Understanding Cell-Cultured Seafood and Its Food Safety Challenges

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SUMMARY

Cellular agriculture, which uses advances in muscle tissue engineering for food production, has been proposed as a complementary method to conventional seafood production systems (i.e., aquaculture and wild-capture fisheries) to ensure a sustainable seafood supply for the expanding global population. Cell-cultured seafood offers many environmental and other advantages over harvesting wild seafood and aquaculture (farmed) production. According to the Good Food Institute alternate protein manufacturers and brands database, 54 companies headquartered in 19 countries are working on some aspect of cell-cultured seafood production. This article describes how cell-cultured seafood is produced and its potential food safety hazards, and it discusses tentative labeling terminology, commercialization challenges, and research needed to advance more cost-effective production, overcome consumer hesitance to buy, and assess societal and cultural impacts. Approaches by Canada and the United States to the regulation of cell-cultured seafood are also described. Regulators, seafood hazard analysis critical control point trainers, and other seafood safety or quality professionals need to understand how cell-cultured seafood is manufactured and its potential food safety hazards so that they can oversee safe production, handling, and regulation.

OVERVIEW

This article summarizes and expands upon a cell-cultured seafood symposium convened at the 2023 International Association for Food Protection Annual Meeting in Toronto, Canada. The following were presenters for the symposium:

- Razieh Farzad, Assistant Professor and Seafood Safety Extension Specialist, University of Florida and Florida Sea Grant, Gainesville, Florida
- Martin Duplessis, Director, Bureau of Microbial Hazards, Food Directorate, Health Canada, Ottawa, Ontario, Canada
- Noreen Hobayan, Director of Quality Assurance and Regulatory Affairs, BlueNalu, San Diego, California

Although chicken is the only cell-cultured animal food product approved for sale, and only by Singapore and the United States, the number of companies and amount of funding devoted to cell-cultured seafood research and development and the escalating need to more sustainably procure fish, crustaceans, and mollusks suggests imminent approval.

The objective of this article is to help regulators, seafood hazard analysis critical control point (HACCP) trainers, and other seafood safety or quality professionals understand how cell-cultured seafood is manufactured and its potential food safety hazards. This will better ensure safe production, handling, and regulation. In addition, potential cell-cultured manufacturers can benefit from knowing what is required to obtain regulatory approval for the sale of their products in Canada and the United States. Lastly, regulators in other countries can use this information to guide the development of protocols for approving cell-cultured seafood in their countries.

Advantages of cell-cultured seafood

According to a World Health Organization report, the world population is projected to reach 9 billion to 11 billion by 2050 *(13)*. This increase in population, along with urbanization, technological advancement, and economic growth, will lead to a 70–100% increase in demand for food resources and pose food security challenges *(41)*. Seafood, as one of the most highly traded commodities within the global food system *(30)*, supplies only 18% of the worldwide demand for animal-based protein *(39)*. Despite the steady growth of the aquaculture industry, a seafood shortage of 50 million tons is estimated by 2050 *(46)*. In addition, the current state of seafood production is not entirely environmentally sustainable, because both wild-caught fishing and aquaculture practices are associated with significant challenges, which suggests a need for alternative methods of seafood production *(29)*.

Cell-cultured seafood, also known as cell-based or cultivated seafood, is an emerging subfield of cellular agriculture, whereby seafood is produced directly from cells obtained by animal biopsy and grown in vitro to replicate the sensory and nutritional profile of conventionally produced seafood *(5)*. Cell-cultured seafood is a promising alternative and has several advantages over traditional methods of production, such as reduction of overfishing and the pressure on wild stocks, as well as ecological damage associated

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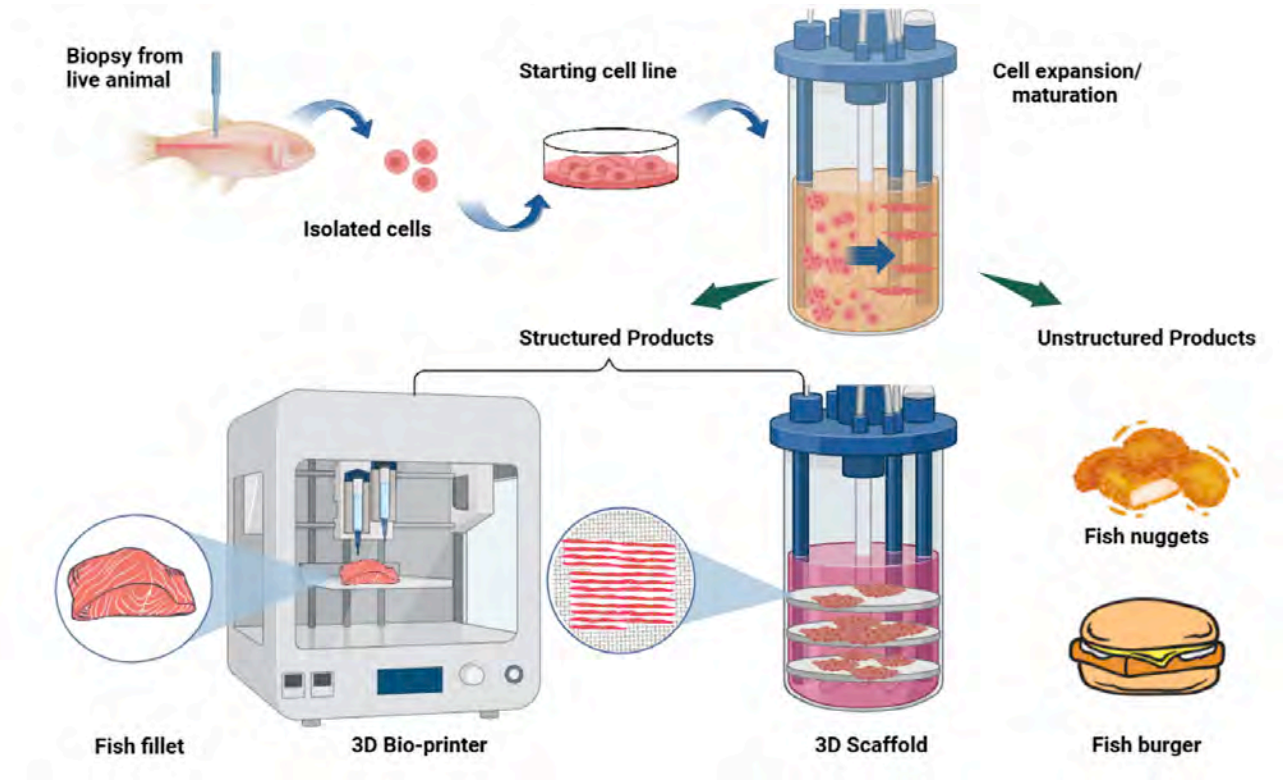

FIGURE 1. Cell-cultured seafood production stages.

with wild harvesting and aquaculture; more humane and sustainable production; no constraint because of weather conditions, fishing limits, or species seasonality; consistent supply; easier traceability; minimized species substitution (economic fraud); less waste; reduction of microbiological risks and spread of zoonotic infectious diseases and foodborne pathogens; no antibiotic use (as in aquaculture); longer shelf life; and enhanced future food security *(29, 34)*.

