Producing Food Products from Cultured Animal Tissues

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Abstract

Cell cultivated meat is a relatively new (only about 20 years old) idea using a technology that has been around for over one hundred years. The technology necessary to culture cells for human consumption is developing at a rapid pace. The concept is relativity simple in that it uses cells of animal origin and raises them in a bioreactor to produce food that closely mimics meat products traditionally derived from harvesting animals. While the concept of producing cell cultivated meat is simple, the implementation has proven to be very challenging. Today, cell cultivated meat is not available for consumer purchase at retail or food service outlets. Still, current efforts are underway to scale up production of cell cultivated meat, but the industry faces several technology hurdles. Those hurdles include lowering the cost of media used to cultivate cells, developing cell lines that can be propagated indefinitely and producing finished products that possess the same palatable and nutritional characteristics of traditionally produced meat products. At the same time that technological challenges are being navigated, other issues such as governmental regulatory oversight, product labeling, and even nomenclature policy must be addressed. Steps are currently underway to develop a regulatory framework for cell cultivated meat and legislation is being considered to provide federal definitions

Photos courtesy of Jnix/Shutterstock and Jess Krieger. Using cells harvested from a cow, scientists are able to cultivate skeletal muscle cells (middle), and then make a burger that was generated by adding those cultivated cells (right).

to ensure clear communication to consumers. In fact, food products produced from cell culture technology are referred to by many names such as: "cultivated meat", "clean meat", "cultured meat", "lab meat", "fake meat", "cell cultivated meat", and "in vitro meat". Finally, because cell cultivated meat is a brand-new platform in food production, food safety cannot be overlooked. As with any novel food, understanding and mitigating potential safety risks is critical. Because the production systems associated with producing cell cultivated meat are so different from obtaining meat directly from animal sources, there may be food consumption hazards that are not present in conventionally produced meat products. Cell cultivated meat may become available in retail outlets within the next 5 years. Before that happens, all these issues and many others must be addressed.

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Introduction

Technologies for Production of Cell Cultivated Meat: Challenges and Opportunities

Culturing cells for human benefit is not a new concept. Likewise, the intention of using those cultured cells to produce food is nearly two decades old (Post 2012, Stephens et al. 2019). Today, the technology necessary to culture cells for human consumption in the form of cell cultivated meat is developing at a rapid pace. Milestones to bring these products to market and available for consumer purchase are being achieved much quicker and media attention has dramatically increased. Still, there are many questions that need to be addressed before cell cultivated meat is ready for the dinner table. In addition to further development of the actual technology needed to produce cell cultivated meat, the safety of these products must be evaluated using the same rigorous standards applied to food today. A framework for the regulatory oversight of these products has been outlined, but as the technology improves, cell cultivated meat products may be developed that will, in turn, raise new questions to be answered with regulatory policy. The nutritional composition and sensory characteristics of cell cultivated meat will need to undergo appropriate scientific investigation to determine the true similarities or differences when compared with conventional meat. In 2012, it was published that for cultivated meat to be successful it needs to exactly mimic and recreate traditional meat in visual appearance, smell, texture, and taste (Post 2012). Finally, success of cell cultivated meat products will depend on consumer acceptability. As with any product made available in the market place, consumers will determine the ultimate success of that product.

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Cell Culture Technology

Animal cell culture refers to the isolation, growth, differentiation, and maintenance of individual cells in a controlled environment. Animal cell culture is a technique that arguably dates to 1885 when Roux first isolated and maintained the chick medullary plates in a warm saline solution for several days (Pham 2018; Roux 1885). However, an in vitro primary outgrowth of frog nerve fibers (Harrison 1907) is often considered as the birth of true animal cell culture (Freshney 2016), since the frog system consisted of individual cells. Subsequently, chick connective tissue cells were successfully cultured, and aseptic techniques were further developed (Carrel 1912). Cell culture was a prominent technique in the development of vaccines for diseases such as rabies and polio through the early to mid-20th century (Plotkin 2014). Overall, cell culture is a classical laboratory technique that arguably began to rise to

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prominence in the 1950s and 1960s through the development of immortalized research cell lines sometimes from tumorigenic tissue, such as the well-known HeLa cell line (Gey et al. 1952).

The technical challenges facing cell culture techniques are that animal cells require a sterile, warm, and humidified atmosphere rich in nutrients. It is also imperative to ensure there are no microbial contaminants. Microbial cells tend to be significantly smaller than animal cells; specifically, bacteria range from 1 to 2 microns in diameter, and animal cells can be approximately ten times larger in diameter than bacteria. The small size of bacteria often makes detection of microbial contamination in animal cell cultures difficult. More importantly, microbes grow at a much faster rate (20 minutes; Gibson et al. 2018) compared with animal satellite cells (approximately 24 hours; Stiens et al. 2000). Therefore, in the event microbes are introduced into an animal cell culture system, they will grow at a much faster rate, exhaust all available nutrients in the cell culture media and potentially result in the death of the animal cells.

