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Original Article

Food Safety and Health Issues of Cultured Meat

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Abstract: The use of cultured meat, also known as in vitro meat, is claimed to be a way of meeting the growing demand for meat worldwide in a safe and disease-free manner, without sacrificing animal and lowering greenhouse gas emissions. However, its economic feasibility is limited by its cost, scale-up complexity, public neophobia and technophobia, and an imperfect knowledge of its impacts on human health. Cultured meat, which is obtained from stem cells using tissue engineering techniques, has been described as a potential alternative to the current meat production systems, which have extensive negative effects. To ensure that a food product is safe for human consumption, it is important to consider all aspects of its life cycle. In this context, the current review analyzes the major elements of the cultured meat life cycle, including the incorrect use of chemicals, such as pesticides or antibiotics, as well as improper processing and storage methods that determine the food safety of cultured meat. The purpose of this review is to determine food safety, health issues, and the potential risks associated with cultured meat production.

Key words: Cultured meat, safety, tissue engineering, novel foods, potential risks

Introduction

Population growth and improvement of socioeconomic conditions worldwide have increased the need for meat and animal-based goods. It is anticipated that the world population will increase from 7.5 billion people currently to 10 billion people by 2050. As a result, the demand for protein may double the existing supply. Considering the unsustainability of traditional meat production methods, scientists have been seeking alternative protein sources (Stephens *et al*. 2018; Gaydhane *et al*. 2018). According to the Food and Agriculture Organization of the United Nations (FAO 2011), 70% more food will be required by 2050 to meet the expanding population needs, which is a huge problem owing to limited resources and agricultural land. For centuries, people have relied on conventional meat production methods (raising and slaughtering whole animals). However, this process is time-consuming and wasteful and causes environmental pollution. Furthermore, outbreaks of Ebola, avian influenza, and other livestock diseases have introduced an element of uncertainty in traditional animal farming. So, it is crucial to establish a meat production plan that is highly efficient, environmentally friendly, and long-lasting (Godfray *et al*. 2018).

Cultured muscle cells have recently started to be tested as a substitute for meat. Muscle-derived cells from slaughtered animals are the primary cell source used to generate cultured meat, which is also referred to as in vitro meat or alternative meat. It is believed that cultured meat technologies can enhance or substantially replace existing animal production practices; therefore, this technology has gained a lot of interest (Xin *et al*. 2021). Among alternative protein sources, cultured meat has the potential to minimize the use of meat from animals in the long run. Cultured meat has also been regarded as a possible way to alleviate cattle farming challenges, as it has the advantages of requiring less water, emitting less greenhouse gases, and reducing pollutant risks. According to Tuomisto *et al*. (2011), cultured meat has approximately 78-96% lower greenhouse gas emissions than

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traditionally manufactured beef, sheep, pork, and poultry, as well as 99% less land use, 82-96% less water use, and 7-45% less energy use, depending on the comparison between the cultured meat product and the type of conventionally produced meat.

To demonstrate the safety of cultured meat, it is necessary to understand its manufacturing process. As this information is gathered and analyzed, it becomes easier to determine potential problems and areas where better practices can be implemented. An assessment of the manufacturing process is required to ensure the safety of cultured meat products. Possible risks should be identified in each phase of the cultured meat manufacturing process and examined for their potential impact on food safety, human health, and environmental consequences. In this review, we discuss the most prevalent manufacturing techniques, tools, and ingredients used in cultured meat production, potential risks associated with product safety, and accessible methods for assessing food safety. Overall, a number of factors must be considered before cell-cultured beef products can be safely manufactured and commercialized.

Cultured meat

A type of meat produced using animal cell cultures grown in vitro is known as cultured meat. (Datar *et al*. 2010). It is a type of cellular agriculture in its basic form. Various terms are used to describe meat produced using in vitro techniques, including clean, cell-based, cultivated, in vitro, synthetic, and lab-grown meat. This type of meat is produced using techniques that do not require the use of whole animals; it is generated in a bioreactor using tissue engineering techniques (Bhat & Fayaz 2011; Stephens *et al*. 2018; Tiberius *et al*. 2019). The benefits of cultured meat outweigh the disadvantages of regular meat, such as cost, animal welfare ethics, resource scarcity, and public health concerns (Bhat *et al*. 2017; Stephens *et al*. 2018). Frederick Edwin Smith and Winston Churchill first proposed the concept of cultured meat as an alternative to regular meat in the 1930s (Arshad *et al*. 2017). In the early 2000s, the National Aeronautics and Space Administration first proposed a laboratory examination of cultured meat with the goal of cultivating myoblasts in suspension culture as a sustainable supply system for long-term spaceflights and space stations (Benjaminson *et al*. 2002; Wolfson 2002).