Given that the science of cell-cultured seafood is still nascent, a true realization of these benefits depends on the development of various technological elements, such as cell lines, optimized cell-culture media, bioreactors, and edible scaffolds, as well as achieving cost-effective product that is viewed as an acceptable substitute by consumers *(38)*. A thorough exploration of these components is necessary to better understand the production process.

How seafood cells are cultured and made into edible products

The manufacturing process for cell-cultured seafood predominantly relies on principles from tissue engineering, cell culture, cellular biology, and biological process engineering that were originally developed for regenerative medicine applications and can be adapted for cellular agriculture *(3, 8)*. As shown in *Figure 1*, the stages in cell-cultured seafood production involve isolating healthy aquatic animal cells capable of differentiating into muscle cells (e.g., muscle satellite cells, muscle stem cells, and induced pluripotent stem cells), culturing the isolated cells under controlled hygienic conditions and promoting their proliferation on a small scale, and scaling up production within a large bioreactor and allowing cells to continue to proliferate and differentiate by adjusting their media and environmental conditions. After adequate multiplication, cells are either harvested and processed into products, such as fish nuggets or seafood burger patties, or edible three-dimensional (3D) scaffolds are employed that offer mechanical support to mimic the native texture of a fillet while providing nutrients for cells to grow. Cells can also be fed into 3D bioprinters to create whole-cut fillet structures. Finally, the products enter downstream processing to be ready for market *(36)*.

Efforts to identify labeling terminology

Since the emergence of cell-based meat technology, numerous terms—such as "lab-grown," "cultured," "cultivated," "cell-based," "clean," "in-vitro," and "slaughter-free" meat—have been employed in both scientific literature and public communications *(2)*. However, which term clearly communicates the nature of cell-based products has

been the subject of considerable debate. Because utilization of multiple nomenclatures may confuse stakeholders, there is a need for clear and consistent terminology. In addition, determining an appropriate name for cell-based products is not only crucial for consumer acceptance but also to ensure adherence to regulations set forth by the U.S. food regulatory agencies *(19)*.

In 2019, the U.S. Department of Agriculture (USDA) and U.S. Food and Drug Administration (FDA) came to an agreement on developing a joint principle for cell-cultured product labeling and claims, ensuring consistent and transparent labeling for meat, poultry, and seafood cell-cultured products *(13)*. In accordance with FDA regulations (U.S. Title 21 Code of Federal Regulation [CFR] 101.3) and USDA guidelines (9 CFR 317.2), use of "common or usual names" for inclusion on package labels is required. However, these U.S. authorities are still in the process of establishing specific food labeling regulations that will be permitted in the future *(18)*.

To facilitate this process, in 2020, the FDA published a request for information to gather public input on cell-cultured seafood labeling. In 2021, the USDA issued an advance notice of proposed rulemaking seeking public comments on labeling meat and poultry products made using animal cell culture technology. Comments were closed in 2021; the agencies are still evaluating data obtained to facilitate publication of formal guidance on this matter *(13)*.

Besides government efforts, several studies and surveys have been conducted in recent years to examine both consumer and industry viewpoints regarding the perception, acceptance, and preference for terminology associated with cell-based products. Survey findings in 2021 from 44 chief executive officers of cell-based food companies indicated a preference for the term "cultivated" among industry professionals *(13)*. This preference primarily arises from the term "cultivated" resonating with consumers and distinguishing cell-based products from conventional ones. Even though consumer acceptance plays a crucial role in the success of the industry, the chosen name for the cell-based product label should not be misleading and must adhere to regulatory criteria *(18)*.

With a focus on exploring potential names for "cell-cultured seafood" products, a study by Hallman and Hallman established specific criteria for determining the common name that not only are appropriate but also meet FDA requirements. The criteria used were identification of the product as a potential allergen; the ability to distinguish it from wild-caught or farm-raised; a preferred term that doesn't evoke impressions that are inconsistent with the products' status as safe, healthy, and nutritious; and a name seen as appropriate and not disparaging to consumers or industry. The outcome of this survey led to the conclusion that the term "cell-based" meets all criteria, with the term "cell-cultured" as the next most suitable *(19)*.

In 2021, Hallman and Hallman further studied the two selected terms, "cell-based" and "cell-cultured," by surveying 1200 American consumers. Similar to the previous study, "cell-based" was the most preferred name *(20)*. However, on March 8, 2021, a letter was sent to the FDA by the Alliance for Meat, Poultry and Seafood Innovation, the National Fisheries Institute, and representatives of leading conventional seafood and cell-cultured meat, poultry, and seafood companies that stated their preference for "cell-cultured" seafood.

Although no specific studies have been conducted thus far to analyze preferred terminology within the scientific community, the most frequently used term is reported to be "cultivated," followed by "cell-based." Similarly, from consumers' perspectives, the term "cultivated" was frequently regarded as the most appealing *(13)*. However, a more in-depth study would be necessary to explore the influence of various languages and cultures on this matter.

Because industry seems to prefer the term "cell-cultured," the authors use it in the remainder of this article. However, if referenced studies use different terms, those are preserved within the reference.

Extent of company and country involvement

The Good Food Institute maintains a dynamic database of companies involved in producing alternative proteins that can be sorted by the method of production (cultivated, plant-based, and fermented), protein type (dairy, eggs, meat, and seafood), and technology focus (end-product formulation and manufacturing, cell-culture media, bioprocessing design, ingredient optimization, etc.), as well as other categories. The database listed 54 companies, headquartered in 19 countries, that are working on some aspect of cell-cultured seafood *(14)*. The countries with multiple seafood company headquarters were the United States (16 companies); Israel (6 companies); Singapore (5 companies); South Korea (4 companies); Canada, Germany, and the United Kingdom (3 companies each); and India and Netherlands (2 companies each). Brazil, Belgium, Chile, China, Iceland, Portugal, Russia, South Africa, Spain, and Switzerland had only one company with headquarters in their country *(Fig. 2)*.

