Safety Issues in the Development of Cell-Cultured Meat

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Abstract. Currently, traditional animal husbandry faces problems such as low utilization of land resources, abuse of antibiotics, and excessive carbon emissions caused by animal husbandry. Therefore, the effectiveness of a new industry to replace traditional animal husbandry has become the main research topic at this stage. In order to meet these needs, cell cultured meat was proposed by scholars, and cell cultured meat technology began to be developed. However, it was found in the research process that there are many safety issues that need to be solved before cell cultured meat is officially put into production. Therefore, the research topic of this article is the safety issues encountered in the cell meat culture process. This study found that: As an emerging food technology, cell cultured meat still faces multiple safety challenges in its industrialization process. Although cutting-edge technology provides solutions for pollution prevention and control and metabolic regulation, the complexity of the production process and the lack of technical maturity still exist. There are still several safety issues. In addition, the industry standards and regulatory systems are still blank, and safety governance faces a double dilemma. In future research, it is necessary to strengthen the production process and specify relevant safety standards.

Keywords: cell-based meat, components, production process.

1. Introduction

Due to the growing population and higher food demand, the demand for animal husbandry is also increasing, but the disadvantages of traditional animal husbandry are also gradually emerging. For example, traditional animal husbandry will lead to the proliferation of antibiotics. The global animal husbandry consumes about 80 million tons of antibiotics each year, which directly leads to a surge in drug-resistant bacteria; excessive carbon emissions, each kilogram of beef production consumes about 25 kilograms of carbon dioxide; the energy utilization rate of meat is also less than one twentieth of that of plant protein. Various health problems are caused by excessive intake of meat, including heart and cardiovascular diseases and colorectal cancer, and the development of artificial meat can alleviate these problems to a large extent. At present, research has also made great progress in the technology of cell-cultured meat. In 2013, the team of Dutch scientist Mark Post successfully cultivated the world's first laboratory beef, marking the feasibility of producing meat through in vitro proliferation of animal stem cells, while plant-based artificial meat technology is more mature and has been commercialized on a large scale. However, there are risks of biological contamination in the process of cultivating cell-based artificial meat. The contamination rate of open bioreactors can reach 15%-30%, and mycoplasma contamination needs to rely on DNA fluorescence staining for detection due to the lack of visible characteristics. Its contamination can lead to abnormal cell metabolism rate of more than 40%; the risk of chemical additive residues, traditional fetal bovine serum (FBS) culture medium contains more than 1,800 protein components, and batch differences may introduce unknown growth factors; and the residual concentration of hormones such as insulin-like growth factor in serum-free culture medium must be controlled at <0.1 ng/mL to meet the standards of the European Food Safety Authority; and whether the use of gene optimization editing will introduce unexpected expression, etc. In 2024, a study showed that in CRISPR-edited bovine muscle satellite cells, about 0.1% of the genomic regions showed non-targeted modifications. The exploration of these issues is very meaningful to the safety of cell-cultured meat. This article mainly studies the safety issues that cell-produced meat may face, and discusses the safety issues that cell-produced meat itself has in the

production process of cell-produced meat and the standards that cell-produced meat needs to be improved in regulations.

2. Safety Issues in the Production Process of Cell-based Meat

This article will first intervene in the discussion of safety issues that may be faced in the production process of cell-based meat. The problems faced in the production of cell-based meat mainly exist in the source of cells, culture medium, scaffold materials and processing. This article will discuss each problem separately.

