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Cellular Seafood: Current Challenges to Overcome for the Development of Cellular Seafood

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Abstract: Worldwide, seafood accounts for nearly 20% of annual animal protein consumption, and its production is critical to ensure global food and nutrition security. The increase in seafood demand has resulted in overfishing and caused a decrease in aquatic species. Aquaculture, or fish farming, includes breeding, growing, and harvesting fish and marine products in a controlled environment. It is the first solution to overfishing. To supply sufficient seafood for the growing demand, the alternative seafood industry provides promising opportunities. Cell-based seafood refers to seafood that is produced using cell and tissue culture without slaughtering the animal. This method, called cellular agriculture, has been adopted from regenerative medicine that uses advanced isolation and cultivation techniques of tissue culture. Cellular seafood should be efficiently produced to serve as a credible alternative to traditional seafood. It should mimic seafood in all its physical sensations, such as visual appearance, smell, texture, and taste. The efficient culture of cells that produce cellular seafood primarily depends on stem cell culture conditions, including incubation temperature and medium composition. Many of these variables should be known and optimized for each species. Overall, this review underscores the importance of further research in cell culture to advance the field of cultured seafood and promote sustainable seafood production.

Keywords: Cellular seafood; stem cells; cultivated meat; lab-grown meat; cell culture

1. Introduction

The global fisheries industry is crucial in providing animal protein for human consumption, accounting for nearly 20% of annual consumption [1-3]. It contains essential vitamins, minerals, and amino acids, which have antioxidant effects benefiting cells and tissues [4]. Seafood also provides high amounts of long-chain omega-3 polyunsaturated fatty acids such as EPA and DHA, which are known to have various health benefits, including potentially reducing the risk of diseases [5-6]. However, the fisheries industry faces significant challenges, including overfishing, habitat destruction, and environmental pollution [7-9]. These issues have led to a decline in the population of aquatic species and raised concerns about sustainability production.

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1.1. Introduction to Cell-Based Seafood as a Sustainable Alternative

Cell-based seafood has emerged as a promising alternative to address the challenges faced by traditional seafood production [10]. Cellular seafood, also known as lab-grown, refers to seafood produced using cell and tissue culture techniques. [11-12]. Cellular seafood, which includes fish, crustaceans, mollusks, and other species, has emerged as a biotechnological field [13]. It focuses on producing seafood from cell cultures rather than relying solely on wild or farmed fisheries. This cell-cultured approach offers several advantages, including reduced environmental impact, improved wild animal welfare, and the potential for sustainable production [10].

1.2. The Importance of the Development of Cell Culture Research

Research in cell culture plays a crucial role in advancing the field of seafood production. Stem cells are essential for the efficient production of cellular seafood since they are used as starter cells [13]. Additionally, optimizing cell culture conditions, such as incubation temperature and medium composition, is critical for the successful cultivation of cells for production [14-15]. However, challenges remain in mimicking all its physical sensations, such as visual appearance, smell, texture, and taste, which are essential for consumer acceptance of cellular seafood products [16-17].

2. Cellular Seafood Production: Challenges to Overcome

The production steps of cellular seafood begin with isolating cells, typically stem cells, from muscle, adipose tissue, or other tissues. These cells, basically stem cells, are then stimulated to multiply using cell culture media, media supplements, and growth factors [10, 13]. Effective cultivation of cells for cellular seafood production depends mainly on cell culture conditions, including temperature and composition of the growth medium, as well as factors such as stem cells [14-15, 18]. Once thawed, the cells are mixed with an edible scaffold that provides structural support [19-20].

The challenge of cultured fish meat lies in the capacity of muscle cells to produce structured proteins, fats, and connective tissues [10]. Scaffolds play a crucial role by supporting cell attachment and directing cell proliferation, differentiation, and organization [21]. Therefore, the scaffold should provide biochemical and biophysical cues to control tissue shape and cell type [22] while also facilitating the distribution of oxygen and nutrients and the removal of toxic by-products produced by cells [23]. This mixture is placed in a bioreactor, where the cells can mature into muscle and fat cells [10]. This process allows for the growth of larger tissues, ultimately forming the final product of cultivated seafood fish meat (Figure 1).