Of those 54 companies, 28 companies (52%) worked with two or more cell-cultured technologies, whereas the remaining 26 companies (48%) focused on one. Thirty-six companies worked on end-product formulation and manufacturing, whereas additional technologies, in order of engagement by a descending number of companies, were cell-culture media (17 companies), cell line development (14 companies), scaffolding and structure (13 companies), bioprocessing design (10 companies), 3D printing (3 companies), and feedstocks (1 company). Because of the dynamic nature of the Good Food Institute database, the stability of the information and the companies listed is unknown.

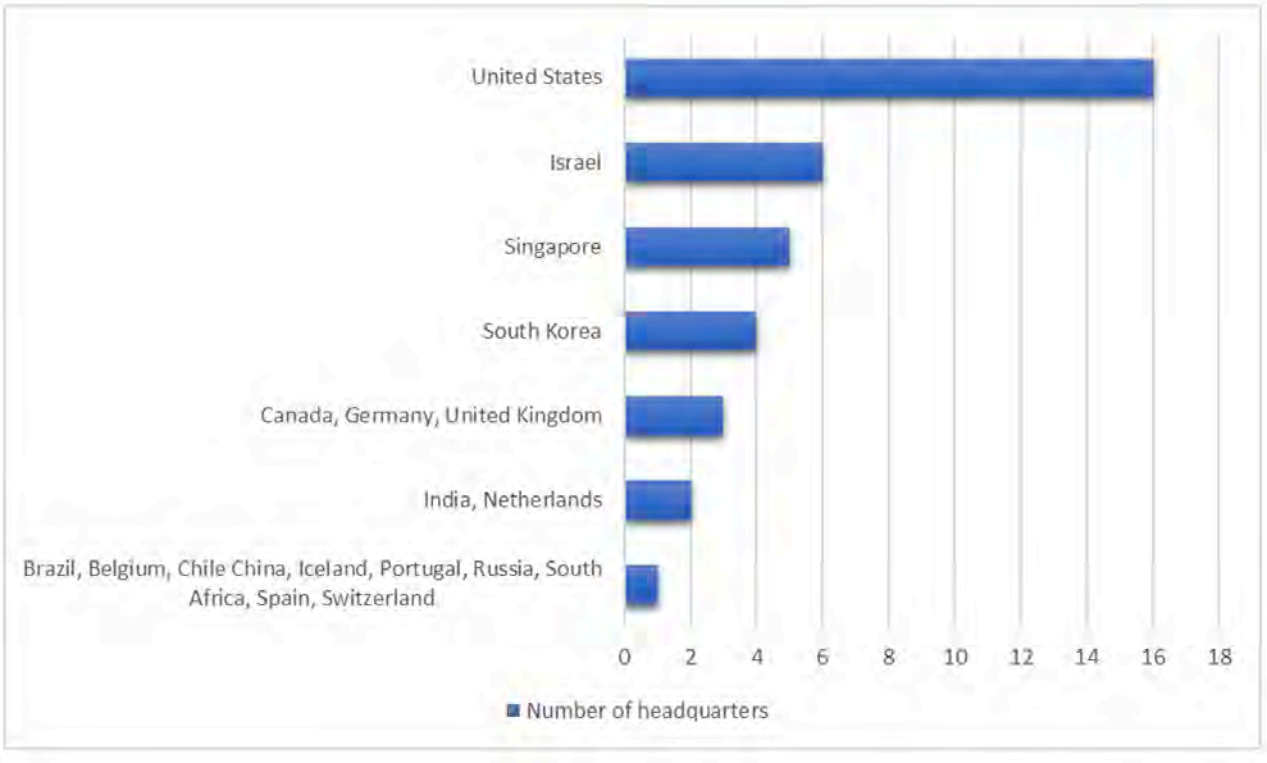

FIGURE 2. Corporate headquarters of cell-cultured seafood manufacturers by country.

POTENTIAL FOOD SAFETY CONCERNS

Consumption of contaminated food could cause a range of diseases, from gastrointestinal issues like diarrhea to more severe conditions such as cancer *(47)*. Therefore, food safety measures must be taken into account to protect public health and uphold consumer trust in the food supply. Cell-cultured seafood production should be subject to the same standards applied to commercially available food products, with additional safety measures on new components and ingredients that are being used for production, such as culture media and scaffolding materials *(13)*.

In the context of food safety, the terms "hazard" and "risk" should not be used interchangeably, because they carry distinct meanings. Hazard refers to a biological, chemical, or physical agent present in food or a condition of food that has the potential to cause adverse health effects *(7)*. Risk is "a function of probability of an adverse health effect and the severity of that effect, consequential to hazard(s) in food" *(27)*, and it is determined by exposure. Therefore, a key step in maintaining food safety throughout the process would be to implement systems to manage food safety risks such as HACCP, hazard analysis and risk-based preventive controls (HARPCs), food safety plan development, and other risk-based preventive control programs *(33)*. Under 21 CFR 123, also known as the seafood HACCP regulation, seafood processors should develop and implement HACCP plans to prevent, eliminate, or reduce to an acceptable level significant species- and process-related chemical, biological, and physical hazards in cell-cultured seafood products.

Even though cell-cultured seafood products have nutritional and sensory characteristics comparable with those of traditional counterparts, the production process is substantially different. This can affect the potential process- and species-related hazards associated with cell-cultured seafood production. For example, traditional species-related hazards such as "pathogens from harvest area," "parasites," "natural toxins," and "aquaculture drugs" do not pertain to cell-cultured seafood production. Therefore, drawing on existing procedures for hazard identification and HACCP plan development, hazards are categorized based on the stages of cell-cultured seafood production. In addition, possible preventive actions or controls and relevant regulations are included. *Fig. 1* provides a "flow diagram" for cell-cultured seafood production. This is usually done as part of "preliminary steps" suggested by the FDA before seafood hazard identification and HACCP plan development.

