Title

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Journal

Journal of Biological Engineering, 18(1)

ISSN

1754-1611

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Publication Date

2024-08-07

DOI

10.1186/s13036-024-00436-1

Peer reviewed

Journal of Biological Engineering

REVIEW Open Access

Cell-cultivated aquatic food products: emerging production systems for seafood

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Abstract

The demand for fish protein continues to increase and currently accounts for 17% of total animal protein consumption by humans. About 90% of marine fish stocks are fished at or above maximum sustainable levels, with aquaculture propagating as one of the fastest growing food sectors to address some of this demand. Cell-cultivated seafood production is an alternative approach to produce nutritionally-complete seafood products to meet the growing demand. This cellular aquaculture approach offers a sustainable, climate resilient and ethical biotechnological approach as an alternative to conventional fishing and fish farming. Additional benefits include reduced antibiotic use and the absence of mercury. Cell-cultivated seafood also provides options for the fortification of fish meat with healthier compositions, such as omega-3 fatty acids and other beneficial nutrients through scaffold, media or cell approaches. This review addresses the biomaterials, production processes, tissue engineering approaches, processing, quality, safety, regulatory, and social aspects of cell-cultivated seafood, encompassing where we are today, as well as the road ahead. The goal is to provide a roadmap for the science and technology required to bring cellular aquaculture forward as a mainstream food source.

Keywords Cellular agriculture, Tissue engineering, Future foods, Cell-cultivated seafood, Culture media, Scaling up

Introduction

The availability of safe, high-quality food for the burgeoning world population continues to be a major challenge in light of the deterioration of natural resources coupled with climate change. To feed the estimated 10 billion people safely and sustainably by 2050, the world will need to produce significantly more food [1, 2]. It is anticipated that global demand for meat will increase by 70% from today, and planetary resources will be insufficient to meet the demand of the world population by 2050 [3]. Within this larger global challenge, aquatic sources provide nutritional protein-rich foods, including omega-3-enriched sources of fatty acids and bioavailable micronutrients [4]. Stagnant levels of fish harvested from open water fisheries and the growing challenges with the sustainability of aquaculture systems are concerns [4]. To adequately feed the growing global population by 2050,

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increases in seafood production of 100% are projected as a need [5]. Decade-wise comparisons of global per capita consumption, capture fisheries production and aquaculture production from 2000 to 2020 based on FAO data are given in Fig. 1. Hence, there is an imperative to establish alternative sources of fish and shellfish to effectively meet the growing global protein demand in the foreseeable future (2020–2050) [2].

Presently, 89% of the aquatic animals produced—equal to 157.4 million tons—are used for human consumption, considering the per capita consumption of 20.2 kg fish per year by 7.8 billion people. The rest is used mainly for non-food uses including fish oil and fish meal production [4]. Future projections for capture fisheries and aquaculture production by 2050 are 98.3 and 140 million tons, respectively [4]. Thus, increases in future fish production will rely mostly on aquaculture production, which is challenging in the context of sustainable production. For fish production to be maintained at a sustainable level, critical efforts will be required to provide larger volumes of feed to support aquaculture, to maintain quality for aquatic environments, to reduce pressure on wild aquatic organisms used for food and provide quality aquatic foods to consumers [6–8]. These challenges prompt the development of alternative sources of aquatic food through cell-cultivated approaches.

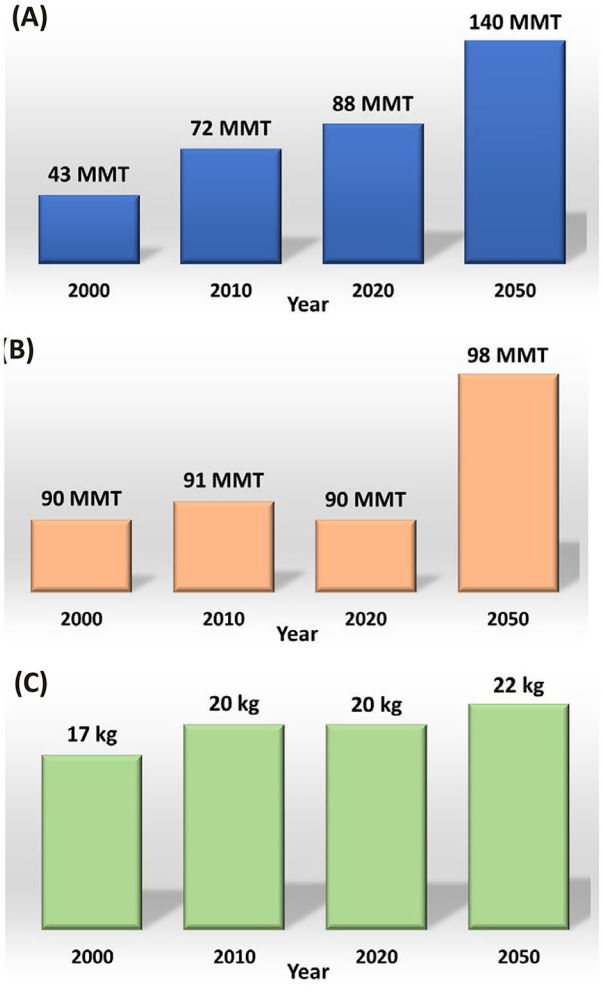

Fig. 1 Trends and projection up to 2050 in total capture and aquaculture production and Consumption based on FAO Data [4]. (**A**) Global aquaculture production (million metric tons); (**B**) Global fisheries production (million metric tons); (**C**) Seafood consumption (kilograms)