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Vertebrate/animal cells were first cultured on open bench tops, which resulted in significant risk and occurrences of microbial contamination. This microbial contamination may have arisen from poor environmental air quality leading to contamination of the cultures. Furthermore, microbial contamination may arise from failure of sterilization, poor laboratory maintenance, and—most frequently—lapses in proper aseptic technique. A major challenge in research laboratories is often delineating the cause of contamination, and it is generally recognized that good aseptic technique revolves around establishing day-to-day procedures pertaining to sterile technique and then strictly adhering to procedures.

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Arguably, the development of sterile disposable plastic ware, which reduced the cost and eliminated the potential failure point of sterilized glass culture dishes, combined with the development of bacterial free air fields (i.e., laminar flow cabinets and biological safety cabinets [Figures 1]), propelled animal cell culture to a mainstream technique to delineate biological mechanisms through the 20th century. The driving forces moving the development of cell culture techniques forward were to produce antiviral vaccines, and as an important alternative to employing animals in biological studies. As cell culture techniques developed through the late 20th and early 21st century, it became increasingly prominent as a research tool, but it also became important as a manufacturing platform to produce molecules that improve human health. The most notable example is the movement of influenza viral propagation from chick embryos to animal cells (Rubio and Eiros 2018). Furthermore, cell culture has become an important production platform for therapeutic monoclonal antibodies, and other therapeutic complex proteins, as well as the promise to produce therapeutic

Figures 1. Biological safety cabinets in a cell culture facility.

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replacement using autologous cells (Atala et al. 2006). Overall, cell culture has matured from a curiosity practiced by a few aficionados, to an experimental modality to better understand biology, to a therapeutic translational biomanufacturing platform. Recently, there has been much excitement about using cell culture technology to produce animal protein (cell cultivated meat) suitable for human consumption. However, there are several technical issues, and definitions that should be considered as the food manufacturing community continues to develop these potential food products.

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Cell Cultivated Meat Production Overview

Cell cultivated meat research has created a novel interdisciplinary practice requiring the expertise of cell biologists (Figure 2), biochemists, chemical engineers, analytical chemists and meat/food scientists to solve a multitude of problems. Scientists working to develop scalable cell culture technologies have coined this new interdisciplinary space cultivated meat science with the objective of ultimately blending biomanufacturing with traditional meat science. The intention is to isolate cells using tissue biopsy from livestock, such as cattle, pigs, sheep, poultry; aquatic, or insect species to develop cell lines that have specific functional or quality characteristics, such as enhanced cell division ability and specific flavor or nutrition. A growth or cultivation bioprocess is developed to produce food from the cell lines (Langelaan et al. 2010), where animal cells are used as a foundation for a final food product with biomanufacturing. This differs from traditional meat production, which reduces an animal carcass into specific products.

Figure 2. Students perfecting cell culture techniques.

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The basic unit of cultivated meat is a viable cell. In order to survive and grow both within and outside of the body, cells need a water-based environment with a supply of nutrients and growth factors needed for various cellular processes and have metabolic waste products removed from the growth environment. Lipids and proteins signal the cell that it should undergo cellular proliferation, or cell division / mitosis, or develop into a cell type found in muscle/meat through a process called cellular differentiation (Allen and Boxhorn 1989; Belal et al. 2018). Differentiation is a process that converts a cell type or a mature cell into its final state. The water-based environment must maintain a specific pH (typically between 7.2 and 7.4), a suitable oxygen/carbon dioxide concentration and appropriate ionic balance. The cells grow best in an environment held at the body temperature of the species from which the cells were obtained along with a continuous supply of oxygen. The natural homeostasis of a living organism provides this environment to cells in the body. In cell culture, this environment must be recreated through the use of external growth systems that include cell culture incubators, bioreactors, and cell culture media.

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As the technology develops, the basic cultivating process of cell cultivated meat products will likely include (1) cell line development, (2) cell cultivation, and (3) tissue cultivation.

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technology develops, the basic cultivating process of cell cultivated meat products will likely include (1) cell line development, (2) cell cultivation, and (3) tissue cultivation (Figure 3). First, cell lines suitable for producing cultivated meat must be developed that can be grown in bioreactors with high fluid volumes. The cells grown in this stage are intended to be incorporated as a raw food material into unstructured-meat products like chicken nuggets and hamburgers or may be used to develop 3D structured tissues such as steak and pork chops.

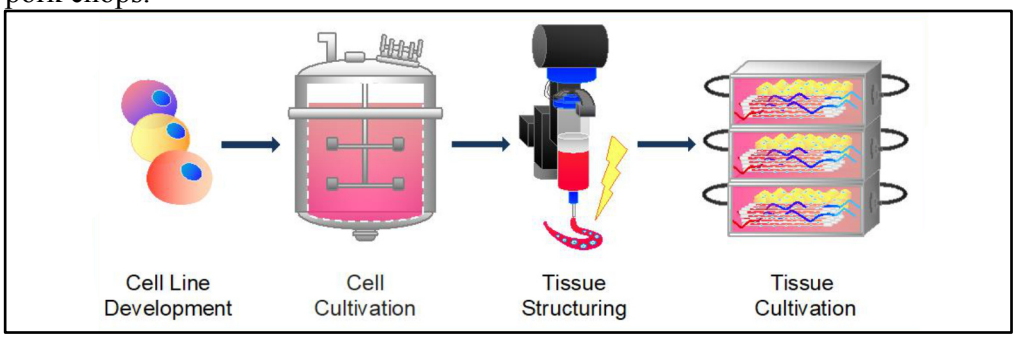

Figure 3. A workflow for meat cultivation.

