, potentially reaching significant market milestones by 2034. While this market's estimated revenue of \$6 billion remains modest compared to the traditional meat industry's \$198 billion sales in 2013 [10], it reflects a rapidly evolving space. Meanwhile, alternative proteins, including lab-grown and plant-based meats, are projected to reach \$140 billion by 2029, highlighting their increasing adoption and investment [11,12].

With over two dozen firms advancing lab-grown beef, chicken, and fish, the industry's trajectory signifies a transformative shift towards sustainable protein solutions. Over 25 companies are now in the race for Cultured/ Clean meat. Key players are represented in the Table 1.

**Table 1:** Key players in the race of cultured and clean meat.

Company	Country
Memphis Meats	US
MosaMeat	Netherlands
SuperMeat	Israel
Just, Inc	US
Integriculture	Japan
Aleph Farms Ltd	Israel
Finless Foods Inc.	US
Avant Meats Company Limited	China
Balletic Foods	US
Future Meat Technologies Ltd	Israel
Appleton Meats	Canada
Higher Steaks	UK
Biofood Systems LTD	Israel
Fork & Goode	US
Meatable	Netherlands
Mission Barns	US
Bluenalu, Inc.	US
New Age Meats	US
Shiok Meats	Singapore
Seafuture Sustainable Biotech	Canada
Wild Type	US
Lab Farm Foods	US
Cubiq Foods	Spain
Kiran Meats	US
Cell Farm FOOD Tech/Granja Celular S.A	Argentina

## Economics and Commercial Factors

Lab-grown meat becoming affordable is a long-anticipated event by environmentalists and animal rights activists, as well as consumers looking for healthier/guilt-free options. However, while ethics, environment and safety are important drivers of adoption, affordability poses the biggest hurdle to consumer preference and commercial success of Clean Meat.

Currently, a pound of lab-grown meat produced by the company Memphis Meats costs approximately 2400 dollars to make, and while this still seems expensive, it is a massive reduction from the over \$300,000 that the meat cost only five years ago. Memphis Meats aims to have the price of a lab-grown burger down to around \$5 within a few years. Meanwhile, a startup called Future Meat Technologies, based in Israel, currently produces around a pound of meat for approximately \$360 and believes they can reduce the cost to somewhere between \$2.30 and \$ 4.50 by the end of the decade.

## D Printing of Clean Meat

3D printing has emerged as a promising solution to some of the key challenges faced in the production of clean meat, offering innovative approaches to improve scalability, tissue complexity, and nutritional customization. Traditional cultured meat production often struggles with the creation of structurally complex tissues that mimic the texture and composition of natural meat. 3D printing addresses this by enabling precise control over scaffold architecture, allowing for the replication of intricate tissue structures such as muscle fibers, fat layers, and connective tissues. Additionally, 3D printing facilitates the incorporation of specific nutrients into scaffolds, enhancing the nutritional value of cultured meat products. By utilizing biocompatible

materials, 3D printing not only supports cell growth but also offers potential for more efficient, cost-effective production, paving the way for cleaner, more sustainable alternatives to conventionally produced animal meat. With 3D printing, along with improvements in production techniques and advances in cheaper media formulations, we expect that the affordability and market acceptance of cultured meat will create a genuine and accessible alternative for consumers who are looking for an ethical, safe, and environment- friendly alternative to meat from slaughtered animals.

## Role of Scaffolds in 3D Printing of Clean Meat

Some of the characteristics required for the materials used in the construction of scaffolds are that they must promote cell adhesion of various cell types, such as muscle, fat, and connective tissue, and allow active cell interaction. In addition, they must enforce mechanical strength, be flexible and expandable, and have a microstructure that facilitates the exchange of essential factors (growth factors and other paracrine mediators) for cell survival, proliferation, and growth. These scaffolds are usually complemented with growth factors and extracellular matrix components that create an environment similar to the natural state. Bio-based materials possess several complementary functionalities, for example, unique chemical structure, bioactivity, non-toxicity, non-immunogenic, biocompatibility, biodegradability, and recyclability, that position them well in the modern world's clean meat materials sector. Using biological materials for clean meat scaffold applications has many intrinsic advantages, such as bio-compatibility, bio- degradability, renewability, sustainability, and non-toxicity. In recent years, from a biological point of view, a broad spectrum of biomaterial-based novel constructs has been engineered for targeted applications in the clean meat sector. These biological materials have been characterized and well organized into specific structures, enabling them to provide a proper route to emulate natural meat - a biomimetic approach. These biological materials are found easily in nature and are suitable for cross-linking with other synthetic counterparts to maximize their efficiency as scaffolds or tissue modeling. Today, many applications use these biopolymers' properties to design more flexible and lasting scaffolds to create clean meat. Some methods to produce in vitro meat involve growing muscle cells cultured on scaffolds using bioreactors. Suitable scaffold design and manufacture are critical to downstream culture and meat production.

