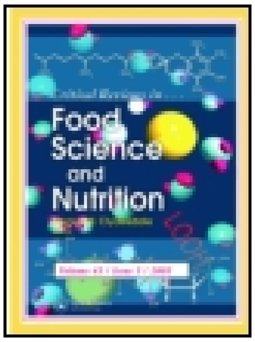

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2020.1815172

REVIEW

3D Printing of cultured meat products

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ABSTRACT
Three-dimensional (3D) printing is a fast-developing digital technology with colossal market scope in food and nutrition technology, providing a platform for establishing unique food products with enhanced sensory and nutritional value for a particular end-user. Cultured meat is the concept of producing meat sustainably in laboratory conditions without the sacrifice of animal life and the excessive use of antibiotics. 3D printing could offer unique solutions for the vital issues of cultured meat production; particularly on regulating the protein, fat, and other nutritional content, along with providing realistic texture. This review highlights the immense benefits of 3D printing technology for the scalable and reproducible production of cultured meat products.

KEYWORDS
alternative proteins; cultured meat; lab-grown meat; plant-based meat; 3D printing; smart proteins

Introduction

Over the last 50 years, global meat production has increased steadily as the demand for meat consumption increases – this growth will continue in the coming decades (Ritchie & Roser, 2017; OECD & FAO 2016). This is however considered unsustainable because conventional meat production is riddled with issues. According to Food and Agriculture Organization (FAO), the total emissions from global livestock contribute to 14.5% of all greenhouse gas emissions and uses 8% of global freshwater (Gerber et al. 2013). To meet the growing demand for animal products, extensive grasslands and forests have been cleared worldwide to raise livestock (Bonnedahl & Heikkurinen, 2018). Health concerns such as nutrition-related diseases and food-borne illnesses arise from conventional meat production due to intensive factory farming and poor animal welfare conditions (Bhat, Bhat, and Kumar 2015). They also contribute to disease outbreaks such as bovine spongiform encephalopathy and swine flu (Sharma, Singh Thind, and Kaur 2015). Regular use of antibiotics in animal farming for increased feed efficiency led to antimicrobial-resistant pathogens, threatening new healthcare crises (National Research Council, 1980; Sharma, Singh Thind, and Kaur 2015).

Moreover, ethics regarding the raising of livestock and the slaughtering of animals have also been questioned. Animal welfare is often ignored in factory farms to keep up with production efficiency (Freeman 2011). Apart from the poor living conditions, the animals' feeding time is systematic and frequent to force them into a desirable size or weight before slaughtering (Potts 2016).

Waste production in animal agriculture contributes mainly to global pollution, often due to consumer's preference for specific meat cuts. In the chicken industry, for example, around 30% of the whole body is discarded, and this percentage is higher in the cattle industry (Welin 2013). This adds to the large amount of food waste produced annually (FAO 2011). Hence unsustainable practices of producing meat by current approaches have raised the need for sustainable methods to produce alternative protein sources to feed our future population (Datar and Betti 2010).

Among other types of alternative proteins, cultured meat is viewed as most realistic since healthy muscle cells are used for food production without compromising nutritional profile and slaughtering animals (van der Weele et al. 2019). Cultured meat has been assigned with multiple names such as clean meat, cell-based meat, cultivated meat etc. (Ong, Choudhury, and Naing 2020). Despite advancements in tissue engineering and 3D tissue culture, it is challenging to replicate meat tissue due to its complex arrangement of different cells, ECM, proteins, nutrients, and growth factors. It has been estimated natural food structures such as mushrooms and jackfruit could match the palatable properties of meat (Gaydhane et al. 2018). With regards to the sensory taste and consumer perspective, various taste testing analysis has been conducted in different geographical locations among different age groups and gender (Bryant and Barnett 2018). Clearly, awareness and tasting experiences have increased the acceptance of cultured meat, and information on personal benefits of cultured meat has been more acceptable than quality and taste. In a recent survey, 58% of the respondents are interested to pay a premium for cultured meat and on average 37% respondents are willing to consider the price of regular meat for cultured meat (Rolland, Markus, and Post 2020). In addition, another report stated the acceptance of cultured meat products sometime vary with the labels and descriptions and hence the importance to educate as well as creating awareness (Siegrist and