Tissue structuring, also called tissue engineering or tissue synthesis, embeds cells within a three-dimensional scaffold, which simulates connective tissue. A scaffold is a matrix composed of edible material that provides a three-dimensional structure for cell propagation to form a specific tissue structure. It can be composed of a protein matrix, such as collagen (Jakab et al. 2010), a polysaccharide matrix, like cellulose from plants, or a blend of both. Protein and polysaccharide scaffolds can be produced from animal, plant, or microbial sources (MacQueen et al. 2019). The living tissue can then be matured in a tissue cultivation bioreactor. Alternatively, cultivated cells may be incorporated into a product that simulates structured tissue, but did not undergo a tissue fabrication step that results in a living tissue matured in a bioreactor.

The objective is to develop three predominant product phases that will result from overcoming technical hurdles at each phase of the cultivation process (Figure 4). The intention of phase I products will be to include small quantities of cells from animal or aquatic species to supplement plant-based meat analogue type products or even conventional type meat products. The animal cells will essentially serve as food additives that enhance the palatability or nutritional characteristics of edible plant protein, such as adding a small percentage of skeletal muscle cells grown from a chicken to a plant-based chicken nugget. Foods from subsequent phases of development of cell cultivated meat are intended to be composed almost entirely of animal cells with very minimal or no

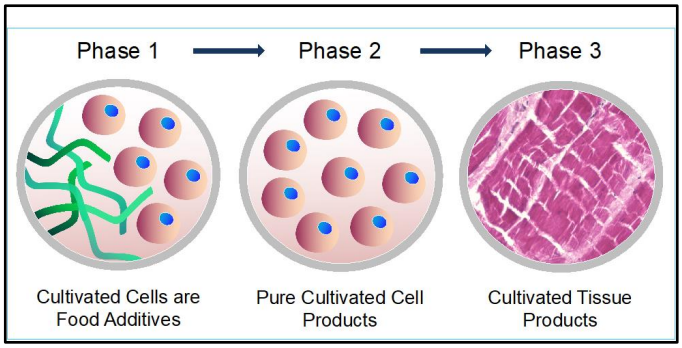

Figure 4. The different product phases of meat cultivation. The image representing cultivated tissue is turkey meat harvested from an animal, provided with permission by Natalie Rubio.

plant-based protein extenders. The third phase of development is intended to result in tissues matured in bioreactors.

Cell Line Development

Cell line development, or cell line engineering, begins with extracting individual cells from a tissue biopsy of an animal. The cells obtained from an animal possess inherit limitations that make them unsuitable for a large-scale bioprocess manufacturing. Aging occurs at the cellular level, and the harvested cells isolated from an animal will also age during culture (DiLoreto and Murphy 2015). The aging process depletes the proliferative capacity of the cells, which necessitates extending their proliferative capacity or immortalizing of cell lines, which bypass's cellular aging. Immortal cell lines can be created through genetically engineering cells (Genovese et al. 2017; Tanaka et al. 2013); by selecting a cell type for expansion with naturally enhanced or indefinite proliferation potential, such as stem cells (Zheng et al. 2006); or depending on spontaneous immortalization of cells through natural genetic mutations that occur during cell culture (Maqsood et al. 2013). Cell lines are stored in a master cell bank, where they are cryopreserved in a state of suspended activity until they are needed (Yaffe and Saxel 1977). Cryopreserved cells can be thawed and re-animated and expanded in bioreactors to initiate the process of cell cultivated meatmanufacturing. Master cell banks tend to range from ten to hundreds of vials of cells and have a biological master file associated with them. This master cell bank is used to populate a working cell bank that can include thousands of vials of cells. For each production run, a vial is removed from the working cell bank to cultivate in bioreactors.

Cells used for cultivation of cell cultivated meat can be derived from various kinds of stem or precursor cells found in animal embryos, bone marrow, or muscle tissue. Induced pluripotent stem cells (iPSCs) can also be used, which, along with embryonic stem cells, can be differentiated into any cell type in the body, such as skeletal muscle cells (Post 2012) (Figure 5A).