## Potential Scaffolds for Cultured Meat Production

Scaffold structures vary mainly in the components used to create the platform. Usually, they are composed of edible entities, such as proteins, polysaccharides, or extracellular matrix components. Scaffold composition can provide mechanical support, biological support, and nutritive value. Specific cell types require different surfaces to grow and will be best grown on scaffolds having properties similar to their natural environment.

Synthetic or natural animal-free polymers such as cellulose chitin/ chitosan, alginate, recombinant silk, Poly (lactic acid) (PLA), and Polycaprolactone (PCL) provide low-cost, consistent scaffolds. Plant protein-based scaffolds are appealing candidates for clean meat due

to their nutritional value, low cost, and cytocompatibility. The well-known and extensively studied extracellular matrix protein, collagen can also be used as a scaffold. Collagen is often derived from bovine, porcine, and murine sources. However, the cost and consistency of animal sources are hurdles for using collagen. To overcome these issues, genetically engineered collagen has been proposed. The formation of novel meat- growing scaffolds, with attention to their biologically compatible properties, flavor, nutritional value, and meat-like texture is desirable.

Hydrogels are universally present biomolecules in human tissues. They are desirable components of a hydrogel as they can mimic the 3D environment of the extracellular matrix of human tissues.

Hyaluronic acid (HA) composed hydrogels are cell-compatible, show favorable viscoelastic properties, have high water retention, and can be synthesized in animal-free platforms. HA is a glycosaminoglycan in the muscle. It participates in wound healing and can regulate cell behavior, such as adipogenesis, angiogenesis, and tissue organization. HA- collagen composites show improved mechanical and biological properties and can be used for cell scaffolding. Collagen and HA are naturally found in the muscle extracellular matrix and therefore they can be used to mimic some of the biochemical and biophysical properties.

Moreover, they are also susceptible to remodeling and degradation by the cells, crucial for cell migration and ECM maturation. As discussed above, collagen is often used in tissue engineering, as it is the most abundant protein in the body. It can serve structural roles in the tissues, as anchoring points for cell adhesion, and facilitate cell migration and tissue development.

Alginate is an inexpensive seaweed-based polysaccharide that forms hydrogels with  $Ca^{+2}$ . It is composed of two monomers, one of which interacts with  $Ca^{+2}$ , making crosslink degree highly desirable. Alginate-HA composites can improve the regenerative properties of the alginate gel while providing improved gelation compared to HA alone.

Chitosan, another hydrogel component is an edible glucosamine polymer, used in skeletal muscle tissue engineering. It is commonly derived from animals, provides a similar structure to glycosaminoglycan, and requires chemical modification to facilitate cell adhesion, biodegradability, and mechanical properties.

The mechanical properties engaged by cells growing on a scaffold are generated by multiple interactions involving the scaffold material and the cells. Generating a sophisticated scaffold with several cell-specific components can mimic natural microenvironments in the skeletal muscle and promote cell growth and differentiation. Most current scaffolds are based on mammalian-derived biomaterials. Alternate scaffold materials can be formulated using non- mammalian sources, namely, salmon gelatin, alginate, and additives including gelling agents and plasticizers. This system composed of non-mammalian edible scaffold material and muscle cells is promising for the production of in vitro meat. The scaffold is a porous material where the anchorage-dependent cells (e.g., muscle cells) can remain viable and proliferate. The scaffold must be biocompatible and have appropriate microstructure and physical properties to enable cell attachment and proliferation. Pore size and stiffness are essential

scaffold design parameters for skin, bone, nerve, and muscle tissues. Specifically for muscle cell culture, the selection of soft porous materials with adequate microstructure and stiffness is important. Non-mammalian biopolymers extracted from algae (e.g., alginate and agar) or fish species (gelatin) can be used in cellular agriculture. Salmon gelatin is an attractive ingredient for preparing edible and biodegradable scaffolds. In addition, due to its physical properties (and lower melting temperature than other mammalian gelatin sources), salmon gelatin can be easily blended with other biopolymers, allowing the formation of copolymers and stable poly-electrolyte complexes [13].