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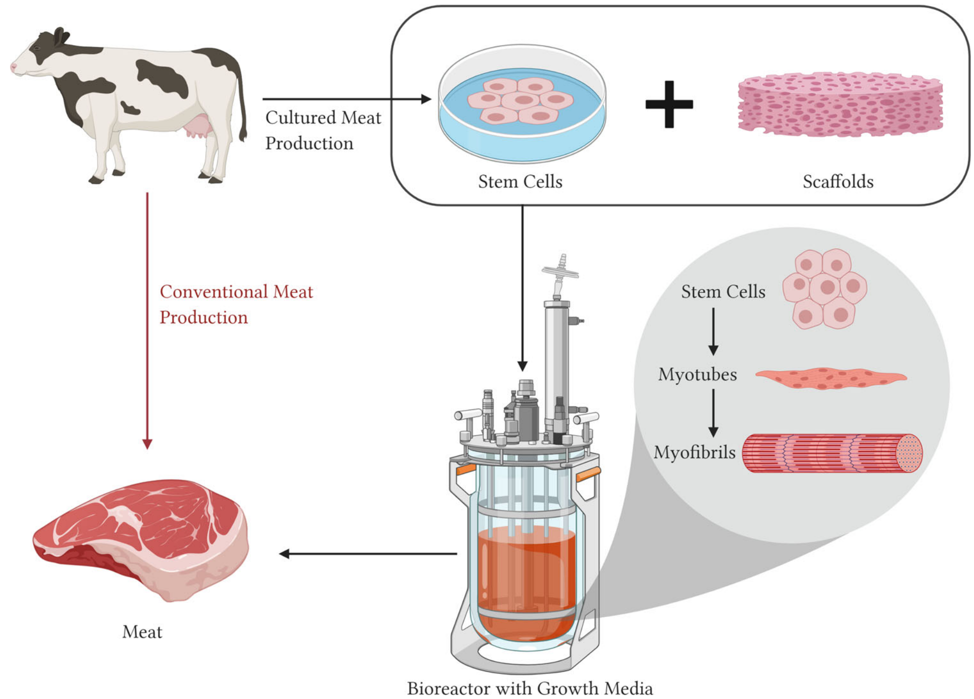

Figure 1. Schematic diagram of the cultured meat production process. Stem cells are obtained from the animal and seeded onto scaffolds before inserting them into the bioreactor filled with growth media to culture meat. Created with BioRender.com.

Hartmann 2020). The use of 3D culture concepts for cultured meat production is limited for laboratory scale and thus the scope of advancement by using 3D printing technology would be a beneficial approach to gain customized cultured meat as well as for large scale production (Neil Stephens, Sexton, and Driessen 2019).

Bioprinting is an innovative process of arranging cellular and acellular components that precisely position and allocate biomaterials with cells to construct complex 3D functional living tissues. Inkjet printing, extrusion printing, and light-assisted printing are three major bioprinting techniques used widely (Kačarević et al. 2018). Among these, extrusion printing is widely used for bioprinting applications. Extrusion printers work based on either pneumatic pressure or mechanical screw plunger to dispense bioink which form continuous filaments (fiber) instead of droplets. The extrusion bioprinters can also be printed with a wide range of viscosities and are approved in compliance with various material styles (Zhang et al. 2017). Extrusion printers have multiple printer heads that allow the simultaneous impression of various bioinks. Additionally, customized shapes, pores, porosity, and cell distribution can be well regulated. All of these advantages help to make extrusion bioprinting technology the most widely used in recent years (Placone and Engler 2018).

3D printing of cultured meat is a novel technology that can potentially alleviate environmental and health issues as it could decrease the demand for animal agriculture, replacing the need for conventional meat production (Rorheim et al. 2016; Bhat, Fayaz, and Kumar 2015a). Despite the lack of evidence on the sustainability gains (Weele et al. 2019) for cultured meat, 3D printing of cultured meat undeniably provides flexibility to meat products as they can be manufactured with nutritional value for specific groups of consumers and fabricate unique textures and shapes (Ben-Arye and Levenberg 2019; Bhat, Fayaz, and Kumar 2015b; Tuomisto & Teixeira de Mattos, 2011).

3D printing is different and sophisticated from culturing meat alone as it prints muscle cells, fat cells, and even extracellular matrix (ECM) supportive cells within the scaffold that supports cell growth and proliferation (Figure 1). Bioink, consisting of cells and biomaterials, is an important aspect of the printing process as it fabricates scaffold structures where muscle fibers are formed to become meat eventually (Bishop et al. 2017; Sun, Zhou, and Huang 2018). After the printing process, the meat will be further matured, usually with bioreactors that provide nutrient transport (Zhang et al. 2018; Bishop et al. 2017). In contrast, cultured meat alone (without 3D printing) is produced by proliferating muscle cells and attaching them to a scaffold or carrier before transferring into a suitable bioreactor with growth media (Sharma, Singh Thind, and Kaur 2015). The scope of this method is inherently limited since highly structured meat like steaks cannot be produced (Gaydhane et al. 2018).

There are few reviews on 3D printing of meat itself (Dick, Bhandari, and Prakash 2019a; Dick, Bhandari, and Prakash 2019b). So far, none of these have elaborated upon the unique strengths of 3D printing for cultured meat products. This review highlights 3D printing approach for cultured meat fabrication and its applications while discussing the challenges faced and its prospects as this technology is increasingly adopted by academia and industry around the world.