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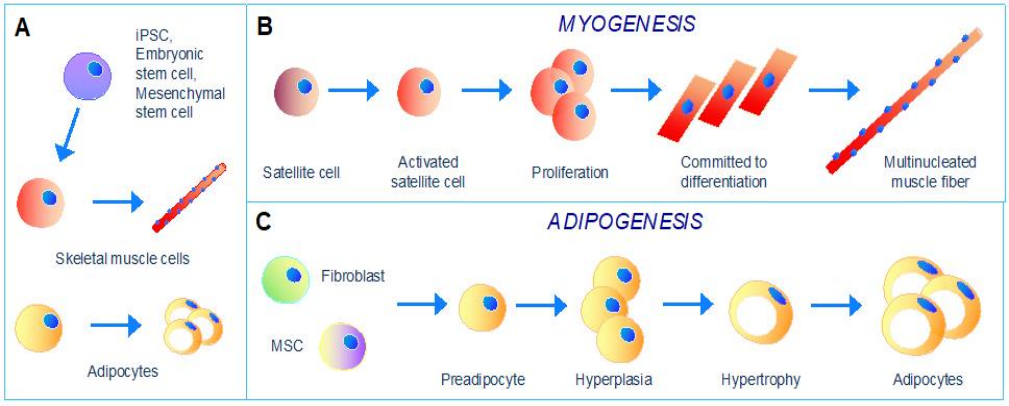

Figure 5. The different cell types used for cell cultured meat cultivation and the growth process for different cell types. (A) Various stem cell sources can be differentiated into cell types relevant for meat products. (B) The process of myogenesis begins with the activation of a muscle stem cell called a satellite cell and ends with a multinucleated muscle fiber. (C) Adipogenesis occurs when cells mature into adipocytes that contain flavorful lipid droplets.

Regardless of the initial cell population, the manufacturing process is intended to result in the production of cells (e.g., muscle and fat) found in conventional meat. The three dominant cell types that influence meat flavor and texture are skeletal muscle cells, intramuscular fat cells, and connective tissue cells called fibroblasts. Cell lines must be able to undergo myogenesis (the formation of multinucleated, contractile skeletal muscle cells [Le Grand and Rudnicki 2007]) or adipogenesis (the development of fat cells containing intracellular lipid droplets [Agley et al. 2013; Mehta, Theunissen, and Post 2019]) (Figure 5B, C). Cell lines that produce connective tissue cells like fibroblasts may also be used, because the texture of meat can be impacted by the connective tissue scaffolding that holds cells together (Krieger et al. 2018).

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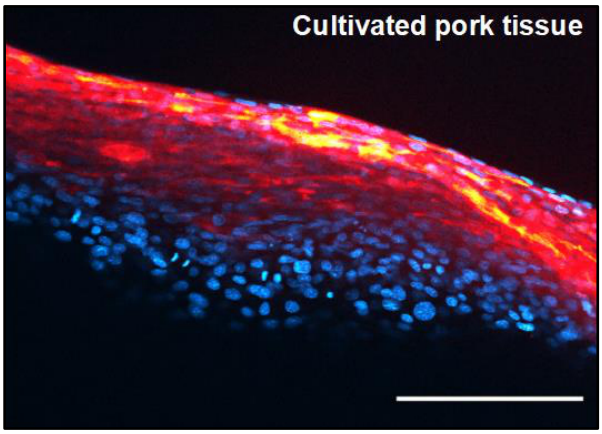

Figure 6. Skeletal muscle cells from a pig are structured into tissue. The red color highlights myosin heavy chain protein, which is involved in muscle contraction, and blue dots show cell nuclei. Image provided by Jess Krieger.

Skeletal muscle and fat cells can be incorporated into both unstructured and structured products.

Skeletal muscle and fat cells can be incorporated into both unstructured and structured products. Unstructured products do not require the additional technology involved for tissue synthesis. Structured products produced from cultivating tissue, (Figure 6), may require additional cell types in order to recreate tissue patterns like marbling and blood vessel networks such as fibroblasts or endothelial cells, which form blood vessels during a process called vasculogenesis (Koffler et al. 2011) (Figure 7).

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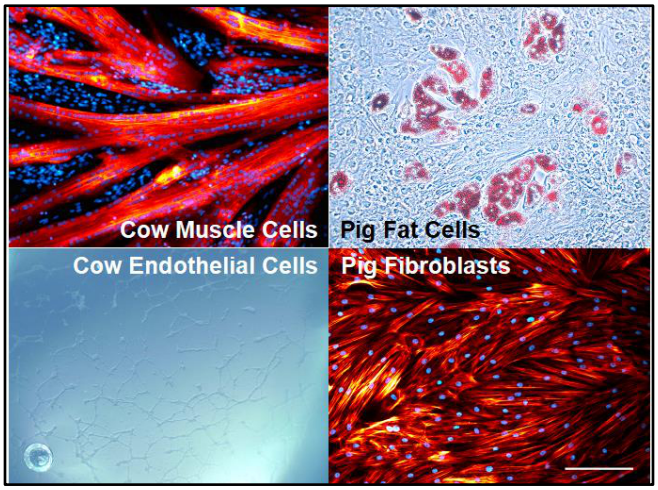

Figure 7. Different cell types required for meat include myocytes and adipocytes. The myosin in the muscle cells is colored red, and the nuclei blue. The lipid droplets inside the adipocytes are stained red. Endothelial cells and fibroblasts may be necessary for structured tissue products. Endothelial cells are observed grouping together into a blood vessel network. The cytoskeletal proteins in the fibroblasts are colored red and the nuclei blue. Images provided by Jess Krieger.