Cell-cultivated seafood

Cell-cultivated seafood has gained attention as an alternative sustainable food production system, where animal cells are grown in vitro using cell culture techniques to form edible seafood products without the need for the animal [9, 10]. Cellular agriculture is one of the key transformative food production systems to help address the above challenges, which originated with the cultivation of goldfish in a study funded by NASA [9]. Cell-cultivated fish production requires the large-scale cultivation of cells to generate large masses of seafood-relevant cells and tissues. These cells and tissues can be used to form unstructured products such as surimi or fish fingers using well-established food processing techniques, or they can be further cultured on three-dimensional biomaterial scaffolds to generate structured products akin to fish fillets. The many advantages to producing seafood from cell cultures rather than using native fish includes improved freshness, food quality and avoiding nonedible components such as bones, skin, shells, and scales as wastes that can negatively impact the environment. Cell-cultivated seafood may also shorten food production cycle time and provide continuous production; cell cultures may require weeks to generate functional foods and may do so in a continuous manner [10].

Fisheries and aquaculture are relatively sustainable food production systems compared to terrestrial livestock, however, due to overfishing, pressure on wild stocks, emerging diseases, antibiotic-resistant bacteria, global warming, and marine acidification with adverse impact on organisms' physiology, loss of biodiversity and species migration, byproducts of production, microplastics, chemical contaminants in waters, and the lack of clean water (Fig. 2), the seafood industry requires alternative and innovative production systems to overcome these current challenges.

Research gaps and challenges

There are several gaps in research and development to be filled in order to progress cell-cultivated seafoods.

Limited seafood cell lines: Producing seafood from fish cell cultures is an intriguing opportunity for cellular agriculture, yet few fish cell lines are currently available

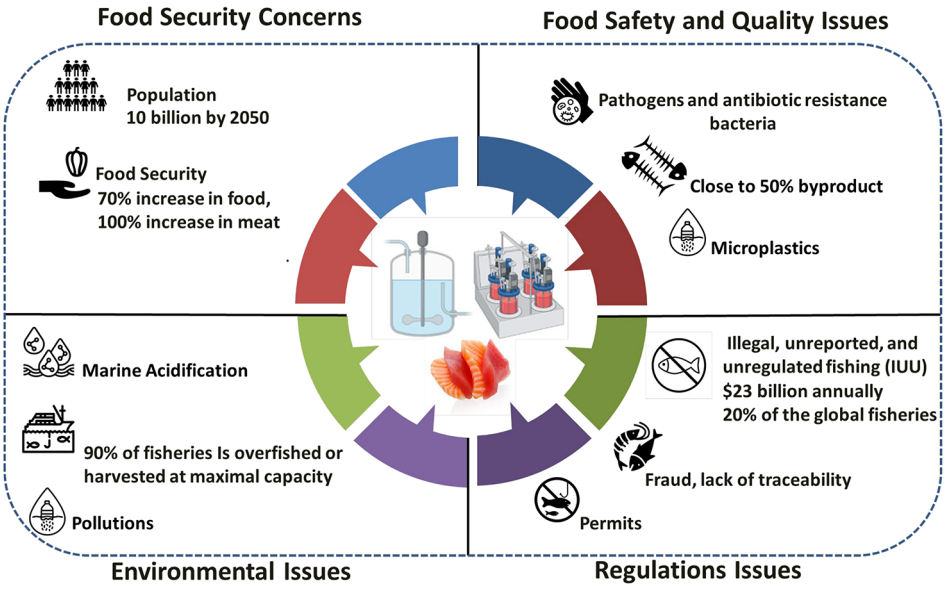

Fig. 2 The main challenges in the seafood industry as driving forces for developing cell-cultivated seafood

that have direct relevance to seafood production [10]. Cell-cultivated seafood processes rely on native seafood sources for harvesting muscle and fat cells, which are then immortalized. Both cell isolation and the immortalization processes remain challenging. For example, access to embryonic stages of many aquatic organisms as a source of stem cells is difficult. The number of cell sources has been expanding thanks to continuous research. Many of these sources, however, still need to be validated in a large-scale culture.

Limited knowledge of seafood cell differentiation: There remains limited knowledge in terms of in vitro fish, crustacean, and mollusk muscle cell or fat cell proliferation, differentiation, and maturation. Omics-based methods, including genomics, proteomics, and metabolomics, are helping to elucidate factors involved in the different stages of differentiation to accelerate cell-cultivated seafood production. Further, a number of studies with fish have provided insights into growth factor requirements and growth conditions [11]. Myogenic precursors from juvenile trout showed higher proliferation and differentiation rates than adult trout myogenic precursors [12], and insulin-like Growth Factor (IGF-1) and IGF-2 stimulated the proliferation in primary cell cultures of myoblasts from rainbow trout [13–16]. Gilthead sea bream (*Sparus aurata*) myocytes were cultured to evaluate the role of IGFs in muscle growth and differentiation via the regulation of myogenic regulatory factors (MRFs: MyoD, Myf5, myogenin and MRF4) expression [17]. At the beginning of the cell culture and during the proliferation, the IGF-2 expression was highest. Additionally, further evaluations indicated that myod2 and myf5 expression (genes involved in early muscle cell proliferation) was increased by IGF-2, whereas IGF-1 increased mrf4 and myogenin expression (genes involved in differentiation) [17].