3D Printing of cultured meat - process

Selection of starter cells

Self-renewing cells that develop cells required to constitute meat (e.g. fibroblasts, endothelial cells, myofibers, adipocytes, chondrocytes, etc.) are the best suitable starting material (Kadim et al. 2015). Stem cells of either embryonic or adult origin are the best source to meet the technical demand needed for cultured meat production. Embryonic stem (ES) cells are one of the best sources since they have potential to (unlimited) proliferate and differentiate to all cell types required for cultured meat production (Roberts et al. 2015). However, ES cell line development from farmed animals is challenging, since there is a high chance of cell contaminant with non-ES cells, and lower efficiency of replated ES cells are major hurdles as undifferentiated pluripotent stem cells lead to potential teratomas upon their respective applications (Pessôa, Bressan, and Freude 2019). Alternatives for advanced cell culture technology to use ES cells for cultured meat production are being explored. Adult stem cells like satellite stem cells and Adipose-derived stem cells (ADSCs) are considerable cell sources for cultured meat applications (Wankhade et al. 2016). Stem cells of mature muscle tissues are called satellite stem cells or myosatellite cells, which are triggered to repair, regenerate, and recover damaged tissues in the mammalian body. Satellite cells isolated from the biopsy are purified using specific cell surface markers, which upon triggering, transforms myoblasts into myocytes and then to myotubes and finally into myofiber (Forcina et al. 2019). Evidence showed inert capabilities of satellite stem cells toward transforming into adipocytes. Similarly, ADSCs harvested from bone marrow and adipose tissues can differentiate into osteogenic, myogenic, chondrogenic, or adipogenic cell lineages. Additionally, the minimal invasive liposuction methods of isolating ADSCs make them suitable for cultured meat production. Liposuction or resection methods also yield higher numbers of viable cells (Simonacci et al. 2019). Furthermore, extensive research is needed to consider these sources for large scale production of cultured meat products.

Growth media components

A growth medium is a nutrient liquid designed and formulated to satisfy physicochemical and physiological cues for nourishing cells to grow on substrates such as scaffolds or matrices. Growth medium is crucial for proliferating and enhancing the cellular differentiation abilities, eventually leading to tissue regeneration and maturation (Moritz, Verbruggen, and Post 2015). Growth factors and serum are important ingredients of growth medium to achieve tissue maturation. Fetal bovine serum (FBS) is widely used for culturing myosatellite cells and is isolated from an animal source either from adult, newborn, or fetus (Dessels, Potgieter, and Pepper 2016). Thus, it is a difficult proposition to use FBS in cultured meat production as it is against the animal welfare, ethics, food safety, and quality concerns in cultured meat applications. The use of antibiotics in a growth medium for effective cell culture is still considered standard practice (Ventola 2015). However, the use of antibiotics in cultured meat production is controversial and could in fact worsen the problems of antibiotic resistance. Researchers are developing ways to replace the use of animal-based serum and antibiotics that satisfy culture meat production (Andreassen et al. 2020; Kolkmann et al. 2020).

Scaffolding

Myosatellite cells and other major cells used for cultured meat development are adherent cells. Scaffolds are the framework for cells to adhere, grow and attain tissue maturity (often by mimicking native three dimensional tissue); thus structured meat products such as steak and meat cuts can be achieved using suitable scaffolding. Scaffolds for cultured meat production must have biologically active, large surface area, contractible (flexible), maximum growth medium diffusion (porosity) to support tissue maturation, and also be edible without showing toxic and allergic responses after being digested/dissociated (Datar and Betti 2010). Elegant methods of mechanically stretching myoblasts are feasible by the use of porous materials made of cellulose, alginate, chitosan, or collagen; which undergo surface modifcations with minimal changes in temperature or pH (Narayanan et al. 2020). Besides these properties of scaffold material, the fibrous nature of a 3D printed scaffold is important to enhance the organoleptic profile of printed meat products. Gelatin and soy protein have proven to satisfy the requirement of fibrous features of meat (MacQueen et al. 2019; Ben-Arye et al. 2020).

Use of conventional 3D cell culture along with scaffolds cannot customize and regulate on cell positioning between the layers of the scaffold, thus lacks in regulating tissue maturation which might show poor nutritional and organoleptic profiles in case of cultured meat. With 3D bioprinting process, we can precisely regulate the cell-cell ratio, cell positioning, and even cell densities of specific types; bioprinting promises to achieve cultured meat without compromising texture and its meat-like profile. To achieve a 3D bioprinted cultured meat, biomimmetic composition of edible materials (which supports the cell survivability and growth) are needed. These compositions of edible materials could be made printable and formulated as meat-ink.

Rheological properties of meat-ink, post-printing deposition, and post-processing (cooking) stability of 3 D printed scaffold are also the key factors of the scaffold to meet the requirement for 3D printing of cultured meat.