2.1. Cell Source

2.1.1. Health status and disease risk of donor animals

As the initial link in the production of cell-based meat, donor animals play a pivotal role in the entire production chain. If safety problems occur at the donor animal stage, even if the subsequent production links are strictly controlled, it is difficult to make up for the defects in the initial link, which may eventually lead to the inability to ensure product safety. Therefore, the health status of donor animals is one of the core elements to ensure the safety of cell-based meat. Donor animals may carry a variety of pathogens (such as viruses, bacteria or parasites), which may be transmitted through cells or culture medium during cell separation and culture, thereby contaminating the final product and posing a potential threat to consumer health. In order to ensure the safety of cell-based meat, it is necessary to strictly control the health status of donor animals from the source and establish a complete donor screening and pathogen detection system. According to research, about 5-10% of donor animals may carry potential pathogens (such as viruses and bacteria), which may be transmitted through cell culture. However, it is also possible to use specific pathogen-free animals as a supply to reduce the risk of cell contamination during this process. Data show that using specific pathogen-free (SPF) animals as donors can reduce the risk of pathogen contamination to <1% [1].

2.1.2. Genetic stability and tumorigenicity risk of cell lines

Genetic stability and tumorigenicity of cell lines are also factors that affect the safety of cell-based meat production. During the in vitro culture process, cells may have abnormal gene expression, or the expression products caused by gene mutations are not the expected products. Cells may acquire the ability to grow indefinitely in vitro, similar to cancer cells. There is no direct evidence that these characteristics can cause food to be unhealthy, but there may be the following risks: mutated cells may metabolize abnormal metabolites and carcinogens, and if they are not completely inactivated and enter the human body, there is a possibility of abnormal proliferation. In long-term cultured cell lines (>50 generations), about 20-30% of cells will have gene mutations or chromosomal abnormalities. When using induced pluripotent stem cells (iPSCs), the risk of tumorigenicity is about 5-10%. Studies have shown that cell lines may acquire unlimited proliferation ability during culture, with a tumorigenicity risk of about 3-5% [1]. Gene editing technology and regular testing of cell genome stability can reduce the risk of abnormal gene expression and mutation, and can also reduce the risk of tumorigenicity; functional experiments and gene expression analysis can further reduce the risk of tumorigenicity to <0.5% [1].

2.1.3. Risk of contamination by exogenous factors

The risk of contamination by exogenous factors is also a safety issue in the production of cell cultured meat. Exogenous factors usually include bacteria, fungi, viruses and mycoplasma. Bacteria and fungi usually come from the air or the operating table is not clean enough; virus and mycoplasma contamination usually come from serum animal donors or operating tables. Studies have shown that the mycoplasma contamination rate in culture media using fetal bovine serum (FBS) is about 10-15%. [1] The use of closed bioreactors can reduce the risk of microbial contamination to more than 95%. The contamination rate of viral contamination is about 5-10%. Through real-time PCR detection, the sensitivity of virus contamination detection can be increased to 99.99% [2]. In general, there is a high

possibility of contamination by exogenous factors during the culture process of cell-cultured meat, but the occurrence of contamination can be effectively controlled through technical means.

2.2. Culture Medium

2.2.1. Risk of virus and prion contamination caused by the use of animal serum

The risk of virus and prion contamination caused by the use of animal serum is that cells are easily contaminated by viruses and soft viruses in the serum when they are cultured in culture medium (Table 1). Serum, as a key component in cell culture medium, often contains incompletely inactivated viruses. Studies have shown that animal-derived raw materials are more susceptible to contamination, and the difficulty lies in the fact that it is difficult to detect contaminated cells unless obvious characteristics have already appeared.

Table 1. Virus contamination during mammalian cell culture [3].

Years of contamination	Contamination (virus/host cell)	Total
2000-2004	CVV/CHO Vesivirus2117/CHO	3
2005-2010	CVV/CHO MVM/CHO Vesuvius/CHO	6
2010-present	MVM/CHO MVM/BHK-21 PCV-1/VERO	3

Prions (such as the pathogen that causes mad cow disease) can be transmitted through animal serum, and their resistance to high temperatures and chemical treatments makes them difficult to remove through conventional sterilization methods. Although there are few direct results to prove this, such risks exist in theory based on the characteristics of animal tissues from which serum is derived.

The challenges of virus and prion contamination are mainly in the detection aspect, which is inefficient or prone to missed detection; the solution can be to use serum-free culture medium; establish a multiple control system, including prevention, detection and virus removal, and promote industry information sharing to reduce risks.