Lack of serum-free media: Serum-free media has been developed for mammalian cells, yet this remains a challenge for cell-cultivated seafood. Cell line development for seafood can require up to 20% serum, making cell-based seafood production unsustainable and expensive. Reducing serum can result in changes in morphology or slower to no cell growth [18]. Reduction of serum in fish cell cultures has been achieved using IGF-2, algal extracts, and protein hydrolysates [18, 19], but elimination of serum without negative impact on growth remains a challenge [18]. More research is required to develop serum alternatives for cellular aquaculture, such as specific plants or bacterial/algal-based products.

Limited genetic tools: Exploring genetic modifications for seafood cells, to accelerate both understanding of cell proliferation and differentiation, as well as to develop cell lines, remains challenging due to the few genetic tools developed for seafood cells. Yet, optimization of immortalization and trans-differentiation processes through genetic modification, including CRISPR-Cas9 [20] editing of fibroblasts that convert them into skeletal muscle or adipose cells, will address some of the cell sourcing challenges for cell-cultivated seafood. Induced pluripotent stem cells are available for adult zebrafish [21], with limits to other publicly available other seafood species. There remains limited knowledge of differentiation pathways in aquatic species other than zebrafish (*Danio rerio*) [21–24]. Genetic tools in other, traditionally consumed, species need to be pursued. Given that these technologies still require genetic modification, consumer acceptance

and reactions to the consumption of genetically modified cells must be evaluated.

Scale Up Demonstration: Compared to mammalian cells, fish cells may be more suitable for bioreactor production due to their tolerance for hypoxic conditions, which reduces the need for active oxygenation; their increased tolerance for different pHs; and in some cases, their growth at lower temperature to reduce energy costs [10]. However, long doubling times are problematic and scale up data remains to be demonstrated.

Lack of available consumer-ready products: The inclusion of heme proteins in plant-based meat increased meat-like flavor and natural color [25]. Similar approaches are needed for aquatic cell-cultured foods to address consumer perceptions. The Peptide Atlas and Protein Map developed from Rohu (*Labeo rohita*) [26] is a useful source for identifying proteins involved in the quality and color of cell-cultivated seafood. Nutrition, flavor, texture, and quality of products and cultural relevance are important parameters that will need to be addressed for cell-cultivated seafood to achieve consumer acceptance as the field progresses. Flavor in conventional seafood is mainly due to the fatty acids, and some amino acids. Developing these flavors in the cultivated meat could be achieved by cell engineering to generate specific amino acids and fatty acids, manipulating cell culture media to contain more marine flavor-based compounds such as protein hydrolysates from marine plants, and adding flavor extracts to the final products.

Cell types for cell-cultivated seafood production

Developing cell-cultivated seafood starts by isolating embryonic stem cells, adult stem cells, or generating induced pluripotent cells from the species of interest [10, 14, 24, 27–30]. Despite efforts to establish cell lines from aquatic organisms (fish, mollusk and crustaceans), the challenge remains to isolate and immortalize viable cells (Table 1).

Tissue selection is the first step for sampling, in the case of fish samples for myogenic cells, this often involves using white muscles with significantly less fat content compared to red muscles, however, the spatial arrangement differs among species (in most fish species, red fibers form a thin lateral superficial sheet just under the skin, whereas white fibers make up the underlying mass of the myotome). In order to isolate cells, adult tissue selection for mollusks plays a crucial role in establishing primary cell culture methods. Mollusks, such as oysters, have diverse tissue types that can dictate the culture conditions and cell dissociation methods [42]. Tissue from three main oyster species, Pacific (*C. gigas*), Eastern (*C. virginica*), and European Flat oyster (*Ostrea edulis*), have been studied for drug, toxicity, and disease research [30–32, 34, 42], including embryo, heart, mantle, digestive gland, gill, ventricle, and adductor tissues (Table 1). Among oyster tissues studied, heart tissue was most frequently selected as it had better potential in establishing a permanent cell line than oyster embryos [31]. These previous studies indicate that the tissue of origin often dictates the success of oyster cell culture [42], along with culture conditions and decontamination treatments.

A significant challenge for seafood cell isolation is contamination from other species, particularly for marine filter feeder bivalves such as oyster, mussel, clam and scallop [42]. Protozoans (*Thraustochytrium* sp.), amoeba, motile zoospores, sporangia, yeast, endospores, and microalgae are common contaminants in marine invertebrate cell culture [31, 42]. Finding optimal antibiotics and antifungal conditions during the initial cell isolation step is also challenging because high concentrations can