Parameters affecting the bioprinting process

Biofabrication techniques such as 3D printing of constructs are being used in food industry applications; printing food could lead to enhanced food quality. Parameters such as printing speed, nozzle diameter, nozzle height, extrusion rate, and infill percentage are critical to achieving geometrical accuracy, consistency, and even precision of the printed structure (Kesti et al. 2016) (Figure 2). Besides these, the mechanical properties of meat-ink are one of the factors to achieve printable meat products. The swelling phenomenon of the meat-ink might impact the fibrous nature of printed meat. Due to variation in nozzle height, expanded printed product, or even deformed layers due to dragging of meat-

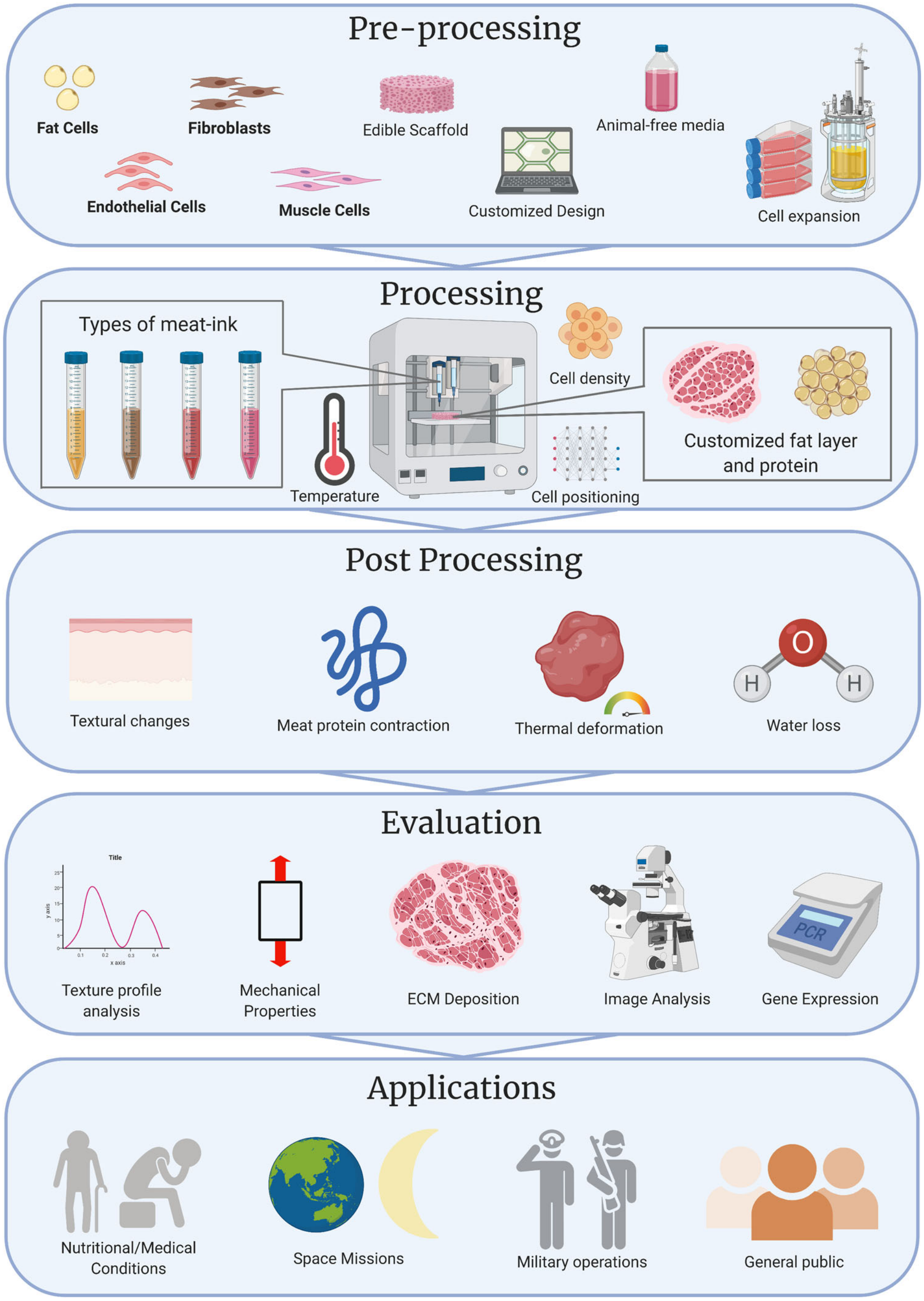

Figure 2. Schematic diagram of major steps considered for 3D printed cultured meat products, their evaluation, and potential applications. Created with BioRender.com.

ink, the post-printing processes could ultimately be affected (Liu, Ho, and Wang 2018). The speed of the nozzle is determined by the rate of movement of the print head and must be controlled by preliminary tests or by measuring the optimum speed. An increase in nozzle speed would result in thinner extrusion of meat-ink with less dragging and hence preventing inter-layer binding and inaccurate end product. Lower nozzle speed results in over deposition of meat-ink at the desired extrusion rate (Tran 2017). However, elevated extrusion rate gives denser prints by overdeposition of ink and reduces empty spaces between the layers. Likewise, different infill percentages affect the printed structure and post-printing process.

Design development – software model

Designing software and printing patterns could determine the nutritional and sensory profiles of the 3D printed meat product. Using different combinations of printing parameters, innovative meat-inks with realistic textures, mouth feels and nutritional profile can be generated (Sun et al. 2018).