Cell Cultivation

The goal of cell cultivation is to yield a large biomass of edible cells originating from a master cell bank of upwards of thousands of kilograms expanded from a working cell bank. The bioprocess must be scalable, meaning it can begin in very small culture volumes, such as a cell culture flask, and be expanded into larger and larger culture volumes, such as a 20,000-liter bioreactor (Allan, De Bank, and Ellis 2019). The

growth process typically consists of an extended growth phase to yield a high biomass of cells which can then be differentiated into cell types found in animal meat.

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Culture media used for cell cultivation to stimulate the cells to undergo proliferation and differentiation consists of a complex of essential ingredients such as essential amino acids, fatty acids, macro and micro nutrients, natural and synthetic growth factors, and anabolic sex hormones. Different cell types require unique growth factor combinations, such as skeletal muscle cells requiring a different media composition than fat cells (Seo et al. 2019). Cell cultivated meat production facilities will require hundreds of thousands of liters of cell culture media or more that may be supplied by a separate media manufacturing process and / or industry.

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There are many types of bioreactors that can be used for cell cultivation, the most common being stirred-tank bioreactors. These systems are already used to biomanufacture proteins used by the dairy industry to make cheese, such as fermentation-produced chymosin from microbes (Barbano and Rasmussen 1992). The cells in stirred-tank bioreactors are grown in a fluid suspension as a single cell suspension, in cell aggregates, or on microcarriers. Microcarriers are spherical units smaller than a millimeter in diameter which serve as an adhesive substrate for cells (Verbruggen et al. 2018). Microcarriers can be made from edible material that can be incorporated into a food product, such as plant derived protein, cellulose, or an inedible material like plastic. Inedible microcarriers must be separated from the cells before they are incorporated into a food product.

Tissue Cultivation

During tissue manufacturing, cells are aggregated into a defined pattern modeled after skeletal muscle tissue. The architecture of skeletal muscle consists of aligned, contractile skeletal muscle fibers; intramuscular fat deposits; nerves; and a complex system of arteries, veins, and capillaries. An extracellular matrix, which functions as a polysaccharide or protein-based “glue”, connects the cells together (Ben-Arye and Levenberg 2019). Tissue structuring can also be achieved through tissue engineering systems, such as bioprinters. First, a tissue model is developed in a software program and a bioink made of media, cells, and structural matrix components is formulated. Then, the bioink is printed according to the model and the cell cultivated meat is matured in a tissue bioreactor (Kang et al. 2016).

The composition of culture media for tissue cultivation is different than in cell cultivation, because the focus is to differentiate or mature a combination of cell types within a tissue matrix instead of optimizing a growth process for a single cell type.

The composition of culture media for tissue cultivation is different than in cell cultivation, because the focus is to differentiate or mature a combination of cell types within a tissue matrix (Levenberg et al. 2005), instead of optimizing a growth process for a single cell type. Maturing skeletal muscle tissue in cell culture scenarios requires additional design considerations for a new class of tissue maturation bioreactors. A tissue maturation bioreactor that can perfuse media through structured meat, induce an exercise regime, and support the survival of larger scale tissue volumes does not yet exist. However, upon development these bioreactors will have features similar to organ transplant care systems. These may be modeled after the systems used to keep donor hearts and lungs alive for recipients, which can connect the vasculature of the organ to a perfusion system that pumps oxygenated blood into the organ (Ardehali et al.

2015). Meat texture is a complex trait influenced by connective tissue and the structure of muscle fibers themselves. One concern with cell cultivated meat is the lack of muscle fiber contraction. This contraction in a living animal stimulates the synthesis of muscle protein. Similarly, bioreactors can stimulate synthesized muscle with electrical stimulation to promote maturation (Davis et al. 2019). Such stimulation may help mimic the texture of meat in cell cultivated meat products.

Two major challenges with tissue manufacturing are the complexity of recreating tissue structure with bioprinters and the associated costs of using tissue structuring and cultivation equipment.

Manufacturing Challenges

Scaling up of cell cultivation is among current efforts of the cell cultivated meat industry, which has several technology hurdles. These hurdles include lowering the cost of media, developing cell lines that can be propagated indefinitely and possess specific palatable and nutritional characteristics; establishing scalable bioprocesses, reducing the operational costs of large-scale biomanufacturing facilities, and disposal, recycling or amelioration of waste products. Two major challenges with tissue manufacturing are the complexity of recreating tissue structure with bioprinters and the associated costs of using tissue structuring and cultivation equipment. Tissue is complexly patterned in the body on the scale of nanometers or micrometers, which current bioprinting systems have difficulty replicating (Kacarevic et al. 2018). A delivery system for oxygen and nutrients to cells deep within the tissue is necessary by design, because passive diffusion of molecules becomes ineffective beyond a cell layer depth of 100 micrometers. The circulatory system accomplishes this in the body, and a vascular network, or equivalent perfusable system for fluid flow, is necessary to perfuse cell culture media through the tissues (Forgacs 2012).