Table 1 Examples of cell line development from aquatic organisms

Species	Tissue Type	Cell isolation method	Reference
Juvenile and adult Rainbow trout (*Oncorhynchus mykiss*)	Muscle	Enzymatic (collagenase)	[12]
European flat Oyster (*Ostrea edulis*)	Heart cells	Tissue explant	[30]
Pacific oyster (*Crassostera gigas*) Hard clam (*Meretrix lusoria*)	Heart, mantle, digestive glands, embryonic stage	Tissue explant	[31]
Eastern oyster (*Crassostera virginica)*	Ventricle	Enzymatic (Pronase)	[32]
Mussel (*Mytilus edulis)*	Digestive gland, gill	Mechanical	[33]
Pacific oyster (*Crassostera gigas)*	Heart, gill, mantle, adductor muscle	Tissue explant	34
Rohu (*Labeo rohita*)	Muscle	Tissue explant	35
Rohu (*Labeo rohita*)	Embryonic cells	Mechanical	36
Australasian snapper (*Chrysophrys auratus*)	Muscle	Enzymatic (Collagenase)	37
Naked carp (*Gymnocypris przewalskii*)	Muscle	Tissue explant	38
Freshwater catfish (*Wallago attu*)	Muscle	Tissue explant	39
Atlantic mackerel (*Scomber scombrus*)	Muscle	Enzymatic (Collagenase)	40
Olive flounder (*Paralichthys olivaceus*)	Muscle	Tissue explant, Enzymatic (Collagenase)	41

damage or kill the desired cells, and low concentrations may not effectively eliminate the contaminating microbes [34, 42].

In order to develop cells suitable for bioprocesses for seafoods, immortalized cells are required. Three methods of immortalization are generally pursued, spontaneous genetic processes, genetic modification approaches such as the expression of the catalytic subunit of telomerase (TERT), or genetic inactivation of p53/p14/Rb [43]. Spontaneous immortalization has benefits and limitations. For example, spontaneously immortalized cells are not considered genetically modified (GM), which allows companies access to European markets that restrict the use of GM foods [43]. However, this immortalization process is not controlled, thus additional genetic changes are feasible. In addition, every cell type has its own susceptibility towards spontaneous immortalization. For example, fish cell lines have a higher susceptibility for spontaneous immortalization due to the high regenerative capacity of the adult stem cell population compared to mammals with more effective DNA repair mechanisms [44]. For cell-cultivated seafood production, spontaneous immortalized cell lines from Atlantic mackerel (*Scomber scombrus*) were developed [40] and a skeletal muscle cell line was confirmed through characterization of muscle stemness and differentiation via paired-box protein 7 (PAX7) and myosin heavy chain (MHC) immunostaining, respectively. Importantly, an adipocyte-like phenotype was demonstrated for these cells through lipid accumulation from the environment, confirmed via Oil Red O (ORO) staining and quantification of neutral lipids, as an alternative path to adipogenesis utilizing adipose-derived cells. Limited antibody markers for fish-derived cells, including adipocytes and myocytes, continue to make cell identification a challenge for the field.

Cell growth conditions

Nutritional requirements in cell culture remain unclear for many cell lines from aquatic organisms. A simple basal medium with added artificial seawater (ASW) or sterile seawater (SSW) helped to provide osmolarity similar to marine habitats [42]. For example, for oyster cell culture media, osmolarity was adjusted to 650–720 mmol/kg[31]. The most common medium used for many aquatic organisms in cell culture is L-15, which contains salts, amino acids, galactose, vitamins, and minerals [30, 31, 34, 42]. However, the L-15 medium contains no proline or taurine, which are present at high levels in the body fluids or tissues of aquatic organisms [31]. Proline and taurine are likely essential components for cell proliferation in mammalian cells [45]. Therefore, adding proline or taurine to oyster cell culture media by using oyster body fluid or tissue extracts could be necessary for supporting cell proliferation. In addition to basal media, many media supplements and growth factors such as fetal bovine serum (FBS), adult organism soft body fluid, embryo or gonad extract, fibroblast growth factor (FGF), insulin, and epidermal growth factor (EGF) have been tested for cell proliferation but with inconsistent outcomes [30–32, 34]. Different cell culture media, supplements, and incubation temperatures used for bivalve cell culture are presented in Table 2.

For oyster cell cultures, penicillin, streptomycin, and amphotericin B are the most commonly used antibiotics. The penicillin concentration for tissue decontamination ranges from 50 to 100 IU/mL, streptomycin ranges from 100 to 500 µg/mL, and amphotericin B from 0.25 to 2.5 µg/mL[31], [42]. These antibiotics were placed into Leibovitz's 15 (L-15) medium, phosphate-buffered saline solution (PBS), or artificial seawater (ASW) for washing the tissue samples. Other antibiotics such as ampicillin, gentamycin, and kanamycin have also been used [42]. It

Table 2 Cell culture media, media supplements, and incubation temperatures used for bivalve primary cell cultures*

Species	Basal Medium	Supplement	Temp	Reference
European flat oyster (*O. edulis*)	seawater	10% Fetal calf serum	20 °C	[30]
	L-15	5% Pacific oyster (*C. gigas*) hemolymph		
	M199			
	BHK21			
	L-15 and sterile seawater			
Pacific oyster (*C. gigas*), White clam (*M. lusoria*)	L-15	10% Fetal bovine serum	28 °C	[31]
		oyster soft body extract		
		oyster gonad extract		
		Pituitary gland: rat, bovine, rabbit, carp		
Eastern oyster (*C. virginica*)	JL-ODRP-4 (chemically defined) artificial seawater	none	25 °C	[32]
Blue mussel (*M. edulis*)	Modified L-15	10% Fetal bovine serum	18 °C	[33]
Pacific oyster (*C. gigas*)	L-15, artificial seawater (1:1)	none	28 °C	[34]
	Opti-MEM reduced serum medium			

*Abbreviations: L15 (Leibovitz's); M199 (Medium 199); BHK21 (Baby hamster kidney-21); JL-ODRP-4 (Serum free media containing yeast isolate)

is important to note that decontamination might vary depending on the source of the tissues, as some tissues have a higher initial microbial load. For instance, digestive glands, gills, and the mantle are prone to more contamination than the heart or adductor muscle because these organs are primarily involved in filtration. In addition, some marine microbes and parasites carry a symbiotic relationship with the animal, leading to more contamination and making it difficult to find optimal decontamination conditions [42].