Cell isolation

In comparison to conventional seafood production, the likelihood of transferring infectious zoonotic and

foodborne diseases is significantly low in cell-cultured seafood production *(12)*. However, procuring cells from healthy animals and maintaining excellent hygiene practices throughout the cell isolation process is crucial to eliminate or minimize contamination and transfer of diseases from the animal source during biopsy.

Using antibiotics is a common practice to prevent microbial contamination during cell sourcing, isolation, and storage. However, antibiotics are either removed entirely during the scaling up of production or diluted to extremely low concentrations, ensuring that their levels in the final products are safe for consumption *(11)*.

Cell line development

An important consideration during cell line development is that culture media can be potential sources of hazards. Media should be well-characterized and screened to be qualified as food-grade substances and possible residuals. A typical culture medium is composed of a complex source of compounds, such as vitamins, amino acids, glucose, inorganic salts, and serum as sources of hormones, growth factors, and attachment factors *(1)*. These components support cell metabolism, maintain proper cell culture conditions, and regulate proliferation and differentiation. Although considerable amounts of these components are washed away during cell harvesting or possibly destroyed by heat treatments, residues may be left and become part of the final food product. This could be considered a hazard, especially in the case of excess growth factors that pose human health concerns; excessive hormones have been linked to procarcinogenic effects. Stepwise risk assessment can minimize residual-related concerns *(25, 33)*.

One primary safety concern related to the use of animal-based serum in media, such as fetal bovine serum (FBS), is the transmission of pathogenic and infectious diseases *(17)*. According to guidelines from the European Medicines Agency *(10)* and the USDA *(40)*, regardless of their geographical origin, bovine-derived serum must be free of specific viruses, such as bovine viral diarrhea virus, rabies virus, and bovine respiratory syncytial virus *(17)*.

Monitoring for early detection of cell infections can help significantly in identifying potential hazards. In addition, the development of animal serum-free media has helped mitigate hazards associated with using animal-based serums *(12)*.

Cell banking

Cell banking involves the creation of a collection of processed cells that are cryogenically stored in containers in a single operation. This process aims to maintain uniformity and stability of the cell content over time. A master cell bank (MCB) provides a standardized source of cells for food production. The cell lines within the MCB are assessed and confirmed to possess consistent and uniform composition *(9, 44)*.

Cell banks must be free of microorganisms, particularly foodborne pathogens, and of zoonotic viruses recognized as hazards to human health *(45)*. Critical food safety risk mitigation regarding cell banking includes the following areas.

Cell identity. Testing cell identity ensures consistency in cell lines. In this step, testing methods designed for cell line authentication, such as short tandem repeat profiling, cytochrome oxidase I gene assays, species identification assay methods such as polymerase chain reaction (PCR), phenotype confirmation, and immune-based assays can be used to in cell identification *(45)*.

Cell purity. Another required safety measure is testing for cell purity to exclude any unrelated DNA in the culture.

Cell sterility. Microbial testing methods such as PCR, product-enhanced reverse transcriptase assays, and immune-based assays can be employed to validate the sterility of the culture from pathogens or identify adventitious viral and microbial agents. *Mycoplasma* species are of particular concern in cell-cultured seafood production. Up to 35% of contamination of cell lines is related to these bacteria *(33)*.

Genetic stability. Another important factor is to check for genetic stability, because genetic drift could occur after multiple passages of cells and result in different cell characteristics *(11)*. Using fresh vials of banked cells can help minimize the effects of genetic drift *(33)*. As noted earlier, gene expression should be tested to discover potential differential behavior of cells.

Stability of culture. Cells must be healthy and follow regular growth patterns *(45)*. Evaluation criteria include cell size, cell morphology, and cell density.

Cell storage. Cryoprotectants such as dimethyl sulfoxide (DMSO) and inulin are often used for storing cells in cell banks. There is evidence indicating that DMSO can have adverse toxicological effects. Thus, it's crucial that these components are thoroughly removed during the production process *(11, 12)*.

Scaffolding and scale-up production

3D scaffolds or microcarriers can be used to provide a structure for cells to attach to and grow into a desired shape. In addition, scaffolding material can provide nutrients for proliferation and differentiation of the cells *(35)*. If scaffolding biomaterial is edible, removal from the final product is not necessary. However, care must be taken in choosing the biomaterial to prevent possible allergic reactions upon consumption. For example, consumers who are allergic to crustaceans could have allergic reactions to chitin or chitosan. This can be addressed by including appropriate allergen labeling on the final product *(12)*.

Scaffolds can also be made out of synthetic material; therefore, chemical and enzymatic dissociation reagents would be required to remove attached cells. These additives are typically expected to adhere to food-grade standards, because they might become integrated into the final product.