The texture of printed food products would be customized by choosing the cocktail of materials with different material properties, printing patterns, printability parameters, and even by adding porous materials to achieve mesostructures toward complex patterns with an additional feature of personalized nutritional values for consumers. It is believed computer modeling can accelerate the processes and product optimization, enabling the industry to mature in years, while reducing the costs of the transformation. Cultured Meat Modeling Consortium (CMMC) was established in 2019 to help scientists envision and enhance the understanding of tissue architecture of various meat types as well as explore modelling of scale-up processes. (Kahan et al. 2019). Additionally, the computer modeling of the scaffolding properties: a solid bulk and a liquid medium within pores are also valuable to program the CAD software connected to bioprinters. The computer-modeling of scaffolds also includes computer fluid dynamics. Using such techniques, the optimum pore size, and branching and flow rate in the scaffold can be determined, and how this can change when a scaffold becomes cell-loaded, and their influence over the overall meat design (Olivares and Lacroix 2013; Choudhury, Anand, and Naing 2018). The hypothetical strategy has been designed to represent the multi-material 3D printed meat such as steak, sausage, and beef patty by using soft meat slurry/paste as a meat-ink along with other food ingredients like salt, garlic, fat layer to address the flavors as well as a nutritional profile of a beefsteak (Dick, Bhandari, and Prakash 2019a). To achieve such designs and final products, the CAD model has to be first designed which later converted into an .STL file by using slicing software, thus creating files that are readable by 3 D printers (Noorani 2017). Liu, Zhang, and Yang (2018), suggested considering different CAD models for the meat-ink portion and fat portion of the final structure, which can be grouped into one multi-material file using slicing software (Liu, Zhang, and Yang 2018).

Bioreactors for scale-up system

Cultured meat products presented for taste testing made use of standard cell culture practices at research-scale. Like biopharma sectors, bioreactors would facilitate scalable production of cultured meat products (Li et al. 2020). Established technologies from the biotechnology and pharmaceutical industries, such as cell therapy, antibody or other biologics production; have proved bioreactors are efficient under regulated conditions to keep cells alive and functional. Similar concepts could be implemented in scale up process of cultured meat products (Specht et al. 2018).

Multiple bioprocessing methods could be useful to bring cultured meat from bench to commercial scale. Among all bioreactors, continuous stirred tank bioreactors are the most widely used for the processing of animal cells as it offers long-term sterility by mechanical stirring, retains a high level of oxygen transfer, and prevents bubbling over air-lift reactors (Allan, De Bank, and Ellis, 2019). Rocking platform bioreactors and vertical wheel bioreactors produce comparable cell growth but at a smaller scale. Single-use platforms like stirred tank bioreactors of capacity 6,000 L are used in existing cell therapy and pharmaceutical industries, which could reach up to 20,000 L (Moritz, Verbruggen, and Post, 2015). Recently, computational and theoretical estimations depicted 2×10^8cells/ml needed in a single reactor of 300 m^3 to produce cultured meat to feed 75,000 people (Li et al. 2020). However, the pharmaceutical industry produces a relatively smaller volume when compared to the estimated volume required for the cultured meat production, thus single-use models may not be economical.

Post-processing feasibility

Analysis of internal and external designs of 3D printed food products is known as post-processing feasibility. 3D printed foods undergo cooking changes which reflect the structure and texture characteristics of their product in printed form. (Dick, Bhandari, and Prakash 2019b). However, limited work has been explored in the post-processing feasibility of 3D printed meat products. Recently, Ariana Dick et al., shared interesting facts on the post-processing feasibility of 3D printed beef. The Dual extrusion-based 3D printing was performed by exploring the fabrication feasibility of 3D printing beef by using beef paste with animal fat infill densities at different layers and achieved 3D printed structure. For 3D printing processing, infill densities of animal fat play a vital role in strength, stability, and structure, which contributes to textural features of printed meat products (Dick, Bhandari, and Prakash 2019a). Besides, the variation of infill percentage of fat would depict the influence of textural changes. Hence is the suggestion for dual extrusion for customized fat in printed cultured meat products. The post-processing parameters such as cooking loss, moisture retention, fat retention, shrinkage as well as textural changes have been studied on 3D printed meat. One of the major challenges in cultured meat is preserving the internal design and structure of 3D printed meat products after cooking. Thermal deformation and contraction of proteins in 3D printed meat products are the main reasons to undergo loss of cooking and shrinkage, which commonly happens during post-processing. Water loss during the cooking of 3D printed meat is one of the major causes to lose the meat matrix and contraction pressure produced by heat would cause the shrinkage of meat proteins as well as water release (Gudjónsdóttir, Napitupulu, and Petty Kristinsson 2019). However, fat content relies on the stability of fat droplets within the printed meat product.