Aside from serum-free media needs, environmental factors such as oxygen, salt, pH, osmolarity, and temperature must be optimized. Fish cells are generally adapted to low oxygen environments with hypoxia-response genes [46]. Some fish cells only grow in 5% carbon dioxide, while others utilize anoxic or standard oxygen tension [47]. A comprehensive study on muscle lactate dehydrogenase (LDH) in warm-water fish and mammalian cells reported significant differences in metabolic activity dependent on pH [27]. Generally, seafood cells grow at lower temperatures than mammalian cells, making them good candidates for producing cell-cultivated seafood with lower energy inputs. There are different fully defined basal media available for seafood cell culture including Eagle's Medium, Modified Eagle's Medium (MEM), Medium 199 (M199) and Leibowitz's 15 (L-15). While there have been significant advances in the development of serum-free culture media for mammalian cell lines, there has been limited progress for fish cells [27]. Serum-free media has been achieved for a few fish cell lines in the past, however, these formulations were not well-defined or were proprietary within companies, resulting in significant challenges in broadening their utility for cell-cultivated seafood. Serum-free media containing lactalbumin hydrolysate, trypticase-soy broth, bacto-peptone, dextrose, yeast isolate, polyvinylpyrrolidone, and non-essential amino acids were studied with different fish cell lines (Table 3), and cell growth and morphology of the cells was similar to those that were grown in serum-containing media [48]. Bioprocessing was utilized to convert different feedstocks including whole oysters (*C. virginia*), whole mussels (*M. edulis*), whole lugworms (*Arenicola marina*), black soldier flies (*Hermetia illucens*) and crickets (*Acheta domesticus*) to protein hydrolysates for growing fish cells [18]. These hydrolysates were cytotoxic for Zebrafish cells (ZEM2S CRL-2147™) at high concentrations (1 and 10 mg/ml) regardless of serum concentration, while, at lower concentrations (0.001-0.1 mg/ml), all of the hydrolysates supported cell growth. Black soldier fly hydrolysates could replace serum and provided a cost-effective source of peptides.

The use of modeling tools also has the potential to foster more rapid identification of key media and related conditions for seafood cell growth and differentiation. For example, through the use of Design of Experiments (DoE) and/or AI, the development of a serum-free medium can be pursued.

Protein hydrolysates from marine byproducts could also provide inexpensive and high quality proteins and amino acids to develop serum-free media.

Differentiation – myogenesis and adipogenesis

There is limited knowledge on the in vitro differentiation and maturation of fish, crustacean and mollusk cells into fat or muscle tissues [40]. To screen for myogenesis in mackerel cells as an example, a variety of methods were utilized [e.g., serum starvation, reduced serum, reduced serum plus additives [50], reduced serum with insulin, 1-oleoyl lysophosphatidic acid (LPA) and transferrin,

Table 3 Feedstocks and processing methods for fish cell growth

Feedstock	Processing-Final products	Cell line	Target	Highlights	Ref
Lactalbumin hydrolysate, trypticase-soy broth, bacto-peptone, dextrose, yeast isolate, polyvinylpyrrolidone, and non-essential amino acids	N/A	Goldfish-derived CAR cells, fathead minnow IFHMJ cells, *Epithelioma papulosum cyprini* lEPC cells, chinook salmon embryo cells, cells from goldfish air bladder IABIII	Cell Growth	growth and morphology of cells grown in serum-free medium close to those grown in serum- containing medium	[48]
Yeast, soy, wheat gluten hydrolysate	Hydrolysates	Channel catfish (*Ictalurus punctatus*) ovary (CCO)	Cell growth	Supplementation with wheat gluten hydrolysate resulted in growth similar to serum-free medium, yeast and soy hydrolysates had inhibitory effects on the cell growth	[49]
Oyster, mussel, Black soldier fly, Cricket, Lugworm	Protein hydrolysis using Alcalase	Zebra fish (*Danio rerio*)	Cell growth, LDH	- High concentration of peptides (1 to 10 mg/ml) cytotoxic; - BSF peptides at lower concentrations (0.001 to 0.1 mg/ml) replaced FBS; - BSF provided cost-effective ($0.915/100 kg cell-cultivated seafood) source of peptides to replace serum.	[18]

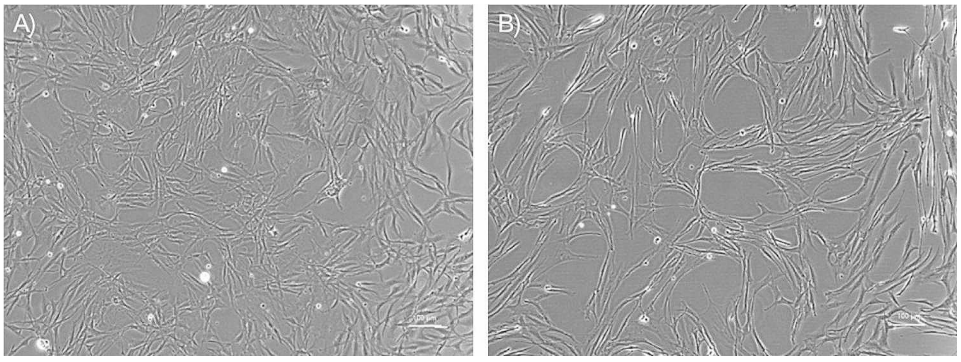

Fig. 3 Example images of mackerel cell lines. Phase contrast images: **A**) Mack1 Passage 147 and **B**) Mack2 Passage 124. Scale bars 100 µm. Photos provided by Michael Saad, based on research from https://doi.org/10.1038/s41598-023-31822-2 [40]