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Governmental and Regulatory Oversight of Cell Cultivated Meat

The development of cell cultivated meat as a potential human food has resulted in considerable debate about how such materials would be regulated in the United States. Under federal regulations, the U.S. Food and Drug Administration (FDA) and the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) have different responsibilities with respect to meat and meat products. The USDA-FSIS oversees meat, poultry, and certain egg products pursuant to the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act. The FDA exercises jurisdiction over all other food products pursuant to the Federal Food, Drug, and Cosmetic Act (FDCA). Importantly, the FDCA authorizes the FDA to oversee the safety of all food ingredients used in both FDA- and USDA-regulated foods.

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The FDA's responsibilities are codified in Title 21 of the U.S. Code of Federal Regulations and cover all foods except meat and poultry and some other items specifically assigned to the USDA. While the FDA does not regulate meat and poultry, the FDA does regulate food ingredients that are commonly used in meat and poultry such as salt, phosphates, and curing agents. The FDA is also responsible for all veterinary products, including livestock feeds, pet foods, veterinary drugs, and devices. In addition, the FDA exercises jurisdiction over biologics, including vaccines, blood, and blood products; cellular and gene therapy products; and tissue and tissue products. Accordingly, the FDA has been the lead federal agency involved in determining the safety of new biotechnological approaches to foods, including genetically modified crops and

animal cloning. Currently, FDA-regulated companies are obligated to understand and comply with the FDA regulations or risk prosecution for violations. The Food Safety Modernization Act, enacted in 2011, resulted in a continuing number of changes requiring more pre-approval by FDA-regulated food manufacturers than in the past.

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Any product containing greater than 3% raw meat or 2% cooked meat falls under the jurisdiction of USDA-FSIS under Title 9 of the Code of Federal Regulations if it enters into interstate commerce. Some features of USDA-FSIS regulation include 100% inspection of animals and carcasses during the harvest activity, pre-approval of labeling for all meat products, as well as Hazard Analysis and Critical Control Point (HACCP; a program designed to ensure food safety) requirement to manage foodborne illness risks. Under this regulatory model, meat and poultry products intended to be marketed in interstate commerce may not legally be sold unless first inspected by USDA-FSIS. This is because both the Federal Meat Inspection Act and the Poultry Products Inspection Act require all meat and poultry sold commercially to be inspected by USDA-FSIS to ensure that the product is safe, wholesome, and properly labeled. The federal mark of inspection communicates to consumers that there is compliance with USDA-FSIS regulations.

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With production methods for cell cultivated meats clearly spanning areas where both FDA and USDA-FSIS have regulatory authority, both agencies have determined that there needs to be formal cooperation between the two. As such, the agencies entered into a joint published "Memorandum of Understanding" (MOU) released on March 7, 2019 (USDA 2019). This agreement stipulates that the FDA will oversee cell collection and propagation up to harvesting as cell cultivated meat, at which point USDA-FSIS becomes the responsible agency. The concept of joint jurisdiction is not new. In fact, the FDA and USDA have a long history of cooperatively working together. As noted above, the FDA takes the lead on safety for all ingredients added to meat and poultry products.

There is the expectation that the USDA will require an inspection system that includes sanitation, physical product inspection, HACCP verification, product testing, and records review, as well as prior label approval before a product may be distributed in interstate commerce.

Similar to harvesting animals for food, there is the expectation that the USDA will require an inspection system that includes sanitation, physical product inspection, HACCP verification, product testing, and records review, as well as prior label approval before a product may be distributed in interstate commerce. In this regard, USDA-FSIS is charged with developing any needed "additional requirements to ensure the safety and accurate labeling of human food products derived from the cultured cells of livestock and poultry…" (USDA 2019).

In many cases, the production process for cell cultivated meat will likely not be vertically integrated. In other words, each step in the production process could be an end point—i.e., the collection, characterization and qualification of cell-lines could be conducted by Company A; Company B could then grow the meat in a cultivator (bioreactor) with media supplied by Company C; Company D could, in turn, market the meat once harvested at Company B. Hazards could conceivably emerge at each step, and especially during the transportation phase. Thus, regulators will need to consider those transitions and consider how best to go about verifying compliance in a manner consistent with the March 2019 MOU outlining where FDA oversight ends and USDA oversight begins.

To iron out the details of how cell cultivated meat will be regulated, in the summer of 2019, the FDA and the USDA formed three Interagency working

groups focused on cell cultivated meat and poultry production. The first working group, led by the FDA, is focused on pre-market safety. This group is tasked with developing the overall pre-market consultation process. The second working group focuses on the transfer of jurisdiction from the FDA to the USDA at the cell harvest stage and will develop specific procedures for transferring inspection oversight. The FDA and the USDA-FSIS are the co-leads for this working group. The third and final working group is focused on labeling and is led by the USDA-FSIS. This group is tasked with developing coordinated principles for product labeling and claims to help ensure consistency and transparency.

The FDA and the USDA-FSIS are engaging industry to help inform the details of how the agencies will ultimately regulate this sector.