Differences between 3D printing of cultured meat against 3D printing of meat paste

Cultured meat production is a new approach that provides an alternative to the conventional production of meat. This new technology has shown potential and has several promising benefits for both the consumer and the environment. Conventional meat productions require time and resources, to allow the livestock to grow till they are ready to be harvested for meat processing. Depending on the type of livestock, this production time can range from several weeks

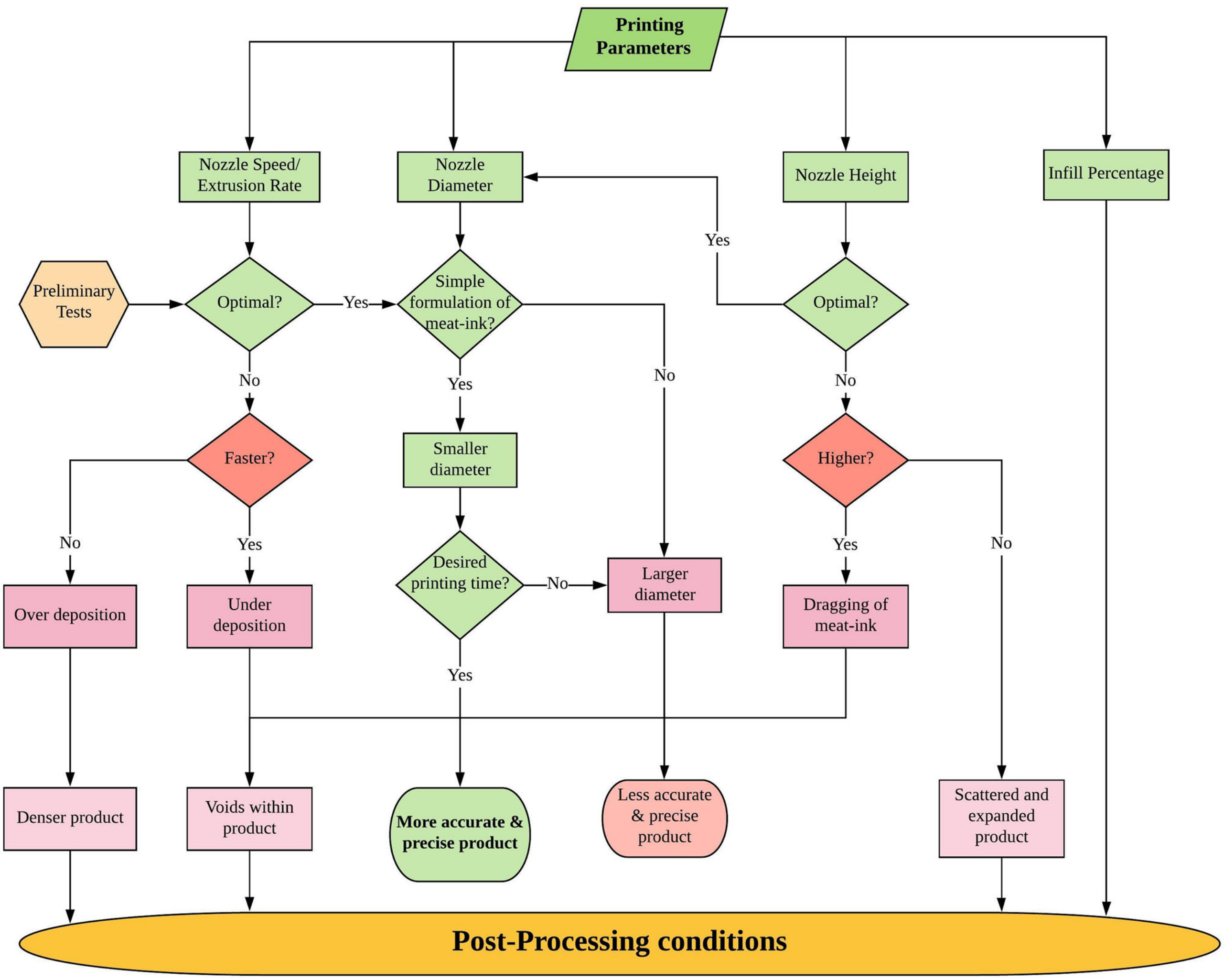

Figure 3. Flowchart of the important printing parameters to consider to obtain a suitable product. Green boxes show the optimal conditions to achieve an accurate and precise product. Created in Lucidchart.

(for chickens) to years (for pigs and cows). Conventional meat could be 3D printed as a paste (which is called as "meat paste" Dick et al. 2019).

Conversely, with cultured meat production, the process of culture to harvest only requires several weeks (Bhat, Bhat, and Kumar 2020; Gaydhane et al. 2018). This saves significant amounts of time and resources compared to conventional meat production if the complexities of *in vivo* tissue growth can be replicated *in vitro*.

Another added benefit of the cultured meat production is the reduced risk of zoonotic, food-borne diseases, and contamination faced by conventional meat production systems due to their nature of production. While both share a similar risk of contamination during the post-harvesting and packing of meat during production, the harvesting of conventional meat products at slaughterhouses facilities potentially exposes the meat harvested to microbial contamination (Sharma, Singh Thind, and Kaur 2015; Bhat, Bhat, and Kumar 2020). Cultured meat on the other hand is performed under sterile conditions with strict monitoring and quality controls in place, thus reducing the risk of microbial contamination during the stage of meat production (Ben-Arye and Levenberg 2019). Additionally, the risk of zoonotic diseases is reduced as there is potentially no handling of livestock during cultured meat production (Rorheim et al. 2016).