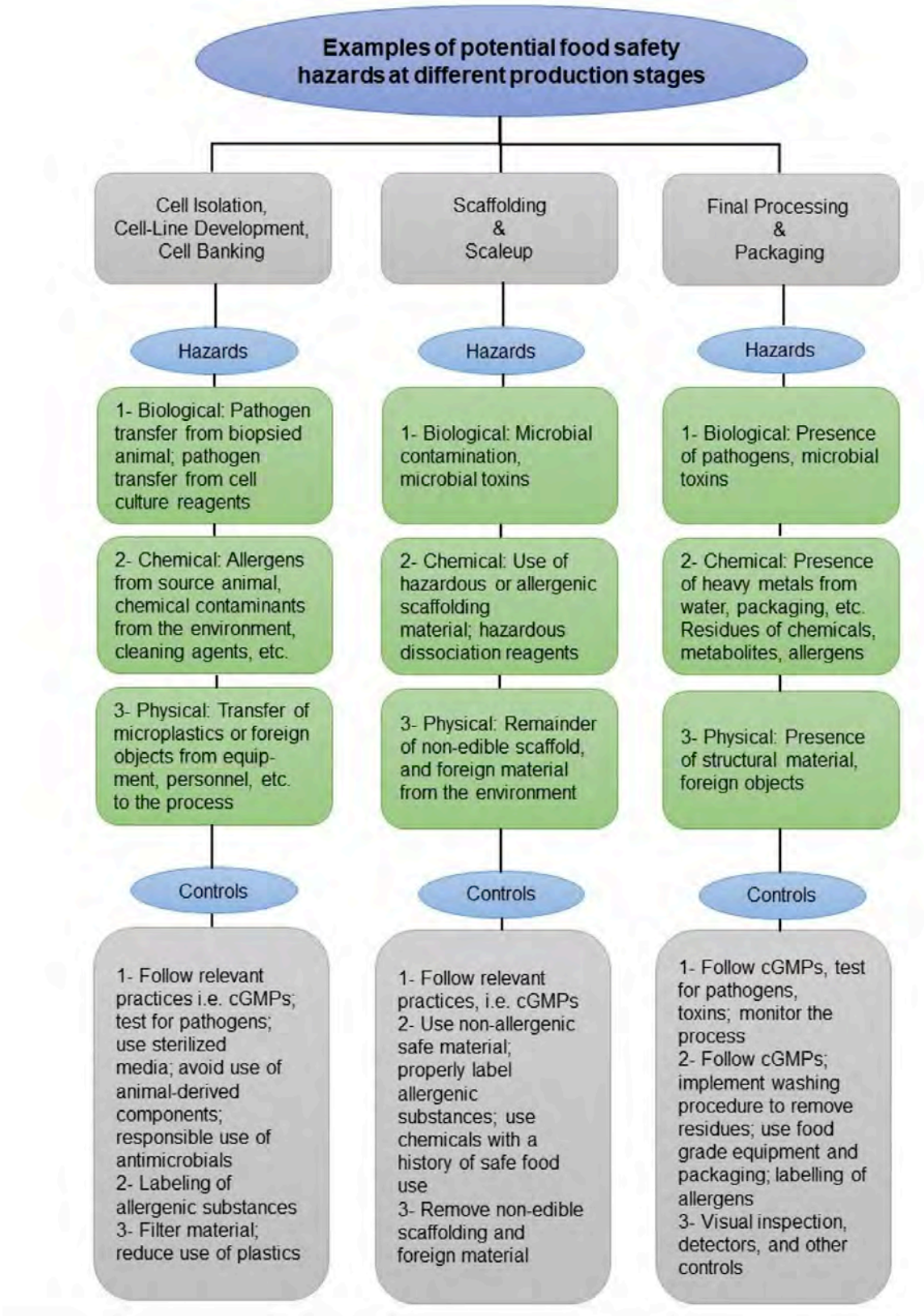

FIGURE 3. Potential food safety hazards and controls in cell-cultured seafood by production stage.

Even so, it is necessary to conduct proper testing to confirm the presence of any substance or novel input for the safety assessment of the final product *(11)*.

Similar to previous stages, microbial contamination can be introduced to the production during the scale-up procedure. Following current good manufacturing practice (cGMP) guidelines, regular testing for pathogens and the use of sterile reagents are recommended mitigation measures *(11)*.

Final processing and packaging

Comparable to traditional seafood production methods, biological, chemical, and physical hazards can be introduced into products during final processing for various reasons, such as use of equipment that has not been cleaned and sanitized following proper standard sanitation operating procedures or poor hygiene of employees. In addition, residues or by-products could carry over from previous manufacturing steps; they must be measured and analyzed to identify potential hazards, possible allergens, and toxins *(11, 33)*.

Foreign material from packaging equipment, personnel, or the production environment can be incorporated into the final products, resulting in physical injuries to consumers. Most of these hazards must be controlled through prerequisite programs, such as sanitation control procedures and GMPs *(13, 42)*. In addition, final product testing of representative numbers of food samples for the presence of pathogens should be used as an additional step to ensure the safety of final products. Introduction of physical hazards, such as metal from packaging material and processing equipment, must be controlled through the firm's seafood HACCP plan. Examples of controls that can be implemented in the HACCP plan include visual inspection of the product and using metal detectors to help reduce these risks.

Figure 3 summarizes potential food safety hazards that can be introduced at various steps of cell-cultured seafood production *(13)*.

REGULATION OF CELL-CULTURED SEAFOOD

Cell-cultured seafood falls within the realm of novel food; thus, the development of new laws and regulations to establish standards and oversee this emerging industry may be required. In 2020, the Singapore Food Agency was the first to approve the sale of cultured "chicken nuggets" *(48)*. In June 2023, two cell-cultured chicken companies received grant of inspection and label approval from the USDA to sell cultivated chicken products *(15)*.

Countries without a premarket framework for novel foods would need to consider an approach to oversight that suits their regulatory context and socioeconomic circumstances. Authorizations may be necessary on aspects such as food safety, quality assurance, and proper labeling, including disclosure of allergens if applicable. The scope of this study focuses on current regulations in Canada and the United States, which have distinct, established premarket oversight approaches.

CANADA'S REGULATION OF CELL-CULTURED SEAFOOD

As of the writing of this article, Canada had not approved or received submissions requesting authorization for the sale of seafood or meat-type products manufactured from cultivated animal cells. Canada already has in place premarket regulations and procedures to ensure that those products, if authorized, are as safe and nutritious as conventional animal protein products for sale in Canada.

Novel food program

In 1998, Canada promulgated Novel Food Regulations (NFRs) as Division 28 of Part B (Food) within the Food and Drug Regulations (FDRs; C.R.C., c. 870). According to B.28.001, a food meets the "novel" definition if one or more of the following applies *(16)*:

1) a substance that **does not have a history of safe use as a food** or
2) a food manufactured, prepared, preserved, or packaged by a **process that has not been previously applied to that food AND causes the food** to undergo a major change or
3) a food derived from a plant, animal, or microorganism that has been **genetically modified to alter its characteristics**

Ingredients produced using cultivated cells from fish and other animals are likely diverse in their development, production, composition, and final characteristics. It is anticipated that these will generally meet the definition of a novel food.

Section B.28.002 requires premarket notification and approval before selling or importing novel foods into Canada. Health Canada's Food Directorate has the authority to administer the novel foods program, coordinate the review of the notifications (submission dossiers) received from petitioners, and issue a novel food authorization in the form of a letter of no objection to the petitioner.

Functions of the novel food program

Health Canada's novel food program has four main functions in place to administer the NFRs and support petitioners with compliance. First, an optional and voluntary novelty determination process is available to companies when they are uncertain whether a food or food ingredient meets the regulatory definition of a novel food (this could be the case for some ingredients other than cultured animal cells). A list of previously determined "non-novel" products is posted online as a resource *(23)*. Non-novel foods do not require notification under the NFRs; however, other regulations could apply (refer to the "Other regulatory requirements" section).