The "premarket assessment" section is actively engaged in communications with start-up companies to better understand various production methodologies. The FDA and the USDA-FSIS are engaging industry to help inform the details of how the agencies will ultimately regulate this sector. The USDA-FSIS also indicated that it does not expect to implement any new regulations when it comes to inspections for cell cultivated meat products, but that conversations are ongoing regarding possible rules or guidance on labeling. At this time, no new regulations are expected from the FDA with regard to premarket safety.

As of this writing, no set nomenclature has been settled upon for meat, poultry, or seafood produced through cellular agriculture.

Finally, the U.S. agencies will be faced with reconciling the U.S. approach to regulations with those applied in other countries. This will be an evolving area with the need to satisfy stakeholders with interests in innovation, safety concerns, transparency in communication, and protection of the identity of ethnically traditional products.

Labeling of Cell Cultivated Meat

"Cultivated meat", "clean meat", "cultured meat", "lab meat", "fake meat", "cell cultivated meat", and "in vitro meat" are all terms currently being used to describe meat produced through cell culture technology. As of this writing, no set nomenclature has been settled upon for meat, poultry, or seafood produced through cellular agriculture. Very recently, however, those closely tied to the cell cultivated meat industry released a story suggesting that "cultivated meat" may be the naming compromise suitable to a majority of vested parties. Cell cultivated meat will continue to be defined with a host of terms until the USDA officially releases a statement providing guidance on labeling requirements.

The United States Cattlemen's Association filed a petition with USDA-FSIS requesting that the USDA undertake rulemaking on beef labeling to clarify the difference between beef derived from cattle and "beef" products created through cell culture technology.

The regulatory conversation around cell cultivated meat began in earnest in February 2018. That month, the United States Cattlemen's Association filed a petition with USDA-FSIS requesting that the USDA undertake rulemaking on beef labeling to clarify the difference between beef derived from cattle and "beef" products created through cell culture technology. To date, the USDA has received more than 6,100 comments on this petition. (USCA 2018).

In October 2019, a bill was introduced in the U.S. House of Representatives with a summary title of the "Real MEAT Act of 2019."

Then, in October 2019, a bill was introduced in the U.S. House of Representatives (U.S. Congress 2019a) with a summary title of the "Real MEAT Act of 2019." More recently, in December 2019 the U.S. Senate's version (U.S. Congress 2019b) of the bill was introduced and referred to the Health, Education, Labor, and Pensions Committee. While this act was primarily introduced to address meat analogue products made from plant proteins, it has specific language that would affect labeling of cell cultivated meat products. One example of this is a statement on page 6 of the proposed House bill that states,

"The term 'beef' or 'beef product' means any product containing edible meat tissue harvested in whole form from domesticated *Bos indicus* or *Bos taurus* cattle."

Because no actual commercial products of cell cultivated meat that generate specific regulatory questions have been produced at this time, a number of hypothetical scenarios are presented here to illustrate the types of labeling-related issues that may arise. These scenarios are focused on use of cell cultivated meat as an ingredient in products that have regulatory restrictions.

The hamburger standard of identity requires the use of "beef" and precludes using certain added ingredients Will cell cultivated meat qualify as beef for purposes of the hamburger standard of identity?

Scenario 1: Cell cultivated meat will be used in ground beef.

Potential considerations:
The hamburger standard of identity requires the use of "beef" and precludes using certain added ingredients (Code of Federal Regulations 2019). Will cell cultivated meat qualify as beef for purposes of the hamburger standard of identity? Or will the USDA require hamburgers sourced from cell cultivated meat to bear clarifying text noting how the beef was produced?

Scenario 2: Cell cultivated meat use in standardized sausage products as an additional protein component (Phase 1: Products blended with plant-based analogues or animal-based products).

Potential considerations:
Would there be limitations in usage? If not, will all protein from the cultivated meat apply toward satisfying standard-of-identity compositional rules or will it be limited? Will the cell cultivated meat be included or excluded in calculations concerning levels of restricted ingredients (e.g., sodium nitrite, cure accelerators, and phosphates)?

Scenario 3: Cell cultivated meat use in ham products (Phase 2: Products intended to be composed almost entirely of animal cells with very minimal or no plant-based protein extenders).

Depending on the physical piece size and geometry of the cultivated meat, would it be considered as whole muscle, chunks, or ground, and how would that apply to ham labeling?

Potential considerations:
The source tissues for cell cultivated meat would likely need to be derived from muscles anatomically qualifying as ham. How will protein from the cell cultivated meat be considered in protein fat free calculations? As with sausage, how will restricted ingredients be calculated? Depending on the physical piece size and geometry of the cultivated meat, would it be considered as whole muscle, chunks, or ground, and how would that apply to ham labeling?

Examples of additional questions that will need to be answered center on (1) allergens that may carry over into cultivated meat from the production system and (2) nutritional composition of cell cultivated meat as compared to conventionally produced meat, especially for cultivated products that contain enhanced levels of micronutrients (for instance, are these to be considered fortified or not?).