2.2.2. Safety of growth factors and hormones in chemically defined culture medium

The safety of growth factors and hormones in chemically defined culture medium. In chemically defined cell culture medium, the safety risks of growth factors and hormones are mainly related to their sources, dosage control, metabolites and long-term exposure effects. Analyze specific risk factors and existing research evidence from a scientific perspective; host cell residues, that is, CHO cell or E. coli DNA/proteins may remain in the production process of recombinant growth factors (such as FGF2 and IGF-1), which poses an immunogenicity risk. Differences in post-translational modifications, i.e., the glycosylation patterns of recombinant proteins produced by eukaryotic expression systems (such as yeast) differ from those of natural proteins in the human body, may trigger abnormal immune responses [3].

2.3. Scaffold Materials

2.3.1. Biocompatibility and degradability of scaffold materials

The biocompatibility and degradability of scaffold materials, the biocompatibility and degradability of cell culture scaffold materials are the core factors that determine their safety. If the material is not designed properly, it may cause problems such as inflammatory response, accumulation of toxic metabolites or immune rejection. First, synthetic polymers (such as polylactic acid (PLA) and polylactic-glycolic acid copolymer (PLGA)) are widely used as scaffold materials due to their degradability and processing properties, but their degradation products may cause local

microenvironment imbalance. For example, lactic acid and glycolic acid produced by PLGA degradation may lead to a decrease in pH, inhibit cell activity and induce inflammatory response. In addition, if metal nanoparticles (such as black phosphorus) are doped in PLGA scaffolds, their degradation products may interfere with the regulation of the immune microenvironment [4].

Natural hydrogels (such as hyaluronic acid HA and collagen) are used in scaffolds due to their high biocompatibility, but their degradation rate and residual components need to be strictly controlled. For example, if the cross-linking agent (such as aldehyde-based polyethylene glycol) of hyaluronic acid hydrogel is not completely removed, toxic substances such as formaldehyde may be released. In addition, sustained-release systems loaded with functional molecules (such as extracellular vesicles EVs) need to avoid uncontrolled release of active ingredients after carrier degradation [5].

2.3.2. Potential toxic residues in scaffold materials

Potential toxic residues in scaffold materials. The scaffold materials of cultured meat play a key role in supporting cell growth and differentiation, but their biocompatibility, degradation products and toxicity of processing residues may pose a threat to the safety of the final product. The following is a systematic analysis of the causes, hazards and countermeasures of potential toxic residues in scaffold materials in combination with foreign literature. First, synthetic polymers, and the toxicity of their degradation products. Synthetic degradable materials (such as polylactic acid PLA, polylactic-glycolic acid copolymer PLGA) are widely used in scaffolds because of their adjustable mechanical properties, but the acidic byproducts produced during their degradation process may damage the cell microenvironment. For example, lactic acid and glycolic acid produced by the degradation of PLGA will reduce the local pH value, inhibit cell activity and induce inflammatory response. If the degradation rate does not match the cell growth rate, incompletely degraded fragments may trigger a chronic immune response. There are also some contaminants in the scaffold material, which may enter the food and cause contamination. For example, during large-scale production, the wear of microcarrier materials (such as polystyrene) may release microplastic particles, and the metal parts of the bioreactor may precipitate heavy metal ions (such as chromium and nickel). These contaminants may enter the final product through the scaffold material and cause chronic toxicity to the human body.

2.4. Processing Process

2.4.1. Safety of additives and processing aids used in the processing process

Safety of additives and processing aids used in the processing process. The production of cell-cultured meat relies on a variety of additives and processing aids to promote cell proliferation, differentiation and tissue formation, but its safety issues are still the core of concern in academia and industry. Growth factors (such as FGF-2 and IGF-1) are core additives for cell proliferation, but their production mostly relies on genetic engineering recombinant technology. If the purification process is insufficient, host cell DNA or endotoxins may remain, triggering immune responses or genetic contamination risks. For example, residual host cell DNA fragments during the purification of recombinant proteins may enter the human circulatory system through intestinal absorption, activate immune responses, and cause human rejection reactions, thereby causing harm to the body.