Cultured meat production process

According to Xin *et al*. (2021), the cultured meat production method is a combination of cellular development and food manufacturing procedures that produce a consumable meat product. It may be categorized into four major stages: (1) collection of target cells, (2) multiplication of cells on a massive scale, (3) differentiation of seed cells into muscle fibers, adipose tissues, or other matured cellular components in myocytes, and (4) integration and processing of all produced cells into meat. Detailed information on each step is as follows:

Step 1: Seed cells are animal cells that have the ability to multiply and form myofibroblasts, adipose tissues, and other cellular components that comprise muscular tissue <Figure 1>. These cells are sometimes referred to as stem cells because of their ability to multiply and form muscle tissue. Different types of stem cells, including embryonic stem cells, induced pluripotent stem cells, skeletal stem cells, and mesenchymal stem cells, have been proposed as potential seed cells (Díaz-Flores *et al*. 2006; Williams *et al*. 2012; Kadim *et al*. 2015; Genovese *et al*. 2017). Transdifferentiation or dedifferentiation of adult cells, for instance, fibroblasts, can produce myoblasts and lipoblasts (Kazama *et al*. 2008; Boularaoui *et al*. 2018). Animal tissue samples can be easily isolated, mechanically disrupted, and purified by flow sorting using surface markers to obtain these cells (Ding *et al*. 2017). Multiple cell types can be employed to generate cultured meat, but each one requires a unique proliferation and differentiation method based on its developmental properties (Stephens *et al*. 2018; Fish *et al*. 2020; Zhang *et al*. 2020).

Step 2: To obtain a large number of cells from seed cells, they must be multiplied after they are obtained. As lab-scale culture is insufficient to meet market demand, a large-scale fermentation device is required (Post *et al*. 2020). The procedure should also employ a cost-effective, non-serum-containing media, and a variety of indicators, including pH, dissolved oxygen and carbon dioxide, proportion of key nutrients, and metabolic product streams, need to be continuously monitored (Allan *et al*. 2019). Medium recycling with continuous disposal of harmful contaminants and replacement of nutrients based on monitoring output is also essential to maximize the use of resources and control manufacturing costs.

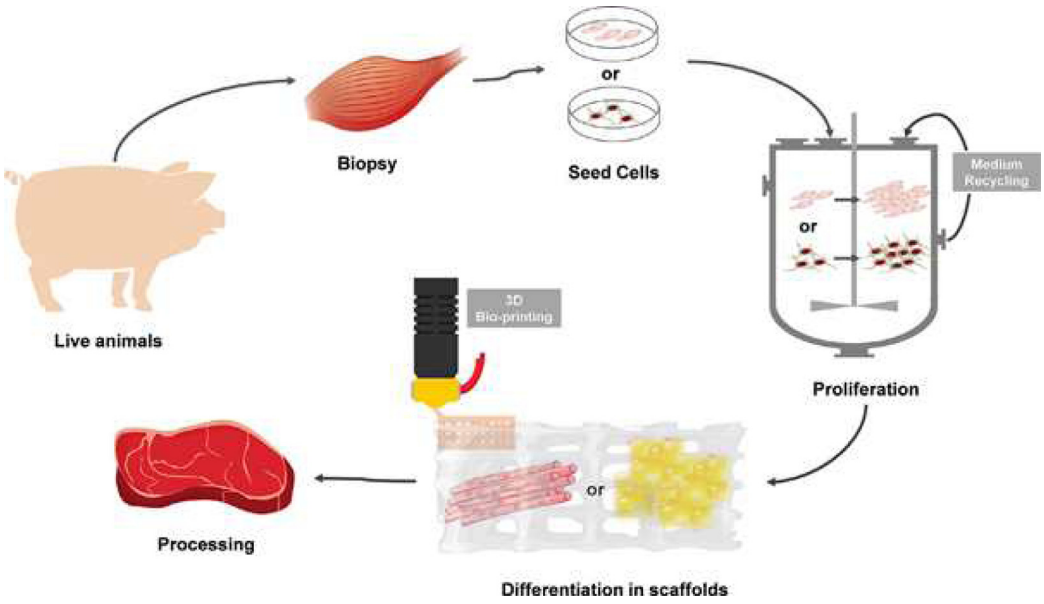

Figure 1. Production process of cultured meat (Xin *et al.* 2021).