Polyhydroxyalkanoates (PHAs) are a group of aliphatic polyesters that are synthesized by a large number of bacteria. PHAs are biodegradable, biocompatible, and have piezoelectric properties that make them suitable for many clean meat applications. These PHAs scaffolds could be a foothold for cells to adhere, grow, communicate, and organize to form the desired tissue. Another important characteristic that PHAs have is the wide range of melting temperatures and glass transition temperatures.

Alginate is a polymer commonly found in the cell wall of brown seaweed and produced extracellularly in some bacteria. Alginate hydrogels resemble the extracellular matrix of the body and can be easily modified into sponges, foams, and fibers, a property that increases the number of applications in Cellular Agriculture. Some desirable properties of alginate scaffolds are their solubility, hydrophobicity, affinity for specific proteins, low toxicity, and biocompatibility. Although it can be a highly flexible material, alginate has naturally poor cell adhesion and poor *in vivo* degradation performance.

A few animal-free scaffolds are de-cellularized plant tissue, chitin/chitosan, and recombinant collagen. De-cellularized plant tissue provides a wide array of structures with varying biochemical, topographical, and mechanical properties; chitin/chitosan-based scaffolds have shown synergistic bactericidal effects and improved cell-matrix interaction. Chitin is a polymer derived from the shells of crabs, shrimp, prawns, and other crustaceans as well as some insects. Chitosan is a de-acetylated derivative of chitin, and this de-acetylation changes in a large degree, the characteristics of one compound compared with the other. Besides being a soluble polymer, chitosan presents properties such as high biodegradability and biocompatibility, non-antigenicity, good adsorption properties, non-toxicity, and bio-functionality. In addition, there were no anti-inflammatory or allergic responses observed in human subjects' ingestion, injection, implantation, and topical application [14].

Collagen and collagen-derived gelatins are used in food and pharmaceutical industries due to their biocompatibility, biodegradability, and weak antigenicity [15]. Lastly, recombinant collagen has the potential to closely resemble native tissue, as opposed to the other two. These benefits, alongside potential scalability and tunability, open the door to applications beyond the biomedical realm, such as innovations in cellular agriculture and future food technologies. Synthetic or natural animal-free polymers such as cellulose, chitin/chitosan, alginate, recombinant silk, Polylactic Acid (PLA), and Poly-epsilon-caprolactone (PCL) provide low-cost, consistent, and tunable scaffolds. The biomaterials should

meet the criteria for cellular agriculture applications, such as being animal-free, found in abundance, biocompatible, versatile, providing nutritional benefits, and already being a part of many commonly consumed products. Although plant and bacterial cellulose share an identical  $\alpha$ -cellulose structure, bacterial cellulose possesses greater crystallinity, a degree of polymerization, and water-holding capacity. The food applications include cultural desserts such as Nata de coco; and functional properties such as gelling agent, stabilizer, and thickener. Moreover, bacterial cellulose has been used to create juiciness and chewiness in emulsified meats. The biocompatibility, low cost, and nutritional attributes make this material a potential candidate for *in vitro* meat production. The antimicrobial and dietary properties, alongside their animal-free nature and abundance, make chitin/chitosan-based scaffolds a likely substrate for cellular agriculture applications [16].

The generation of Cell-based meat (CBM) requires a three-dimensional (3D) scaffold to provide support to the cells and mimic the extracellular matrix (ECM). The scaffold needs to be edible and have suitable nutritional value and texture. Textured soy protein—an edible porous protein-based biomaterial can be used as a novel CBM scaffold that can support cell attachment and proliferation to create 3D-engineered bovine muscle tissue.

Vascularized skeletal muscle tissues can be generated inside 3D scaffolds by co-culture of muscle cells, endothelial cells, and supporting cells. Since the scaffold constitutes a main component of the final product, it should resemble the composition and properties of meat. Textured soy protein (TSP) is a porous, food-grade; inexpensive by-product of soybean oil processing invented in 1960. It is frequently used as a raw material in meat substitutes, due to its texture and high protein content (>50%), which both improve its nutritional value and provide anchor points for cell adherence; therefore, it can be used as a scaffold. Its porosity is an important scaffolding feature that facilitates tissue development throughout the volume of the 3D scaffold. TSP is a dry, porous, protein-based material that expands and becomes softer when soaked in water. TSP can be tailored to various sizes and shapes, which will be useful for scale-up processes (for example, adjustment to bioreactor geometry) in future CBM production. These properties render TSP a suitable candidate for processes aimed at engineering tissues for human consumption. Scientists created a bovine muscle tissue on an edible scaffold made of TSP [17].