Table 4 Scaffold considerations for cell-cultivated seafood

Category	Parameter	Research questions
cell responses	Stiffness	Does the relationship between scaffold biomaterial stiffness and effects on proliferation, differentiation, and maturation vary across species?
	Topography	Groove scaffolds promote cell alignment and myogenic differentiation, with the magnitude dependent on groove width [61]. Is the optimal pattern for inducing myogenesis different across species?
	Functionalization	Do scaffolds for fish and aquatic invertebrates require different types of functionalization (e.g., peptides) compared to cells from terrestrial animals?
product impact	Texture	What biomaterials for scaffolds have positive or neutral effects on texture of cell-cultivated seafood products? For those that have a negative impact (e.g., too stiff), can they be treated to improve texture or increase to their degradation rates for improved sensory appeal?
	Cooking	How do various biomaterial scaffolds respond to cooking? What treatments will bring thermal properties toward those of fish or invertebrate extracellular matrix proteins?
	Geometry, form factor	How closely does the geometry of cell-cultivated seafood products need to replicate that of conventional seafood products to be acceptable from a sensory and consumer perspective? What manufacturing methods can best achieve that target?

reduced serum medium with insulin-like growth factor 1, reduced serum medium plus additives with IGF-1; medium with extracellular signal-regulated kinase inhibitor (ERKi) [51]]. Myogenic potential was assessed via RT-qPCR using primers based on genome sequences from southern bluefin tuna (*Thunnus maccoyii*) (e.g., myogenic differentiation 1 (*MYOD1*), myogenin (*MYOG*), (troponin T type 3a (*TNNT3A*)), along with immunohistochemistry. Differentiation via paired-box protein 7 and myosin heavy chain immunostaining was observed in a continuous muscle cell line developed from Atlantic mackerel (*Scomber scombrus*) [40]. The cell line also exhibited an adipocyte-like phenotype, which was confirmed via Oil Red O staining and quantification of neutral lipids. MEF2A, Mrf-4, MyoD and Myf-5 expression was reported in a muscle cell line developed from a freshwater fish (*Labeo rohita*) during differentiation of muscle cell culture [52]. However, more detailed studies need to be carried out to facilitate selection of the right cell type for cultivated aquatic food development. Images of mackerel cells are provided in Fig. 3.

Scaffolds and tissue engineering

While suspension culture-based approaches may be sufficient for unstructured seafood products like surimi, tissue-like products that replicate some of the complexity of muscle tissue, including texture/mechanics and mouthfeel after cooking and oral mastication, will require more sophisticated methods to impart structure to the final product [53]. A variety of approaches are utilized that mainly rely on scaffolds to facilitate the transport of oxygen, nutrients, and waste products as tissues mature (Table 4). Approaches to scaffolding and tissue engineering for cell-cultivated meat have been reviewed elsewhere [54–57]. The differences in requirements for scaffolds for seafood vs. terrestrial meat can be divided into two broad categories: those related to the cell requirements and those related to the effects of the scaffold on the organoleptic properties of the final product. Because scaffolds play a crucial role in delivering cues to the cells as they proliferate, differentiate, and mature, scaffolds that are appropriate for use with cells from one taxonomic group may not be optimal for another. Therefore, optimization of scaffold stiffness [58], topography [59–61], or surface functionalization [62] may require significant differences between terrestrial animals, fish, and aquatic

invertebrates. Scaffolds can also impact the acceptability of the final product due to texture, taste and flavor. For example, the melting temperature of fish collagen differs from that of collagen from terrestrial animals, with important impacts on cooking fish muscle [63], thus, the thermal properties of scaffolds for cell-cultivated seafood will need to be carefully considered. In addition, the 3D geometry of muscles from terrestrial animals, fish [63], crustaceans [64, 65], and mollusks [66, 67] are different and need design considerations with scaffolds for whole-cut cell-cultivated products.

One of the earliest investigations into cell-cultivated meat or seafood was a NASA-funded study that demonstrated the in vitro expansion of goldfish muscle explants co-cultured with brown bullhead fibroblasts [9]. While research into cell-cultivated seafood over the subsequent two decades has lagged behind that of cell-cultivated terrestrial meat, several recent studies have demonstrated progress.

While this is an early example of a scaffold-free cell-cultivated seafood prototype, there is precedent for the use of scaffold-free techniques in both academic [68, 69] and commercial [70] efforts at producing cell-cultivated terrestrial meat, but less so for seafood-related goals. Recent advances with scaffold-free alternatives were reported for livestock-derived adipocyte cell cultures in 2D, that could also be applied to seafood cell cultures; the 2D systems were consolidated into 3D tissues via post-cell growth aggregation using food grade cross-linking enzymes like transglutaminase or a gelling agent (e.g., alginate) [71].