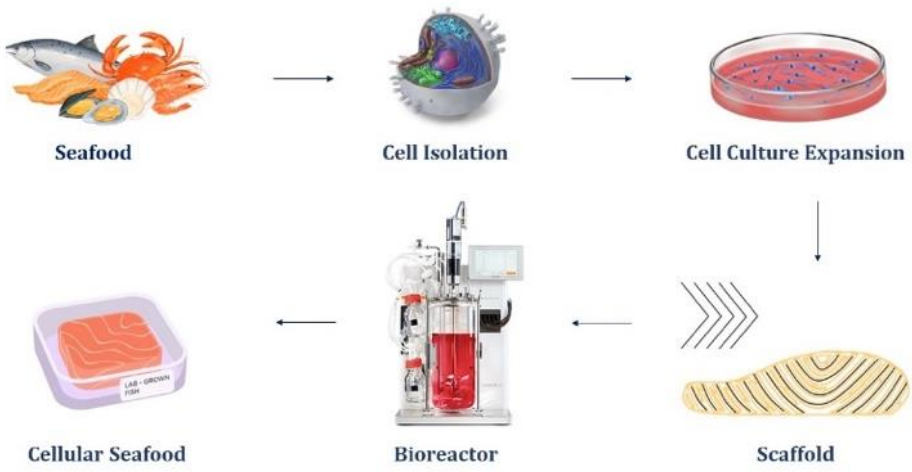

Figure 1. Workflow of cellular seafood production

2.1. The Importance of Stem Cell Isolation

Stem cells play a crucial role in cellular seafood production by differentiating into various cell types, which helps regenerate and grow fish tissue. Fish stem cell isolation methods are like those used for mammalian cells [10, 13]. However, there is a significant challenge in identifying the cell surface proteins that differentiate these progenitor cells and muscle cells [24-26]. Mammalian antibodies often do not cross-react with fish-derived cells due to low sequence conservation in extracellular proteins, which limits isolation and identification options [13]. Currently, isolating mesenchymal stem cells (MSCs) is more accessible than isolating stem cell niches for crustaceans and mollusks. Also, Fluorescence-activated cell sorting (FACS) is effective for isolating stem cells [10, 12]. Most fish tissue culture studies use primary stem cells isolated from fish muscle or adipose tissue due to a lack of fish cell lines [27-28]. To aid in the rapid isolation of these cells with minimal damage, magnetic beads combined with muscle progenitor-specific antibodies can be employed.

Table 1. The culture conditions for various seafood species [10]

	pH	**Oxygen**	**Carbon dioxide**	**Temperature**
Fish	7.2–7.4	Well-adapted to tolerate low oxygen environment under the water	Depends on pH buffering capacity of basal media	Cold-water fish, like salmon, thrive in temperatures ranging from 20 to 23 °C, while warm-water fish, such as zebrafish, prefer temperatures between 26 and 30 °C.
Crustaceans	6.8–8.1	The dissolved oxygen concentration (DO) of 3.8–6.4 mg/l is critical for aquatic animals. Water temperature directly affects DO concentrations and biological tolerances. Adult blue crabs can tolerate low DO levels, with an LC_{50} of less than 1.0 mg/L. However, larval and juvenile blue crabs are more sensitive, with an LC_{50} ranging from 4.08 to 6.44 mg/L.	Some do not state the need for carbon dioxide incubators. It may depend on the tissue type	Between 18 and 28 °C
Mollusks	7.4–7.8	Adult mussels, such as the eastern elliptic, exhibit resilience to hypoxia, as they can maintain normal oxygen levels even under stress at 2–3 mg/L. In contrast, juveniles are more susceptible to low dissolved oxygen levels (<4 mg/L).	Generally, they do not require carbon dioxide	.

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Table 1 (continued..)