Step 3: Differentiation into myoblasts or other matured muscular tissue cell types is carried out after the appropriate number of cells has been obtained. It is important to assess the maturity level of the produced cells at this stage since features such as protein structure, fatty acid composition, and vitamin levels are influenced by cell maturity (Liu 2019). The myofiber diameter, length, and protein content of living animals can vary considerably depending on their growth conditions and may be significantly lower than those of actual muscle fibers. Despite this, muscular stem cells are thought to have considerable myogenic capacity to differentiate (Park *et al*. 2016; Braga *et al*. 2017; Lamarche *et al*. 2021). Thus, optimizing differentiation conditions and increasing the maturation of differentiated cells in accordance with the principle of in vivo muscle tissue formation is essential.

Step 4: In the final phase of cultured meat manufacturing process, all harvested matured cells are processed in various ways, such as shaping, dyeing, and seasoning, before being transformed into the finished cultured meat product (Zhao *et al*. 2019). As the usual culturing method can only produce a thin double cell layer, it is necessary to combine myofibers and adipose tissues, and possibly collagenous cells, to obtain a marbled and textured meat (Stephens *et al*. 2018). Furthermore, the shaping step can be incorporated into Stage 3, which involves co-culturing different cell types in a biomimetic three-dimensional (3D) atmosphere created by a scaffolding or hydrogel (Tuomisto 2019). In addition, improvements in 3D bioprinting technology allow the creation of large-scale muscle tissue composites using mitochondrial hydrogels (Kang *et al*. 2016). Finally, after food treatment, for example, addition of heme proteins and flavoring compounds, the finished product is obtained.

Food safety and health issues in the cultured meat production process

Thorough examination of the raw materials, intermediate products, and final products of cultured meat production systems is necessary to detect potential safety risks. These are identified at each process step; certain risks may be present at any point during the process, whereas some may be particular to a single process step or even a single manufacturing method.

According to the findings of a recent exploratory qualitative study conducted by Ketelings *et al*. (2021), certain aspects of cultured meat demand greater attention from scholars in order to assure the maximum protection for consumers. Recently, research on cultured meat mostly focuses on manufacturing operations (such as cell collection, growth medium composition, biological agents, and digesters), as well as consumer perceptions and legal requirements. However, to bring cultured meat to the consumer's plate, studies on the various safety elements of cell-cultured meat are vital. In the following subsections, we discuss in detail the sources of potential hazards and their impact on food safety.

1. Source animal

The first step in the cultured meat production process involves selecting the source animal. For the end product to be safe, the animal must be disease free. For instance, animal leukemia viruses, such as the bovine leukemia virus, are commonly detected in cattle (Polat *et al*. 2017) and can spread to humans through infected meat consumption (Buehring *et al*. 2019). Researchers believe that some viruses may be capable of spreading and/or surviving in certain circumstances (Graves & Ferrer 1976; Gillet *et al*. 2004). It is also important to investigate the existence of zoonotic viruses (viruses capable of spreading from animals to humans) and their propensity to persist or multiply during cultured meat production. After choosing a disease-free animal, crucial steps are cell selection/screening and modifying/ adapting cell lines.