Second, petitioners developing novel food notification packages can benefit from presubmission consultations with Health Canada regulators. Consultations are voluntary but strongly encouraged; they allow petitioners to ask questions about compliance requirements and seek feedback on their proposed approach to address safety and nutrition quality endpoints in their submissions. A high-quality submission can reduce the need for additional questions during assessment. A presubmission consultation can be requested by emailing the Food Directorate's Submission Management Information Unit at smiu-ugdi@hc-sc.gc.ca.

The program's third function is the assessment of mandatory novel food notifications, described later in more detail. In keeping with Health Canada's commitments to transparency in decision-making, the fourth function of the program is the posting of completed assessment summaries (called decision documents) at novelfoods.gc.ca (after review by the petitioner to confirm that no confidential business information is included). Published summaries provide reference material to companies contemplating or preparing premarket notifications.

Information needed for novel food safety assessment and timeline

Given the anticipated complexity and diversity of foods produced from cultured animal cells, safety assessments must be done case by case. The assessment is conducted according to Health Canada's *Guidelines for the Safety Assessment of Novel Foods (21)*. Authorization decisions are made by the Food Rulings Committee, composed of Food Directorate senior management, and are based on the scientific and technical information reviewed in the safety assessment. The provided information should address the following safety and nutrition endpoints (similar to any novel food); however, additional information to support product characterization and safety may be requested.

Novel product development and characteristics. This should include all steps involved in selecting, isolating, and storing cells; biomass production; and commercial food production, as well as intended uses and descriptions of unique characteristics.

Molecular characterization. This aspect applies to foods that are genetically modified (GM), regardless of the methods and technology used. The submission dossier should explain the technologies, methods, and development steps used to introduce DNA-level changes; show evidence of the genetic modification that was achieved and its stability; describe the characteristic or characteristics that were changed and explain the mode of action; and provide evidence of the resulting phenotype. The potential for unintended modifications to be introduced should be carefully addressed, as well as the potential production of unintended expression products—that is, search for open reading frames (ORFs) in all six frames across the modified loci and the junctions with the host genome and search discovered ORF sequences for similarity to known toxins and allergens. For cases in which genome sequencing data are used to support molecular characterization, guidance previously developed for GM plants *(22)* can serve as a useful reference for conducting the analysis and presenting results.

Manufacturing process. A description of the manufacturing steps must include all input substances and their role or technical function. This informs an understanding of potential microbial or chemical hazards that could be in the product and how they are controlled and monitored. The submission should also address which substances used in manufacturing, such as culture media or scaffolding, remain or leave residues in the final product.

Microbial hazards. Potential hazards, including known food pathogens, endogenous viruses, and potential zoonotic microbes, should be considered. Confirmation that manufacturing can consistently produce a microbially safe product can be documented by microbial testing of three nonconsecutive batches showing compliance with specifications (certificates of analysis can be provided as supporting evidence). International standard testing methods and/or validated in-house methods with relevant limits of detection can be used.

Chemical hazards. The potential for chemical contaminants, whether natural toxins or environmental or process-induced contaminants, should be considered. Product specifications for contaminants can be set, along with validation that these can be met by testing using appropriately sensitive methods for the given chemicals. Certificates of analysis for at least two different batches can be supplied as supporting evidence.

Dietary exposure. Anticipated frequency and level of consumption by the general population and sensitive subgroups inform how a novel food may affect the nutrient intakes of Canadians, as well as their exposure to potential toxins, antinutrients, contaminants, or novel substances determined to be in the food. This can be estimated based on consumption data for the food's conventional counterpart in the Canadian Community Health Survey.

Toxicology considerations. For substances of unknown safety that may be introduced into the food supply, toxicological testing is required. A case-by-case approach should be used to determine the appropriate toxicological tests to be carried out. Knowledge of the source organism (and its potential to harbor toxins) and chemical analysis of the food are considerations in determining the need for toxicological testing. Conventional studies of toxicity, including chronic or developmental toxicity, genotoxicity, or carcinogenicity, may need to be conducted on the final product or its components.

Allergenicity considerations. The allergenicity health risk associated with a product containing known allergens, such as seafood proteins (fish, crustaceans, and mollusks are all

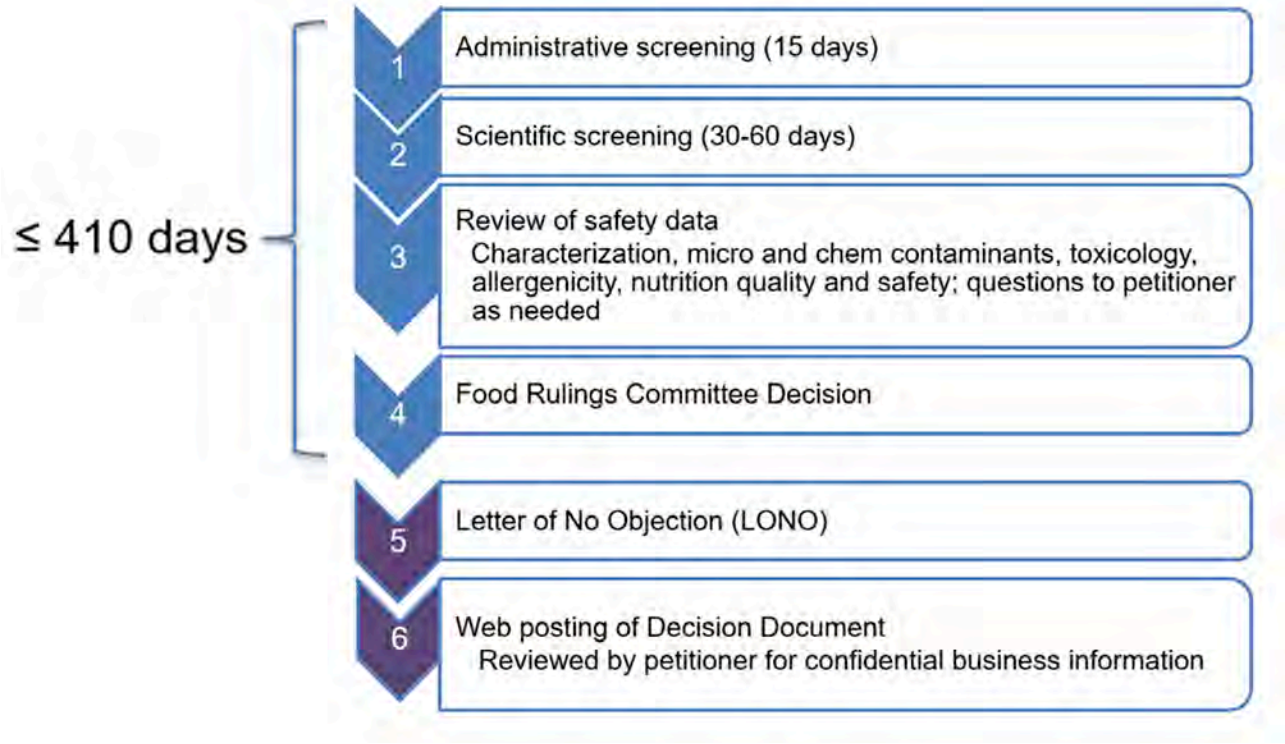

FIGURE 4. Steps in Health Canada's safety assessment of a novel food premarket notification (submission dossier) and authorization.

priority food allergens in Canada), can be managed through labeling, as with conventional foods. Risk mitigation or management for new allergenicity risks that might arise with cell-cultured foods will be considered case by case.