Signaling molecules/GF		Antibiotics
Most effective at 80 ng/mL IGF-II, 20 ng/mL bFGF (added three days after culture initiation)		Before cell isolation, eggs were treated with 400IU/mL penicillin and 400ug/mL streptomycin and disinfectants: buffered iodophore (1:10) and malachite green (0.01 mg/mL). Contamination (yeast, fungi, protozoa) still occurred even though shrimps were pre-treated with 1000IU/mL penicillin and 1000ug/mL streptomycin, fungizone (25ug/mL) and antifungal Nystatin (100ug/mL) overnight 100IU/mL penicillin and 100ug/mL streptomycin were added to culture media routinely.
bFGF, EGF, TGF-beta, insulin, and IGF-1 were tested. 20 ng/mL bFGF-treated cells were subcultured for > 90 days without a feeder layer.		In holding media: Penicillin 10,000 units/mL, Streptomycin 10,000 ug/mL, Amphotericin B 500 ng/mL, Gentacmicin 1 ug/mL
None		100U/mL Penicillin, 100ug/mL Streptomycin, 0.06ug/mL Chloramphinicol
None		Penicillin 10,000 units/mL, Streptomycin 10,000 ug/mL, Amphotericin B 500 mg/mL
Not specified		1x antibiotic mixture (Life Technologies) containing: Gentamicin 250ug/mL Amphotericin B 250ug/mL Penicillin 1000 IU/mL Streptomycin 1000ug/mL
MPS medium (Tong and Miao, 1996)	20% heat inactivated FBS; chitosan (2 g/L); shrimp nerval module extracts (100uL/flask)	Sodium bicarbonate (0.75 g/L) Sodium pyruvate (0.55 g/L)
L-15	10% FBS	Five g/L NaCl and 1 g/L glucose were best for cell attachment and growth. Also tested were a few carbohydrates, amino acids, L-ascorbic acid, Buffalo rat liver (BRL)-conditioned medium, and selenium.
Artificial or natural seawater (27 psu), 22 amino acids, sugars, vitamins, cholesterol, phenol red.	10% FBS	150 mg/L glutamine (added just before use)
L-15	20% heat-treated FBS *Tested 5, 10, 15, 20% but used this*; 1% prawn hemolymph serum after initiation of primary culture	1 g/L glucose, 5 g/L NaCl
2x L-15 (*Used 5 media formulations, HBSCM-5 performs best); HBSCM: Haemolymph based shrimp culture medium Formulated from commercially available L-15 powder medium	15% FBS	1g/L glucose 0.1g/L L-proline

2.2. Optimization of Cell Culture Conditions

The efficient production of cellular seafood depends on optimizing cell culture conditions, including media, temperature, pH, oxygen levels, osmolarity, and nutrient availability. These factors can significantly impact cell growth and differentiation, affecting the quality and quantity of cellular seafood produced [14-15, 18]. Researchers are actively studying these variables to develop optimal culture conditions for different seafood species (Table 1 and Table 2). Growing enough viable muscle cells is challenging due to their adherence and dependence on optimal growth conditions, including adequate nutrients and a suitable microenvironment [29-30]. As a result, one of the significant challenges in cultivated seafood production is developing cell culture media that can sustain production. The ideal growth media should be cost-effective, sustainable, food-grade, available in large quantities, and, most importantly, effective in maintaining cell proliferation and promoting differentiation

Table 2. List of culture conditions of various crustacean cell cultures [30]

Crustacean Species	Tissue Type(s)	Osmolality/ Salinity	pH	Incubator Conditions
 **Chinese White Shrimp** *(Fenneropenaeus chinensis)*	Embryonic tissues from 8-10 dpf fertilized eggs	Osmolality was adjusted to 2.4% using NaCl.	7.0 - 7.2	22°C
 **Giant Tiger Prawn** *(Penaeus monodon)*	Lymphoid tissue. Cells from gills, ovaries, hepatopancreas, heart and muscle	470-500 mmol/kg	7.63 - 8.1	28°C (+1°C), 5% CO_2
	Lymphoid organ, Heart, Hepatopancreas, Hemocytes, Nerve cord, Nauplii, Eyestalk, Muscle, Testis, and Ovary	720 +/ -10 mOsm/kg	6.8	25°C
 **Giant Freshwater Prawn** *(Macrobrachium rosenbergii)*	Hepatopancreas, Eyestalk, Heart tissues	470–550 mmol/kg	7.2	Highest growth rate: 28°C
 **Pacific White Shrimp** *(Litopenaeus vannamei)*	Hemocytes, Heart, Lymphoid tissue, Hepatopancreas, Gill, Eyestalk, and Muscle	730 +/ -20 mOsm/kg	7.2	28°C

2.3. Challenges in mimicking all the physical sensations

One of the significant challenges in cellular seafood production is the physical sensations of traditional seafood, including its visual appearance, smell, texture, and taste; to be an acceptable alternative, cellular seafood should be efficiently produced and mimic seafood fish meat in all of its physical sensations of traditional seafood, Researchers are currently investigating various techniques to achieve these aims.