The strict processing conditions during the cultured meat production allow for the control and modification of the final meat product during meat production instead of post-production processing, which is used currently in conventional meat production systems (Sharma, Singh Thind, and Kaur 2015).

To better evaluate and understand the differences between cultured meat production and conventional meat production, a summary table below highlights their differences (Table 1).

Promising applications of 3D printing in cultured meat space

The 3D printing value of cultured meat is based on its premise of broader potential advantages. The scale, distribution, and interconnectivity of pores of the scaffold which are necessary for the transport of oxygen and nutrients could not only be controlled via 3D printing technology but even the positioning of cells and biomaterials can be regulated (Seol et al. 2014). This is difficult to achieve in other technologies that affect the structure of the meat to be produced.

Table 1. Differences between cultured meat production VS conventional meat production.

Cultured Meat Production	Conventional Meat Production	Ref
Production time		
• Cultured meat requires several weeks to culture and harvest	• Conventional livestock take weeks to years before meat can be harvested	(Bhat, Bhat, and Kumar 2020; Gaydhane et al. 2018)
Risks of zoonotic and foodborne diseases		
• Lower risk of diseases as potentially no handling of livestock is required in cultured meat production	• Handling of livestock increase risk of transmission of zoonotic diseases	(Ben-Arye and Levenberg 2019; Rorheim et al. 2016)
Contamination source		
• Potential contamination from substrates used during cultured meat production	• Microbial contamination from during processing of slaughtered meat or diseased livestock	(Bhat et al. 2019; Bhat, Bhat, and Kumar 2020)
Land Use and resource consumption		
• Bioreactors are space efficient and may be stacked to reduce land use	• Large land space required for livestock farming	(Bhat, Bhat, and Kumar 2020)
Animal Cruelty Concerns		
• Potentially slaughter free method of meat production	• Livestock slaughtered for meat production are subjected to poor living conditions	(Rorheim et al. 2016)
Controlling Meat composition		
• Ability to manipulate the composition during cultured meat production	• Typically done during post product processing with less control compared to cultured meat	(Rorheim et al. 2016)
Infrastructure required		
• Skilled expertise and adequate laboratory facilities required	• Less skill expertise and advanced facility required	(Petetin 2014; Bhat et al. 2019)

The 3 D printed cultured meat can be a "highly structured meat product" that mimics conventional meat which lead to greater consumer acceptance (Figure 3).

Personalization is a highly-promising offering of 3D printed cultured meat. The military, for example, can benefit by this as this allows meat to be customized based on each soldier's nutrition and energy requirements (Liu, Zhang, Bhandari, and Wang, 2017). The shelf life of meat can also be extended for long military operations. This is similar to the application in space missions. NASA has funded research in 3D food printing to determine the capability of printed food to meet the requirements of food safety and nutritional stability for long space missions while using the least amount of spacecraft resources (Liu et al. 2017; NASA 2013). Moreover, 3D printing of cultured meat in space has been proven to be feasible by a recent experiment carried out by Aleph Farms and 3 D Bioprinting Solutions (Smith 2019).

3D printed cultured meat can also be used to improve the consumption of certain groups of interest, especially people who have difficulties obtaining the proper amount of nutrition or medical conditions such as dysphagia (Sher and Tutó 2015). This computer-assisted technology can offer textured and appealing meat that can provide nutrition for the elderly or patients with chewing and swallowing difficulties (Portanguen et al. 2019; Liu et al. 2017). The printed cultured meat can also be designed to include healthier content such as certain types of vitamins since there has been a trend of children and adolescents not consuming sufficient vitamins and minerals (Portanguen et al. 2019; Bhat, Fayaz, and Kumar 2015b). Moreover, this would be convenience for people with allergies as they no longer have to worry about dietary restrictions when meat can be designed without specific allergens (Lipton, 2017). On a global scale, 3D printed cultured meat can be applied as a solution to solve issues such as famine since it can maximize the resources available and collate them into a customized structure of meat (Portanguen et al. 2019).

Current challenges of 3D printing in the cultured meat industry

Over the past decade, 3D bioprinting has rapidly transformed from niche concept to widely used technology for various biomedical applications. The bioprinting technologies have developed clinically amenable platforms, paved the way for the creation of high-performance models for the discovery of drugs, and developed tools for understanding different tissues/organs development (Kengla et al. 2017). Likewise, 3D printing of cultured meat could accelerate the creation of personalized meat. However, some challenges remain.

Scaffolds that support cells and provides structural features to printed meat tissue is one of the major challenges to achieve mechanical stability with the edible feature as well as sustain adequate cell growth, differentiation and tissue maturation of cultured meat products. (Gungor-Ozkerim et al. 2018). Scientists are exploring strategies for scalability, cost-effectiveness, shelf-life and printable features of novel materials to be used in 3D printed cultured meat production (Jovic et al. 2019). (Dick, Bhandari, and Prakash 2019a; Ben-Arye et al. 2020; MacQueen et al. 2019).