Recent grants from The Good Food Institute (GFI) have been focused on creating small, edible scaffolds called micro-carriers. These micro-carriers will organize the cells into “building blocks” that can be further arranged to form thicker cuts of meat (Marcel Machluf, Technion, Israel Institute of Technology, Israel). This can produce minced meat to 3D cuts such as steaks and chicken breasts, appealing on the plate. Recreating meat cuts with complex structures, such as steak or chicken wings, requires the development of a 3D scaffold to support multiple cell types such as muscle, bone, skin, and fat. The structural scaffold found in animal meat consists mainly of collagen fibers.

Creating fiber-like scaffolds with varying degrees of stiffness has been funded by GFI at UCLA (Amy Rowat, UCLA, USA). These scaffolds will help arrange the cells into meaty fibers, with muscle cells and fat cells to co-exist. Scaffolds from algae and fruits are

being envisaged as potential raw materials for creating cultured meat. Developing such scaffolds will enable reliable scaling-up of muscle and fat cell culturing in bioprocessors.

A recently founded startup from Australia, Cass Materials, has developed a novel edible scaffold for cultivated meat production, addressing one of the key challenges facing the cellular agriculture industry. Perth-based Cass Materials has harnessed the natural fermentation processes discovered in the production of nata de coco - a coconut jelly commonly used in desserts in the Philippines - to manufacture a nanocellulose fiber matrix within which meat cells can adhere and grow. These nanocellulose fibers are also produced in scoby, the symbiotic culture formed during the fermentation of kombucha and several other naturally fermented foods and beverages. This scaffold from Cass is tasteless, so as not to taint the flavor of meat, and is a dietary fiber, which could improve the nutritional value of cultivated meat. This means there is protein and dietary fiber in each bite, making it a valuable nutrition balancer.

The scaffolds discussed in the paper highlight several key bio-safety features and nutritional advantages essential for clean meat production. These scaffolds are designed to be biocompatible, biodegradable, and non-toxic, ensuring a safe environment for cell growth and differentiation. They provide mechanical support while mimicking the natural extracellular matrix, which is crucial for proper tissue development. Many scaffolds are made from materials with high protein content, such as plant-based proteins and extracellular matrix components like collagen, which not only enhance tissue formation but also contribute to the nutritional profile of the final product. Furthermore, these scaffolds offer the potential for further nutritional supplementation, allowing for the incorporation of essential vitamins and minerals into the meat, thereby enhancing its dietary value and making it a more nutritious alternative to traditional meat.

Specifically, the scaffolds mentioned above provide an ideal platform for mineral and vitamin supplementation, primarily due to their porous and adaptable structure. This allows for the incorporation of various nutrients directly into the scaffold material during the production process. For instance, hydrogels and protein-based scaffolds can be engineered to hold and release minerals like calcium, magnesium, and zinc, as well as vitamins such as vitamin D and B12. These nutrients can be added to the culture medium or embedded within the scaffold, where they are gradually released as the cells grow, ensuring that the cultured meat is nutritionally enhanced. Additionally, the scaffold's ability to mimic the extracellular matrix allows for the controlled release of these nutrients, optimizing their absorption and retention by the growing cells. This enables the creation of clean meat products with tailored nutritional profiles that can be enriched with essential vitamins and minerals to meet specific dietary needs.

In conclusion, the advancement of 3D-printed scaffolds in clean meat production offers a transformative opportunity to tailor meat products to a wide range of tastes, nutritional requirements, and dietary conditions. By manipulating scaffold materials and incorporating specific nutrients, it is possible to create cultured meat that aligns with individual dietary needs, such as higher protein content, reduced fat, or enhanced vitamins and minerals. While Clean Meat can be a popular substitute for meat, the ability to customize composition can

be particularly beneficial for patients with specific health conditions, such as those requiring low-sodium diets, higher iron intake, or specialized nutrition for recovery and muscle regeneration. The ability to precisely control both the composition and texture of cultured meat opens up new possibilities for personalized nutrition, making it a versatile and sustainable food source that can be adapted to meet the health needs of diverse populations.

## Author Contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. Nikita Naik reviewed and edited the manuscript.

## Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the research, authorship, and/or publication of this article:

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