The use of 3D bioprinting to produce cell-cultivated meat products has been a focus [72] due to the level of control over structure, and this strategy was also applied for the formation of cell-cultivated large yellow croaker (*Larimichthys crocea*) prototypes by printing with a bio-ink consisting of gelatin, alginate, and primary croaker satellite cells into a tissue-like structure [73]. Microcarriers (MCs) as scaffolds in suspension are also utilized towards cell production goals and scalability in cell-cultivated seafood production, providing large surface/volume ratios. These can have a temporary role or become part of the final product when developed from edible sources [74]. Cells grown in the 2D environment inside or on the surface of the MCs can provide a smooth transition from flasks and bioreactors to finalized 3D tissue outcomes [74]. Different types of marine polymers could be used for cell-cultivated seafood including hydrogels from algal sources, chitosan extracted from marine exoskeletons, and gelatin from underutilized species such as jellyfish, fish skin and seafood byproducts, which can also provide specific colors and flavors. In addition, extracellular matrix proteins and lipids can be integrated into the process via scaffolds and can have a significant role in the sensory and textural properties of fish meat. A recent study illustrated that some of the established lipid structure approaches, such as oleogels, could be integrated with cells cultured on microcarriers to form 3D structures simulating meat products [73]. These approaches of combining structured fats with fibrous tissue scaffolds could enable the development of muscle-like fish products, however, there have been few studies reported in the literature to date. Cellular aggregates as self-scaffolding outcomes can also be pursued as a robust option for increasing biomass.

Scale-Up

Scaling-up using bioreactors for the 3D cell production environment is a major bottleneck for the cell-cultivated meat industry. Most approaches being pursued are based on variations with stirred tank bioreactors derived from pharmaceutical industry designs, with a focus on cost reductions via simplified designs or those requiring lower energy impacts. These systems apply to cultivated meat and seafood alike. Other approaches generally being pursued in the field include hollow fiber-based bioreactors. In all cases, the costs of scaling are related to media, microcarriers, clean rooms, bioreactor hardware and labor. Innovative approaches will be required to reduce the cost of scaling up. For example, there are many unutilized nutrients and growth factors, which could be recovered and returned to the bioreactor after removing cell metabolites. This could be achieved using different approaches such as growing plants on the spent media to generate additional biomass for use in the production process, utilizing microbial communities for metabolic support to reduce inhibitor byproducts, along with more traditional selective membranes to isolate, recover and re-use key growth factors. Reductions in ammonia can be pursued using microorganisms and chemicals, which can help sustain cultures with reduced media changes or specific nutrient feeding. Glutamine substitutes including α-ketoglutarate (αKG), glutamate (Glt) and pyruvate (Pyr) had a positive impact on cell proliferation and differentiation by reducing the rate of ammonia production [75]. For instance, proliferation media containing αKG improved primary bovine fibro-adipogenic progenitor cell proliferation, while significantly reducing ammonia production rate due to the antioxidative and ammonia scavenging properties of αKG [75].

Food safety

In the US, both the Food and Drug Administration (US FDA) and the United States Department of Agriculture (USDA) have established a joint agreement to address cell-cultivated meat and seafood safety and regulations. The FDA oversees cell collection, cell banks, cell growth and differentiation for all the seafood organisms, while

the USDA/Food Safety and Inspection Service (FSIS) evaluates the products after harvest onwards for catfish. Codex Alimentarius also recently initiated programs on developing Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices (GMP) for cell-cultivated meat and seafood.

Complementary to regulatory organizations, the Food and Agriculture Organization (FAO), and the World Health Organization (WHO) developed the first comprehensive food safety document that covers cell-cultivated seafood [2]. This document outlines the food safety risks including zoonotic risks from cell lines and the production environment, biological contamination risks from initial cell sources to production, and risks from unwanted residues and novel inputs during production and processing of cell-cultivated meat products. These risk factors are combined with a food safety plan to address the challenges and regulatory requirements of both the FDA and the USDA along each step of cell-cultivated seafood production (Fig. 4). Critical Control Points are biological, chemical, allergen and physical issues that need to be used for developing preventive controls.

In the cell culture environment, bacteria can rapidly outgrow the animal cells, with additional hazards from other organisms including viruses, prions, fungi, protozoa and parasites. *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Aeromonas hydrophyla*, *Vibrio spp.*, and *Mycoplasma spp.* are some of the most common bacterial contaminants in foods. Chemicals may be added intentionally or unintentionally to the production process and can pose food safety risks. These chemicals include antibiotics, drugs, sanitizers, cryoprotective agents, leachable chemicals (including plasticizers), surfactants, and anti-foaming agents. The leachable chemicals can originate from sensors and piping in the production process. There are approved chemicals listed by FDA that can be used for cell culture, but for new production processes these potential contaminants will need to be tracked. Physical hazards include objects, debris, plastics, and microplastics.

A major issue with seafood is the allergens, with different types of proteins and allergens in fish and shellfish. For example, the major allergens in fish are parvalbumins, while in shellfish, tropomyosin, arginine kinase, and myosin light chain are the main allergens [76]. Cellular aquaculture has the potential to reduce allergenicity in seafood by selectively growing specific cell types (e.g., myogenic and adipogenic cells) to avoid allergenic components. This can also be achieved through genetic modifications, such as using RNA Interference (RNAi) techniques to knock out causative genes [77]. Additionally, the incorporation of food-grade additives like creatine or ethylenediamine tetra-acetic acid (EDTA) into the cell culture media may offer a route to address allergen-related issues by modulating the expression of parvalbumin, thereby reducing allergenicity [78].