2. Microbiological contamination by bacteria, toxins, and viruses during the manufacturing process

Compared to conventional meat, cultured meat is less vulnerable to infection, degradation, and spoilage because of the aseptic and regulated environment in which the production takes place (Genovese *et al*. 2011). Different types of pathogenic bacteria found in gastrointestinal tracts and excrements of living animals can contaminate conventional meat, making it unsuitable for human consumption (Rhoades *et al*. 2009). Thus, cultured beef may have a longer shelf life; nevertheless, contamination can occur at any point throughout the manufacturing process and must be regulated. In order to avoid contamination, extra attention is required during critical manufacturing procedures. For example, bacteria (including mycoplasma), fungi, and viruses may be present in raw materials and additional chemicals, which if introduced into the cell culture, may cause cell contamination. Because of this, ensuring the identification, quality, and (where possible) cleanliness of the ingredients, as well as evaluating their safety, is critical for ensuring the quality of the end product. Impurities must be avoided during cell storage, handling, preparation, transfer, interaction with infectious materials, or immersion in water baths to ensure that pollutants are not introduced into the cells (Fountain *et al*. 1997; Cobo *et al*. 2005; Thirumala *et al*. 2009). To improve the safety and control of hazardous pollutants, fully enclosed equipment is preferred. Special attention should be paid to plastics, strainers, covering equipment, packing materials, and cleaning agents, which contain leachable particles that can migrate into food products and leave residues if not properly cleaned.

3. Composition of the cell culture medium

While cell culture is a critical step in cultured meat production, selecting the significant culture medium is essential. Medium and serum are required for cell proliferation and differentiation. Owing to the possibility of residual medium and serum presence in the final product, the origin of the culture media and sera is a major concern. Growing cells require a variety of nutrients. A typical culture medium comprises amino acids, vitamins, sodium chloride, glucose, growth factors (hormones), and other nutrients. Among these, growth factors are the most significant components. It is generally accepted that the absence of growth factors prevents cells from multiplying; hence, the use of growth factors in cell cultures is crucial. Therefore, determining the composition of the medium and identifying possible threats is an integral aspect of the safety evaluation process.

Myosatellite cells are commonly cultured with fetal bovine serum (FBS), which is derived from bovine adults, infants, or even fetuses in the early stages of pregnancy (Dessels, Potgieter, & Pepper 2016). Therefore, the use of FBS in cultured meat production is a challenging approach because it contradicts animal welfare, ethics, food safety, and quality concerns. FBS consists of thousands of ingredients. For commercial media, the exact composition can vary from batch to batch because many formulations are not adequately described or their identification is not generally accessible (van der Valk *et al*. 2010; Gstraunthaler *et al*. 2013). This ambiguity impedes the capacity to track by-products that may pose a risk to human health if they remain in the final product. An acceptable way to assess residues in a product is to conduct residual testing using toxicological standards.

However, according to researchers, it will soon be possible to replace animal-based serum and antibiotics used in the manufacturing of cultured meat with synthetic alternatives (Andreassen *et al*. 2020; Kolkmann *et al*. 2020). Several experiments conducted using serum-free media with the inclusion of other proteins (Shiozuka & Kimura 2000) or novel media such as AIM-V (Fujita *et al*., 2010), Sericin, and UltroserG (Portiér *et al*. 1999; Fujita *et al*. 2010) have shown encouraging outcomes. For example, AIM-V has demonstrated higher dynamic tension during the differentiation stage than that in medium with serum. To lessen the reliance on animal products, additional research must be performed to determine how to eliminate sera from the entire culturing process.

The development of an optimal medium composition

containing hormones and growth factors is also an important field of endeavor. Growth factors facilitate cell development and proliferation. Purified growth factors or hormones derived from plants, animals, or transgenic bacterial species that produce recombinant proteins can be added to the culture media (Houdebine, 2009). In some species, co-cultured hepatocytes can also create insulin-like growth factors that stimulate myoblast proliferation and differentiation, as well as myosatellite cell proliferation (Cen *et al*. 2008).

4. Residual antibiotics in the final product

Initially, non-sterile conditions are used for the collection of tissues or cells from living or newly slain livestock. This processing step is vulnerable to microbiological invasion; hence, antibiotics or other drugs may be required to inhibit the growth of bacteria, fungi, yeast, or other pathogens in this environment (Cobo *et al*. 2005). In addition, to prevent infection in cell cultures, it is a usual practice to add antibiotics or a combination of antibiotics and antimitotics to the cells, especially in long-term cultures. Nevertheless, the use of antibiotics in cultured meat production is controversial and may actually exacerbate the problem associated with antibiotic resistance.