In terms of appearance, color plays a vital role in how we perceive the freshness and quality of seafood. It is affected by both achromatic (colorless) and chromatic properties measured by reflection (absorption and scattering) on the surface of meat [31]. Natural pigments such as anthocyanin, carotenoids, and curcuminoids can be used as coloring agents for red-fleshed fish flesh (e.g., salmonid species) or red shrimp species. Advanced technologies such as artificial intelligence and machine learning are increasingly used to mimic meat product colors by analyzing molecular structures and how each ingredient behaves independently and in combination [32]. However, achieving the highest quality color remains challenging due to various intrinsic and extrinsic factors affecting food color [33]. Moreover, the dimensions of a typical muscle fiber in terms of appearance are 1 to 40 mm in length and 20 to 100 μm in diameter. Still, so far, the structural fiber of the alternatives is produced only at the micron level [34-35].

The textural profile, which includes attributes like firmness, juiciness, springiness, and cohesiveness, is a crucial parameter for determining the quality and acceptability of food products [36]. Consumers are often willing to pay more for products with superior textures. Achieving the correct fiber structure is essential for a product to be considered fish-meat-like. To develop an alternative product with a similar structure, a thorough analysis of fish meat's structure is necessary; the selection of raw ingredients and techniques should be appropriate to mimic the fibral, connective, and adipose structure of the imitated product based on the desired properties of the product [32].

Texturization of an alternative typically involves processes like extrusion to rearrange protein ingredients into a fibrous structure that mimics the technical and functional aspects of fish meat. Plant protein, being globular-shaped, presents a challenge as fish meat is fibrous. Developing a fibrous structure in plant proteins during processing involves several key events, including unfolding, cross-linking, breakdown, and gelatinization. Plant proteins undergo various processing techniques such as extrusion, spinning, and applying shear force to be texturized. These processes help unfold and cross-link the proteins, ultimately contributing to the desired fibrous structure [17, 37]. Proteins provide nutrition and contribute to essential functional properties of the product, such as emulsification, gelling, and water/oil-absorbing capacity [32].

The smell and taste of fish meat are crucial components of its overall eating experience, resulting from the stimulation of taste receptors in the oral and nasal cavities by various bioactive compounds [38]. Fat and protein are key components that contribute to fish meat's smell and taste. Fish meat contains over 1000 different smell and taste components responsible for its specific flavor. As a result, fish meat has smell and taste profiles: a fish meaty smell and taste derived from amino acids and water-soluble reducing sugars, a species-specific smell and taste due to differences in fatty acid composition and aromatic water-soluble compounds [32]. Despite advances, optimizing the scent and taste of fish meat alternatives remains a significant challenge [16-17, 30, 39-41].

2.4. Cost and Feasibility of Cellular Seafood Production Processes

Factors such as the cost of growth media, scale-up of cell culture processes, intellectual property issues, and regulatory hurdles affect the commercial availability of cellular seafood [42]. A feasible solution for cellular meat production needs to be cost-effective and ideally produced locally. Availability of affordable growth medium is crucial, but current options like bovine fetal calf serum are expensive [43]. For a large-scale facility costing $60 million annually producing 540,000 kg of meat, the estimated cost to produce 1 kg of cell-cultured meat is $63. The main cost components include the cell-culture medium, bioreactors, and labor, which collectively make up over 80% of the total production cost. Despite ongoing technological advancements, particularly in reducing the cost of the cell-culture

medium, the industry still faces uncertainties in achieving cost competitiveness and sustainable returns on investment [30].

Several companies have emerged as key players in working on the production of cell-based seafood in recent years [44] (Figure 2). In the United States, UPSIDE Foods (formerly known as Memphis Meats) has been at the forefront of studying lobster and other crustaceans. They acquired Cultured Decadence in January 2022, a company focused on lobster and other crustaceans. This acquisition further solidified UPSIDE Foods' position in the field.