2.4.2. Effect of the processing process on the nutritional content and structure of cell-cultured meat

The effect of the processing process on the nutritional content and structure of cell-cultured meat. As an important direction of alternative protein technology, the regulation of nutritional content and structure during the processing of cell-cultured meat directly affects the safety and market acceptance of the product. The impact of the processing on nutrients lies in the fact that heat treatment and chemical treatment during processing may lead to the loss of micronutrients such as vitamins and minerals. For example, the sodium chloride solution used in the washing step after cell harvesting may increase the sodium content, while high-pressure homogenization may destroy heat-sensitive

vitamins (such as vitamin C). Studies have shown that heme iron in traditional meat needs to be achieved through exogenous addition or gene regulation in cell-cultured meat, otherwise it may cause the risk of iron deficiency.

3. Safety Issues of Cell-based Meat Products

In addition to certain safety issues in the production process of cell-based meat, cell-based meat products themselves also have certain safety issues such as cell-based meat nutritional issues, whether there are allergens, and toxic substances in cell-based meat.

3.1. Nutritional Components

3.1.1. Whether the nutritional composition of cell-based meat is reasonable

o Whether the nutritional composition of cell-based meat is reasonable, the protein content and composition of cell-based meat are highly dependent on the culture medium formula and cell culture conditions. For example, the use of genetically engineered cell lines (such as C2C12s muscle cells) can enhance cell proliferation ability, but may change the amino acid spectrum of the protein. Studies have shown that compared with traditional beef, some cell-cultured meat products have lower levels of essential amino acids (such as leucine and lysine), which need to be balanced through culture medium optimization or exogenous supplementation [6]. The fat content of cell-cultured meat is usually lower than that of traditional meat, and the fatty acid ratio may vary significantly due to the different lipid precursors added to the culture medium. For example, in the case of apple cell-cultured biomass, EFSA found that its fatty acid composition was significantly different from that of natural apple fruit, and it was necessary to regulate the metabolic pathway through synthetic plant hormone analogs to optimize the fatty acid ratio. In addition, the use of xenogeneic serum or synthetic growth factors (such as FGF, IGF-1) may introduce non-natural fatty acids, and toxicity assessment is required to ensure safety. Some cell-cultured meat products use sodium chloride solution to wash cells during processing, which may lead to a significant increase in sodium content. For example, when evaluating apple cell-cultured biomass, EFSA found that its sodium content was more than 10 times that of natural apples, but potential health risks can be avoided by limiting daily intake (such as 10 mg/day)

3.1.2. Digestive characteristics and nutritional safety

With the rapid development of cell-cultured meat technology, its digestive characteristics and nutritional safety have become core issues in food regulatory science. This paper systematically analyzes the degradation mechanism and potential risks of cultured meat in the gastrointestinal environment by integrating cutting-edge research results. First, in terms of protein digestion efficiency, existing studies have confirmed that cultured meat is biologically equivalent to traditional meat. Test data based on the international standard in vitro digestion model (INFOGEST) showed that the digestibility of myosin heavy chain in cultured meat was 82.3±4.7%, which was not significantly different from 85.1±3.9% of traditional beef (P=0.12) [7], indicating that it can efficiently release essential amino acids for the human body. Secondly, the study of tissue structure stability revealed the breakthrough progress of 3D bioprinting technology: the bionic muscle scaffold showed controllable disintegration characteristics in simulated gastric fluid (pH=1.2), and its half-life (t1/2) was 2.1±0.3 hours, which was highly consistent with the 2.4±0.5 hours of traditional muscle tissue [7]; scanning electron microscopy dynamic observation further confirmed that the scaffold fibers maintained directional dissociation during digestion and no abnormal fragments were produced [8]. Finally, the nutrient release kinetics analysis showed that the release curves of essential amino acids such as lysine and leucine in cultured meat were highly correlated with those of traditional meat (R^2=0.92), but the free fatty acid release rate was reduced by 12-15%[9]. This difference may be directly related to the fact that the intramuscular fat deposition technology in cultured meat has not

yet been perfected, suggesting that lipid metabolism regulation will become a key direction for future technology optimization.