Nutritional considerations. To determine whether a novel food is as nutritious as similar products already on the market, the product's nutrient composition (crude protein, fat, ash, fiber, fatty acids, amino acids, vitamins, minerals, known antinutrients, and bioactive substances) is assessed relative to a suitable comparator. Potential implications of differences in nutritional quality would need to be addressed.

Because assessments are comprehensive, they can take up to 410 calendar days to complete *(Fig. 4)*.

Other regulatory requirements

Aside from the NFRs, developers and manufacturers are responsible for meeting premarket regulatory requirements that may apply to the specific product, as well as general and postmarket provisions that apply to all foods sold in Canada. The mandates for these regulations fall to different government bodies, including Health Canada, the Canadian Food Inspection Agency (CFIA), and Environment and Climate Change Canada (ECCC). Examples of regulations that can apply to cell-cultured foods include other provisions of the FDRs (e.g., regarding food additives, fortification, and nutrition labeling), the Safe Food for Canadians Regulation (e.g., trade, licenses, preventive controls, traceability, compositional standards, inspection, packaging, and labeling), the Feeds Regulations (regarding animal feed), and the New Substances Notification Regulations (Chemicals and Polymers) and the New Substances Notification Regulations (Organisms), both regarding environmental and human health impacts. At petitioners' request, regulators from Health Canada, the CFIA, and ECCC responsible for these aspects can be included in presubmission consultations to address questions.

With respect to product labeling, Health Canada administers regulations and standards relating to the health, safety, and nutritional quality of food sold in Canada, under the Food and Drugs Act, including labeling. The CFIA administers non-health and safety food labeling regulations related to misrepresentation, labeling, advertising, and standards of identity under the Food and Drugs Act and establishes and administers regulations under the Safe Food for Canadians Act (SFCA).

Like for all foods, certain mandatory food labeling rules apply to products of cellular agriculture. This includes general prohibitions against label information that is false and misleading, as stated in Section 5 of the Food and Drugs Act and Section 6 of the SFCA. Other requirements may also apply to cell-cultivated foods, such as composition and common names that are set out in the regulations. For

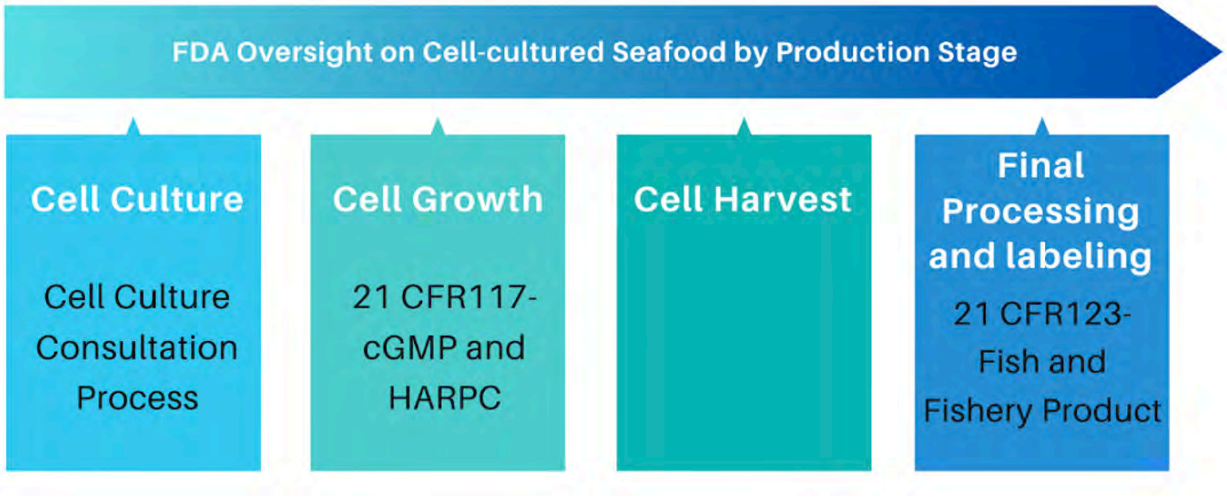

FIGURE 5. FDA guidance and regulations pertinent to cell-cultured seafood production stages.

example, the common name must describe the food, and it must not be mistaken for another food that is defined in regulations or in a standard. Regulated parties are responsible for complying with all labeling requirements, and the CFIA verifies compliance.

U.S. REGULATION OF CELL-CULTURED SEAFOOD

In March 2019, the USDA's Food Safety and Inspection Service (FSIS) and the FDA established a formal agreement on how they would collaborate in regulating cell-cultured foods *(43)*. Under this agreement, regulatory oversight depends on the animal species serving as cell sources and the agencies' current jurisdictions. The FDA's current jurisdiction includes all animals to be used as food until slaughter, whereas the FSIS regulates slaughter, processing, packaging, and labeling of meat and fish in the order Siluriformes (catfish), under the Federal Meat Inspection Act (FMIA), and poultry, which falls under the Poultry Products Inspection Act (PPIA). Products that don't fall under FMIA or PPIA, i.e., seafood (except for catfish), game meat, and food intended for animal feed, are under the FDA's purview both before and after slaughter.

Because of the previously mentioned jurisdictions, the FDA oversees cell collection, selection, and growth of all cell-cultured animal products before harvesting them from bioreactors. However, after harvest, cell-cultured meat, poultry, and catfish products are regulated by the FSIS, whereas the FDA regulates all noncatfish seafood and game meat, as well as food intended as animal feed both before and after bioreactor harvest.