Shiok Meats, based in Singapore, has made significant strides in the cell-based crustacean market. They have focused on shrimps, crabs, and lobsters, showcasing their first cell-based shrimp dumpling in March 2019. In 2020, they filed a patent for isolating and cultivating muscle and fat cells from crustaceans. Shiok Meats continued to innovate, showcasing the first lobster prototype in November 2020 and the world's first cell-based crab in August 2021. Their efforts culminated in the opening of Singapore's first cultivated crustacean mini-pilot plant in November 2021. CellMEAT, a company based in South Korea, entered the market in 2019, focusing on Dokdo shrimp. In October 2022, they held a tasting event for a cultivated shrimp dish made with their flagship Dokdo Shrimp, indicating progress in their development efforts.

These companies represent the forefront of research and development in the cell-based crustacean industry, paving the way for a potential revolution in how crustaceans are produced and consumed.

2.5. Health Risks Associated with Consuming Cellular Seafood

Consumer perceptions regarding the effects of cell-based meat production often differ from reality. While meat consumption has been associated with nutrition-related diseases, foodborne illnesses, and antibiotic-resistant pathogens due to antibiotic overuse, cell-based meat production aims to address these issues by avoiding antibiotics and growth hormones. There is a gap between public perception and the actual practices involved in cell-based meat production conducted in aseptic environments to prevent contamination. Preservatives like sodium benzoate, commonly used in processed meat products, are added to protect growing cells from yeast and fungus. While antibiotics are often used in cell culture to prevent bacterial infections, patents for industrial cell-based meat production aim to achieve this without antibiotics. The necessity of antibiotics or antibacterial in routine muscle cell culture remains to be seen [45-50].

The fisheries industry faces significant challenges, including overfishing, habitat destruction, and environmental pollution. Cell-based seafood has emerged as a promising alternative to address these issues. Currently, there are two main challenges to overcome in developing cellular seafood: *i)* The challenge of cellular seafood lies in the capacity of muscle cells to produce structured proteins, fats, and connective tissues. Most fish tissue culture studies use primary stem cells isolated from fish muscle or adipose tissue due to a lack of fish cell lines.

Additionally, identifying the cell surface proteins that differentiate progenitor cells and muscle cells is a significant challenge. Challenges in cultivated fish meat production include developing cell culture conditions that can sustain production; *ii)* Challenges remain in mimicking all its physical sensations, such as visual appearance, smell, texture, and taste, which are essential for consumer acceptance of cellular seafood products. Despite advances, optimizing the scent and taste of fish meat alternatives remains a significant challenge. In addition, establishing continuous cell lines, improving growth conditions, and ensuring consumer acceptance are crucial steps towards successful commercialization. Regarding this, companies such as UPSIDE Foods, Shiok Meats, and CellMEAT are pioneering work in the cell-based seafood industry worldwide. Therefore, further research and innovation are needed to overcome these challenges and realize the potential of cultured crustacean meat production.

3. Future Perspective and Recommendations

The development of cellular seafood holds excellent promise as a sustainable alternative to traditional seafood production methods. Future research efforts should address the critical challenges

identified in this review to realize this potential. Firstly, advancements in cellular agriculture techniques are needed to enhance the capacity of muscle cells to produce structured proteins, fats, and connective tissues. This may involve further understanding the regulatory mechanisms controlling cell differentiation and protein synthesis in fish muscle cells. Secondly, research should aim to develop optimized cell culture conditions that can sustain the production of cellular seafood on a commercial scale. This will require the development of novel cell culture media and bioreactor systems that can support the growth and differentiation of fish muscle cells into mature tissue. Thirdly, there is a need for continued innovation in mimicry to enhance cellular seafood products' visual appearance, smell, texture, and taste. This may involve using advanced technologies to create products that resemble their traditional seafood production. Finally, while challenges remain, the development of cellular seafood has the potential to revolutionize the seafood industry and provide a sustainable alternative to conventional seafood production. Continued research and innovation in this field will be essential for realizing this vision.

Conflict of Interest

The authors declared no potential conflicts of interest concerning the publication of this article.

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