To minimize the risk of antibiotic presence in the finished product, several manufacturers are decreasing or eliminating the use of antibiotics altogether or only utilizing them in earlier stages of the production process and then rinsing, cleaning, and purifying the cells and tissues at various points. In most cases, antibiotics that are now permitted for use in food-producing livestock are also allowed for humans (National Research Council 1999). As they are present at lower levels in food, they are less likely to cause allergic reactions compared to direct ingestion (National Research Council 1999). The current assumption is that if antibiotics are used in cultured meat manufacturing processes, they will only be used at low concentrations. Since the end product from cell-cultured meat manufacturing procedures will have to be characterized, it is necessary to determine the types and amounts of antibiotics used during the manufacturing process, as well as whether appropriate human health safety data are available to support their usage.

5. Safe use of cryoprotectants for cultured meat preservation

The cryopreservation method uses deep freezers and liquid nitrogen to preserve cells for long periods of time at cryogenic temperatures. In situ muscular stem cell identity and myogenic powers steadily diminish over time, and the use of effective cell banking is necessary to preserve essential characteristics. To retain the self-renewal and myogenic characteristics of muscle stem cells after they have been cultured in vitro for an extended period of time, cryopreservation-based cell banking is necessary. In vitro cultivation of thawed frozen muscle stem cells or dissociated muscle tissue cells can preserve their quality until they are needed for further research. During cryopreservation, water crystallization in the medium and cytoplasm must be minimized. Cryoprotectants including dimethyl sulfoxide, ethylene glycol, and sucrose are used in freezing procedures. Additionally, the slow-freezing technique is widely used in animal cell storage (Freshney, 2015). Two cryoprotectants, inulin and sorbitol, are now utilized as food processing enhancers and have been shown to be acceptable at specific quantities in foodstuffs (MacDonald & Lanier 1997; Savini *et al*. 2010). Cryoprotectants, on the other hand, can be harmful when utilized during cell storage. Dimethyl sulfoxide, a prominent cryoprotectant, has been established to be hazardous when used in medical applications (Hornberger *et al*. 2019). A standard methodology for the preservation of cultured meat has not yet been defined. The cryoprotectant employed in the freezing procedure will have to be tested for safety to determine any harmful effects caused by it, irrespective of the method used.

Food safety management methods

1. Safety concerns during the manufacturing process

The safety of cultured meat is dependent on the ability to characterize and assess the finished product, as well as on a production method that focuses on product safety. Various areas of production need to be controlled to minimize the risk of contamination in the end product, whether cooked or served raw. This subsection includes methods and procedures from relevant areas that could be applied to the cultured meat production line [particularly, the hazard management system, Good Manufacturing Practices (GMP), Good Cell Culture Practices (GCCP), and Good Tissue Practices (GTP)] to produce products that are safe, uniform, and of high quality.

Management systems can assist in the identification of potential hazard sources. Hazard analysis and critical control point (HACCP) management systems are widely used in the food industry, encompassing all stages of production and distribution, as well as marketing and the preparation of food for consumption. In the food processing industry, the

HACCP technique is a systematic evaluation of each step of the process that identifies every possible threat or contaminant origin. A regulation or process is implemented for each potential danger (biological, chemical, or physical) to prevent or limit the occurrence of contamination. Specifically, a thorough documentation of every processing step and identification of probable contaminants aids in the identification of the types of impurities and other undesirable pollutants that should be examined in the final product.

To ensure consistent product quality and safety, standard operating procedures (SOPs) from the food industry, meat processing, and pharmaceutical and medical disciplines can be implemented for cultured meat production. The GMP are a collection of commonly accepted guidelines for producing food that ensure a consistent output (Regulation 2023/2006/EC; 21 C.F.R. § 117). For in vitro work, the GCCP establish minimal criteria and recommend the best practices to ensure the quality of the final product, as well as recommend using antiseptics, eliminating antibiotics, adopting SOPs, and monitoring of the quality of nutrient supplementation and additives. Furthermore, documentation is highlighted as a critical component of quality control and a means of ensuring the safety of the final product. Currently, GTP are typically applied in the medical manufacture of human cells and tissues, but some aspects may also be applied to cultured meat. They are likely to be particularly relevant for the prevention of infectious disease agent contamination (i.e., contamination from viruses, bacteria, fungi, parasites, and prions), while ensuring that cells and tissues maintain their function (Price & Coecke 2011; U. S. Food and Drug Administration 2011).