Food Safety Awareness

All foods for human consumption must be evaluated for potential biological, chemical, and physical hazards.

As with any food, and particularly with novel foods, understanding and mitigating potential safety risks is critical. In accordance with the FDA and the USDA policies, all foods for human consumption must be evaluated for potential biological, chemical, and physical hazards. All USDA-regulated establishments must create and maintain a HACCP plan, and all FDA-regulated establishments must create and maintain a Hazard Analysis and Risk-Based Preventive Controls Plan. Both types of programs involve identifying and managing potential hazards that may arise in food production.

It is likely that cell cultivated meat and poultry products made in a bioreacor themselves have a risk to become contaminated with adventitious agents during handling, packaging, transportation, etc.

The identification and management of these hazards is an essential component of ensuring the safety of the food supply—and underpins the ability of consumers to have confidence in the safety of their food. To that end, the conversation around potential food safety risks in cell cultivated meats is intriguing as its often assumed that cell cultivated meat products are free of all potential food safety risks because their slaughter-free production system is different from that of conventionally produced meat products. While it is fair to say that cell cultivated meats are missing some of the potential food safety risks associated with conventionally produced meats because living animals are not slaughtered and muscle tissues derived from those animals are not consumed, cell cultivated meat products are not free of potential food safety hazards. It is likely that cell cultivated meat and poultry products made in a bioreactor themselves have a risk to become contaminated with adventitious agents during handling, packaging, transportation, etc. Furthermore, because the production systems associated with producing cell cultivated meat are so different from obtaining meat directly from animal sources, there may be food consumption hazards that are not present in conventionally produced meat products (Post and Hocquette 2017).

Food safety risks for conventionally produced meat and poultry products are generally placed into two categories: (1) “pre-harvest” (prior to animal slaughter) and (2) “post-harvest” (after animal slaughter).

Food safety risks for conventionally produced meat and poultry products are generally placed into two categories: (1) “pre-harvest” (prior to animal slaughter) and (2) “post-harvest” (after animal slaughter). Applying these categories to cell cultivated meats, “pre-harvest” may be said to cover the period of cell collection (from living animals), regulation of cell banks, as well as early stages of cell growth and differentiation. “Post-harvest” may be said to focus on the period spanning the growing of the meat in the bioreactor, harvesting from the bioreactor, and subsequent processing and handling. One could argue that the “post-harvest” food safety risks associated with cell cultivated meats are similar to those observed in conventionally produced meats, especially when it comes to processing (e.g., grinding, injecting, marinating, packaging, storage, etc.) and transportation of finished products. The March 2019 FDA-USDA MOU makes clear that the Agencies intend to apply the current robust food safety regulatory framework to cell cultivated meat production. To that end, the joint regulatory plan unveiled by the USDA and the FDA in 2019 proposes a regulatory framework whereby the FDA will oversee cell cultivated meat production up until the point of harvest from the bioreactor, after which the USDA will take the lead.

Management of “pre-harvest” food safety risks associated with cell cultivated meat products is likely to be largely different from conventionally produced meats.

Although the current goal for cell cultivated meat production is to produce a finished product that is indistinguishable in palatability, appearance, safety, and nutrient value from conventionally produced meat products, the process by which cell cultivated meats are produced is fundamentally different. Thus, management of “pre-harvest” food safety risks associated with cell cultivated

meat products is likely to be largely different from conventionally produced meats. As with any cell culture, the potential for contamination (bacterial or otherwise), cell modification, or mutation is present (Langdon 2004). While there are laboratory standards that are intended to minimize the likelihood of these risks in research or medical settings, there currently is no food safety framework to mitigate the public health risk of such occurrences. Similarly, the reagents and technologies (e.g., cellular growth factors, antibodies, buffers, antibiotics, etc.) often involved in the production of successful cell cultures for medical and/or research purposes may not be approved for use in food production. It is likely that these challenges are manageable, and industry awaits further clarification from the FDA on how it plans to oversee cell collection and propagation for food production purposes.

The management of "pre-harvest" food safety risks associated with cell cultivated meat products is likely to be largely different from conventionally produced meats.

Conclusion

In 2012, Dr. Mark Post offered three motivations for developing cell cultivated meat (Post 2012). Those three things were an increase in global meat demand could limit production capacity, an increase in societal concerns for animal welfare and public health, and an increase in awareness of environmental impact due to conventional livestock production. Cell cultivated meat may become available in retail outlets within the next 5 years. Before that happens, these and many other questions, issues, and challenges must be addressed. Among those are: What will be the actual production process of cell cultivated meat? Will these processes be scalable to satisfy consumer demand? Will plant materials be incorporated with animal materials? What will be the standard of identity for cell cultivated meat? How will the regulators work to ensure product safety? How will the production, distribution and consumption of cell cultivated meat vary internationally? What impact will cell cultivated meat production have on the environment? And finally, will consumers even want to eat hamburgers, chicken nuggets, and fish fillets produced in such a novel way and perhaps pay a premium to do so?

Cell cultivated meat may become available in retail outlets within the next 5 years.

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