Existing conventionally produced seafood regulations that apply to cell-cultured seafood include 21 CFR 117 (cGMPs and HARPCs for human foods) during the cell growth stage and 21 CFR 123 (fish and fishery products HACCP regulation) during the final processing and labeling stages. *Fig. 5* depicts the FDA's guidelines and regulations relevant to stages of cell-cultured seafood production *(24)*.

The Bioterrorism Act of 2022 also requires all U.S. food facilities, including those producing cell-cultured food, to register their facility with the FDA and requires importers to give the FDA advance notice of shipments of imported foods. Imported cell-cultured seafood must also adhere to existing requirements that pertain to imported wild-caught and/or aquaculture seafood.

Voluntary FDA consultations

Unlike Canada, the United States has no mandatory premarket or precommercialization notification requirement. However, the FDA encourages voluntary premarket consultations early and often during the development of cell-cultured foods (which can be arranged by emailing AnimalCellCultureFoods@fda.hhs.gov). Consultations are conducted product by product and can be challenging because of lack of guidance documents. However, consultations help overcome hurdles associated with the commercialization of cell-based products. Meeting with the FDA early in the development process and thereafter helps cell-cultured food industries ensure food safety by evaluating potential risks and managing them. As of the writing of this article, no consultation had been completed for cell-based seafood *(13)*, but two consultations had been completed for cell-cultured chicken products.

For cell-cultured seafood products, the scope of the consultation first entails evaluation of cell collection from the animal. Then, a review of techniques used for cell line development is conducted. For example, what are the media inputs, what kinds of modifications are performed,

and how is cell banking done? Next, the FDA looks at cell growth and differentiation stages. Finally, harvest from the bioreactor and final processing steps are assessed.

Informal approval

At the conclusion of this entire process, the FDA generates a scientific memo that serves as a summary of the assessed elements based on the firm's consultation package. If there are no further questions related to the food safety of the product or manufacturing process, the FDA issues a "no question" letter and publishes a public-facing version of the consultation *(13)*. There is no requirement to either consult with or seek approval of cell-cultured seafood or other animal products before sale. However, prudent companies would be wise to do so to foster goodwill with regulatory agencies and bolster consumer confidence.

As mentioned previously under the "Efforts to identify labeling terminology" section, there is no regulatory framework in place to determine how cell-cultured foods should be labeled, and the FSIS and FDA have yet to decide on nomenclature. However, all cell-cultured meat and poultry labeling must be preapproved by the FSIS.

COMMERCIALIZATION HURDLES

As cell-cultured food products receive approval, there is increasing anticipation regarding when these products will become available in the market. Given that cell-cultured food production technology is still in its early stages of development, significant challenges must be addressed to facilitate the transition from pilot scale to market commercialization. Alternative products often do not match the nutritional value or culinary attributes of their conventional counterparts. This could be a barrier to consumer acceptance, especially in countries where seafood consumption is tied to cultural roots *(26)*. A recent study on consumer preference of cell-cultured seafood products suggests they are more likely to attract customers when, in addition to being sustainable, they have sensory, nutritional, and health benefits similar to those of their conventional counterparts *(28)*. The high cost of producing cell-cultured seafood is also a significant barrier that positions these products in high-end markets. The world's first cell-cultured beef burger was reported to cost U.S. $325,000 to produce *(6)*. Several reasons for the high cost of cell-based meat products are mentioned later.

Using culture media to provide cells with the necessary nutrients to grow is costly mainly because of the addition of FBS. FBS is harvested from blood drawn from a bovine fetus after slaughter and contains essential growth factors and amino acids. The composition of FBS varies from batch to batch, making it difficult to replicate its formulation. It is estimated that the use of culture media accounts for as much as 55–95% of the final production cost. In addition, there are ethical concerns about using animal-based serum in cell-based food production, which suggests the need for serum-free media to mitigate the environmental and ethical impacts of cell-cultured seafood production *(31)*.

The need for sterility is another challenge contributing to higher production costs. The existing infrastructure for cell-cultured food production is based on the same aseptic techniques used in the pharmaceutical-grade production of therapeutics. It involves single-use plastics, sanitizers, and sterilizing agents, hence increasing costs *(8, 32)*. A shift toward more renewable energy sources is also needed to reduce costs. Recent life cycle assessment studies show that cell-based food production is highly energy-intensive *(37)*. Another critical factor in cell-based food production is the scale of available bioreactors. Current limitations in the size and availability of large-scale bioreactors can affect the industry's ability to efficiently produce significant quantities of cell-based food, posing a challenge to scaling up and commercialization *(4)*.

As technology advances, production processes will become more streamlined, and the cost of cell-cultured seafood is expected to decrease over time.

RESEARCH NEEDS

Despite significant progress in cell-cultured seafood production, there remains a knowledge gap in developing highly efficient production systems. More research and development are required to bridge this gap. For example, compared with mammalian cells, fish cell culture is relatively understudied, except for zebrafish (*Danio rerio*), a popular aquarium fish. Unlike immortalized cell lines, which can go through several production cycles, primary cells have a shorter life span and introduce variability in the process. Therefore, primary cells are not considered reliable sources for large-scale applications. In addition, thus far, most in vitro cell culture studies have focused on livestock animals, rather than fish and seafood; there is a need to develop genetically stable, immortalized cell lines for various fish species *(34)*.

Similarly, although there have been successful developments of serum-free media for numerous mammalian cell lines, there is a shortage of commercially available serum-free media specifically designed for fish cells. Considering the key challenges in developing effective serum-free media, some studies suggest the adoption of genetic engineering techniques to modify cells so that they can proliferate and differentiate in vitro without the need for exogenous growth factor signals. Tools such as clustered regularly interspaced short palindromic repeats (CRISPR) gene editing, can help enhance the tolerance of cells to various stressful conditions associated with media suspension and large-scale growth conditions by modifying cell signaling pathways *(38)*.

As previously mentioned, research on optimizing large-scale bioreactors that could facilitate effective proliferation and differentiation of cell lines is of great importance. This would help reduce the cost of production and

enhance affordability. More in-depth investigation on the socioeconomic impact of cell-cultured seafood production is also needed to explore the contribution of cell-cultured seafood to nutrition security, types of employment likely to be created and/or lost in low- and middle-income countries, impact on aquatic ecosystem health, and marketing strategies in different regions and various cultures *(28)*.

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