As discussed earlier, cell source is an important key factor toward production of cultured meat without violating animal ethics. Stem cell separation from farmed animals is challenging due to the limited scientific exploration for cell maintenance, differentiation and expansion toward large scale capacities (Cidonio et al. 2019). The replication of the complex hierarchical structure of interconnected vascular networks to the smallest capillaries is one of the major roadblocks in cultured meat production (Ben-Arye and Levenberg 2019; Poldervaart et al. 2014).

Additionally, there are challenges in scaling up of cultured meat products to meet the needs of industrial production. Culturing and expansion of 8×10^{12} muscle cells to produce 1 kg of protein would require traditional stirred bioreactors with capacity of 5,000 liters (Stephens et al. 2018). Although this volume is not uncommon in bioprocessing, processing at this scale has not been tested in tissue engineering. On the otherhand cost effectiveness of culture medium used, existing culture media and its components such as growth and differentiation factors are too expensive. Strategies are being proposed to bring down cost (Bhat et al. 2019; Petetin 2014).

Morever there are regulatory challenges as well. The methods of cultured meat production are still confined to lab-scale production and not on a commercial scale. This has resulted in different regulatory requirements depending on the country, which poses different challenges to the cultured meat industry (Ong, Choudhury, and Naing 2020; Kreis et al. 2019). For example, any meat and meat product treated with recombinant bovine somatotropin (rBST) is banned in the EU market whereas it allows for sale in the US market and is approved by the FDA (Petetin 2014). In the context of cultured meat for the development of cell lines, the use of genetically modified cells, would result in additional review and approval by the regulatory bodies involving genetically modified animals (Stephens et al. 2018).

3D Printing meat companies

A wide range of edible items have been printed by the world of culinary art, ranging from cakes to pizzas. The goal of 3D printing meat is to solve major problems: reducing greenhouse gas emissions, providing consumers with a safe source of protein, and encouraging a sustainable approach to satisfy meat demand (Sousa 2019). Israeli company *Aleph Farms* recently teamed with *3D bioprinting solutions*, to conduct a 3D printed meat test in a microgravity environment. The process started with the extraction of muscle, blood, fat, and cow tissue support cells. Extracted cells were mixed with hormones and nutrients that encouraged the fast growth of cells to produce muscle tissue. The astronauts were able to grow and 3D print the muscle tissue using cells provided by *Aleph Farms* (Carlota 2019). *MeaTech* aims to produce complex cultured meat by integrating advanced 3D printing technologies. Stem cells are isolated from the animal's umbilical cord which is further divided and expanded toward specific cells and 3D printed into cultured meat in predefined design, shape, and structure (Darrah 2020). They plan to develop sustainable farming without harming animals through an industrial meat production process. *Novameat* offers a vegan product that imitates a true steak's taste and appearance. They use a filament made from ingredients such as peas, seaweed, and rice which provides meat-like consistency and also healthy nutrients (V., 2020). Novameat aims to extend its range of items into burger patties, tuna, and chicken. *Redefine Meat,* aims to produce animal-free meat identical to meat cuts such as steakswith its proprietary 3D printing technology, meat digital modeling, and advanced food formulations (Vialva 2019).

Conclusion

Research on additive manufacturing (AM) technologies has been ongoing for decades and has been widely applied across various fields; however 3D printing of cultured meat remains distinct from 3D printing of meat due to the state of cells and its print ink formulation. This technology requires suitable starter cells and growth medium for cell expansion before bioprinting the meat-ink and scaffolds. The 3D printed product would then be analyzed through post-processing to ensure its stability and structure. 3D printed cultured meat production offers a variety of potential benefits and applications in, for example, the military and space, which motivates further research and proves its value in the global market. Conventional meat production continues to raise environmental and health issues as the human population increases. Most importantly, cultured meat allows the sustainable production of slaughter-free meat where no animals are slaughtered during the manufacturing process. Cultured meat production technology is in its infancy and is still developing. Though the issue of using serum-free media and antibiotics has been addressed via technical advances, the production of whole cuts of meats has yet to be achieved. The increasing number of 3D printing meat companies has shown that more interest and attention has been given to developing an alternative technology to conventional meat production and that commercialization is attainable shortly. However, 3D printing of cultured meat and in general the cultured meat industry needs to overcome the challenges concerning the culture medium, stem cell source, and scalability. These challenges need to be addressed while analyzing its sustainability gains to allow cultured meat to be widely commercialized and achieve its initial goals. Additionally, despite support from the scientific community, animal rights groups and environmentalists, there may be the issue of consumer perception of unnaturalness in its production which will define the consumption of cultured meat in near future.

Acknowledgements

This research is supported by Bioprocessing Technology Institute, A*STAR. The authors would like to sincerely thank Dr David Yeo Chen Loong (BTI A*STAR) for critically proofreading the mansucript.

Funding

This research was supported by Bioprocessing Technology Institute, A*STAR.

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