2. Safety of the final product

To ensure that the final product is safe, it will be necessary to determine the types and amounts of residues, by-products, and metabolites present in the final product, as well as a safety evaluation of the inputs and the final product itself.

Physiochemical and proteomic assays are available to evaluate the production of novel products, revealing variations in protein, peptide, amino acid, and metabolite levels relative to traditional meats (World Health Organization, 2008). Some newly produced or changed proteins may have an effect on the stability or natural characteristics of the product, consequently affecting its toxicological or allergy risk. The evaluation of a unique protein's toxic effect or allergenicity may be based on its amino acid sequence resemblance to recognized toxins or allergens (Ladics *et al*. 2011). Cell-cultured products can be analyzed by genetically and biochemically to evaluate the amount of any genomic variations and detect the unexpected consequences which could contribute to the development of metabolites not ordinarily observed in meat (Stout *et al*. 2020).

Microbial challenge analysis may be an appropriate strategy to identify the potential impact on food safety as well as physicochemical changes that may occur throughout the manufacturing and storage of a food product. Following intentional contamination of food, it is processed or stored in test conditions and then evaluated (Komitopoulou 2011). In addition to product safety, this evaluation can assess micro-organism elimination treatments' efficacy. Performing a compositional evaluation is potentially an important part of a safety comparison. Contaminants, nutritional content, and allergens can all be examined throughout the process. The combination of a cell-cultured product's micronutrients can serve as a benchmark for comparison with traditional products (Williams 2007; U. S. Food and Drug Administration 2011).

In vitro tests can be a useful first step for safety assessment. In addition to avoiding or reducing animal experimentation, these procedures are more economical and resource-efficient than alternative evaluation metrics. Components are tested in vitro instead of whole foods since test chemicals are dissolved in solutions. Additional study is essential to define whether certain in vitro quality testing can be performed to entire products efficiently. Analytical testing may be necessary for companies that use innovative medium or additives to detect certain abnormalities, metabolic by-products or other undesired pollutants in the finished product. If, for instance, any drugs, growth hormones, or blood proteins employed in cell proliferation and differentiation are present, they should be evaluated and proven scientifically. Molecular approaches (e.g. polymerase chain reaction and enzyme-linked immuno-sorbent assays) are effective methods that can be used to characterize microorganisms. Biosensors can also analyze and identify harmful microorganisms in meat products (Sionek *et al*. 2020). A variety of standard toxicity testing procedures can be employed to evaluate cell culture production methods. It is widely accepted that all ingredients into food should be of food-grade quality, following particular parameters and standards to Codex Alimentarius. For this reason, toxicological studies may be necessary to prove the safety of the finished products.

Overall, the safety of the cultured meat product depends on the ingredients utilized in manufacturing and the finished product's composition. For example, a cultured meat product

containing both cultured meat and other elements such as binders, seasoning compounds, and organic elements must be examined for safety. Section 402 of the Federal Food, Drug, and Cosmetic Act considers a product contaminated if it carries or includes any food enhancer that is harmful under the Section 409 of the Act (Federal Food, Drug, and Cosmetic Act 1938). Products are considered contaminated if it has been prepared, packaged, or stored in unsanitary conditions, causing contamination or harm to the consumer's health (21 C.F.R. 117.1). Food Safety and Inspection Service should take appropriate enforcement steps to prevent or remove contaminated or mislabeled consumer food products produced with animal tissue from market (FDA CFSAN 2020).

Conclusion

The purpose of this review was to investigate the food safety and health risks associated with cultured meat in order to confirm its safe introduction in the market. This review also resulted in a more comprehensive understanding of the quality aspects that should be included in cultured meat evaluation. Regulatory agencies must conduct inspections at the facilities where cells obtained from live animals and poultry are handled in order to ensure their safety. These establishments must obtain inspection grants from regulatory authorities and follow regulations, including sanitation and the creation and implementation of HACCP systems. The inspectors should check batch records created during cell culture to ensure that the cellular products are safe, wholesome, pure, and accurately labeled in accordance with the regulatory requirements. The application of existing methods and concepts from adjacent industries can aid in the development of a safety framework for cultured meat production. In addition to focusing on how to produce cultured meat, researchers should focus on threat evaluation and interpretation within these sections to ensure that cultured meat is introduced safely into the food supply.

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Conflict of Interest

No potential conflict of interest relevant this article was reported.

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