3.2. Allergens

As a new type of food, the potential allergic risk of cell-based meat needs to be paid special attention. Animal serum used in early production processes may introduce exogenous allergens. For example, fetal bovine serum contains β-lactoglobulin, but after 2022, mainstream companies have adopted plant-based culture media, and this problem has been effectively controlled. However, some studies have found that special proteins such as troponin C may be expressed during the culture process, which may cause allergic reactions in consumers. However, after being improved by gene editing technology, the allergenicity of such proteins can be reduced by about 70% [10].

3.3. Toxic Substances

The toxins in cell-based meat itself are mainly divided into endogenous toxins and exogenous toxins. Endogenous toxins refer to toxins produced by cell metabolism or stress response; the byproduct toxins produced by metabolism mainly inclu de lactic acid and ammonia, etc. Long-term accumulation exceeding a certain concentration may cause harm to the body of the ingestor. As shown in Table 2, Choi et al. (2021) found that when the glucose concentration exceeded 25 mM, the lactic acid content in the culture medium of bovine muscle cells could reach 8.2 g/L, exceeding the standard limit of traditional meat processing (<5 g/L) risk threshold (taking bovine bone cells as an example). Exogenous toxins mainly come from culture medium and scaffold materials, which have been explained in Section 1.3 of this article.

Table 2. Experimental data of 300L food reactor by Choi et al. (2021) [11].

Toxin Type	Safety limit	Cultivation Exceeding Standard Rate
Lactic acid	<5g/L	68%
ammonia	<2Mm	42%

4. Cell Meat Safety Assessment System

The applicability of traditional food safety assessment methods to cell meat. Traditional food safety assessment methods mainly focus on microbial contamination, chemical residues and allergen detection. These methods have certain limitations in evaluating cell meat. For example, traditional methods usually rely on the detection of bacteria and viruses, while the production process of cell meat involves cell culture and genetic engineering, which may lead to new safety hazards. In addition, traditional assessment methods fail to fully consider the diversity of cell sources and the complexity of culture medium components, which may affect the safety of the final product. Establish a unified safety assessment standard for cell meat. At present, the standards for cell meat are not unified enough worldwide. For example, the regulatory frameworks of the US FDA and the EU EFSA for cell meat have not been fully unified. (Develop safety assessment methods for cell meat. Traditional food safety assessment methods (such as microbial detection and chemical residue analysis) cannot fully evaluate the safety of cell meat, especially those involving cell sources and gene editing. New means need to be developed to detect the safety of cell meat.

5. Conclusion

This article summarizes and analyzes the problems that may be encountered during the cultivation of cell-cultured meat, and determines that there are still many safety issues that need to be resolved during the cultivation of cell-cultured meat. These problems have been alleviated to a certain extent through relevant technical means, but have not been completely resolved and lack corresponding laws and regulations. Due to the complexity of the production process and the immaturity of the process, cell-cultured meat is easily contaminated by external pollution sources during the cultivation process,

and it is also easy to metabolize and produce new pollutants, leading to safety problems in the production process. Strengthening quality supervision during the production process, refining the management of each step, and adopting new technical means can effectively solve these safety problems. This study fills some gaps in the safety of cell-cultured meat cultivation, and has certain research significance for those who are interested in studying the safety of cell-cultured meat and producing cell-cultured meat. It provides a comprehensive reference for future testing of cell-cultured meat and research on the safety of cell-cultured meat. However, this study did not explore whether cell-cultured meat is more likely to deteriorate and cause safety problems during the storage process than traditional meat. In the future, we can start work on the preservation of cell cultured meat.

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