Regulations

Cell-cultivated seafood industries need to comply with preventive controls rules established by the Food Safety Modernization Act (FSMA). According to the FDA, "Generally, domestic and foreign food facilities that are required to register with Sect. 415 of the Food, Drug, & Cosmetic Act must comply with the requirements for risk-based preventive controls mandated by the FDA FSMA as well as the modernized Current Good Manufacturing Practices (CGMPs) of this rule (unless an exemption applies)". Traditionally, the conventional seafood industry is regulated by the FDA, except for catfish

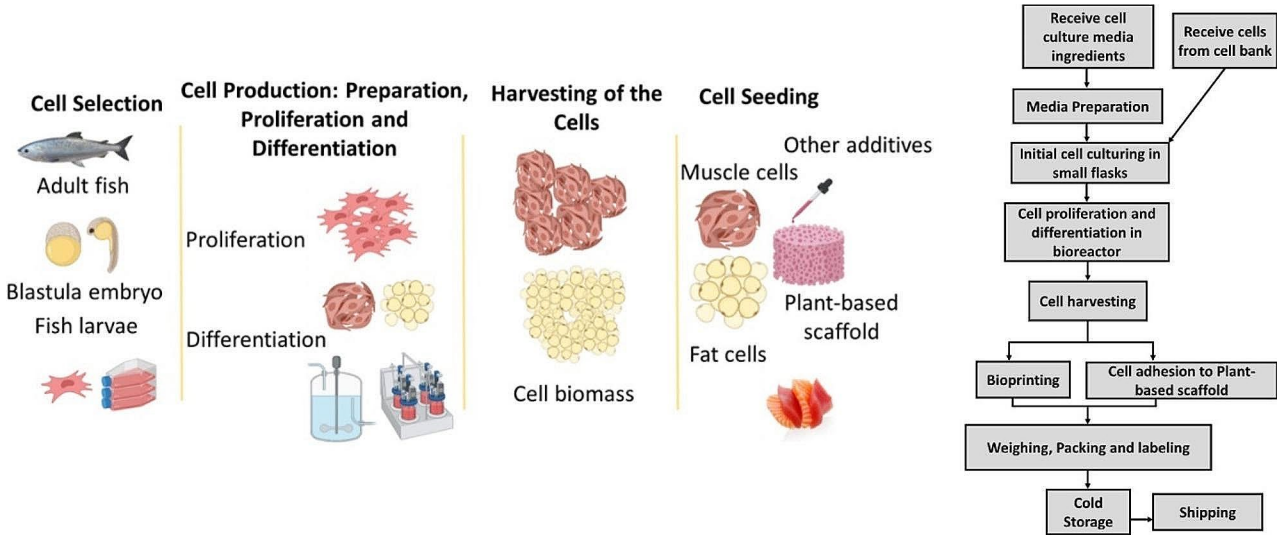

Fig. 4 (**A**) Cell-cultivated seafood production steps; (**B**) Cell-cultivated seafood production critical control points

(*Siluridae*), which along with meat products are regulated by USDA [79].

Cell-cultivated seafood production is considered a novel or alternative food production system. Thus, labeling is also an important part of the regulations for food products. Developing a common terminology to increase transparency is required for clean labeling. There was a comprehensive study for seafood products indicating that two "common or usual names," "Cell-cultivated Seafood" and "Cell-Cultured Seafood," met regulatory criteria [80]. By displaying these two phrases on packages of frozen Atlantic Salmon, both "Cell-cultivated" (60.1%) and "Cell-Cultured" (58.9%) enabled participants to differentiate cell-cultivated seafood from "Farm-Raised" and "Wild-Caught" fish [80].

There is a need to develop reliable test kits and rapid detection sensors to validate the safety of cell-cultivated seafood products. Testing methods are essential for assessing allergenicity in seafood products, including those produced through cellular aquaculture. These methods need to encompass not only the cultured cells themselves but also the biomaterial scaffolds employed in the process [81, 82]. *In silico* assessments can determine sequence homologies and identify structural similarities of newly expressed proteins to existing allergenic examples [83] while other testing methods approved by the EFSA and the FDA for allergenicity verification include the pepsin resistance test and immunochemical cross-reactivity testing with Immunoglobulin E (IgE) from the serum of allergic individuals [84, 85].

Traceability of cell-cultivated seafood will also be a major topic as is the case with conventional meat products. The conventional seafood industry is highly fragmented with very little connection from the point of harvest to the point of consumption. In contrast, cell-cultivated seafood could be easily traced back to the source of production.

Socioeconomics

One concern with cell-cultivated seafood is that in the future, by developing this novel food production system, the declining need for animals, including fish and crustacea, could negatively impact the fishing industry and the associated communities [86]. However, cell-cultivated seafood is strategically positioned to complement traditional methods like wild-caught species and aquaculture farming, to support sustainability of these communities well into the future. Moreover, the capacity to harvest and culture cells from unconventional seafood sources provides new possibilities for these communities, simultaneously enriching food choices available to consumers. Figure 5 summarizes some of the benefits and challenges/concerns associated with the cell-cultivated seafood industry.

Industrial scale

Businesses involved in cell-cultivated meat, including seafood, have been gaining significant importance across the globe, reflected in investments of about $2.8 billion since 2016 among 156 companies dedicated to

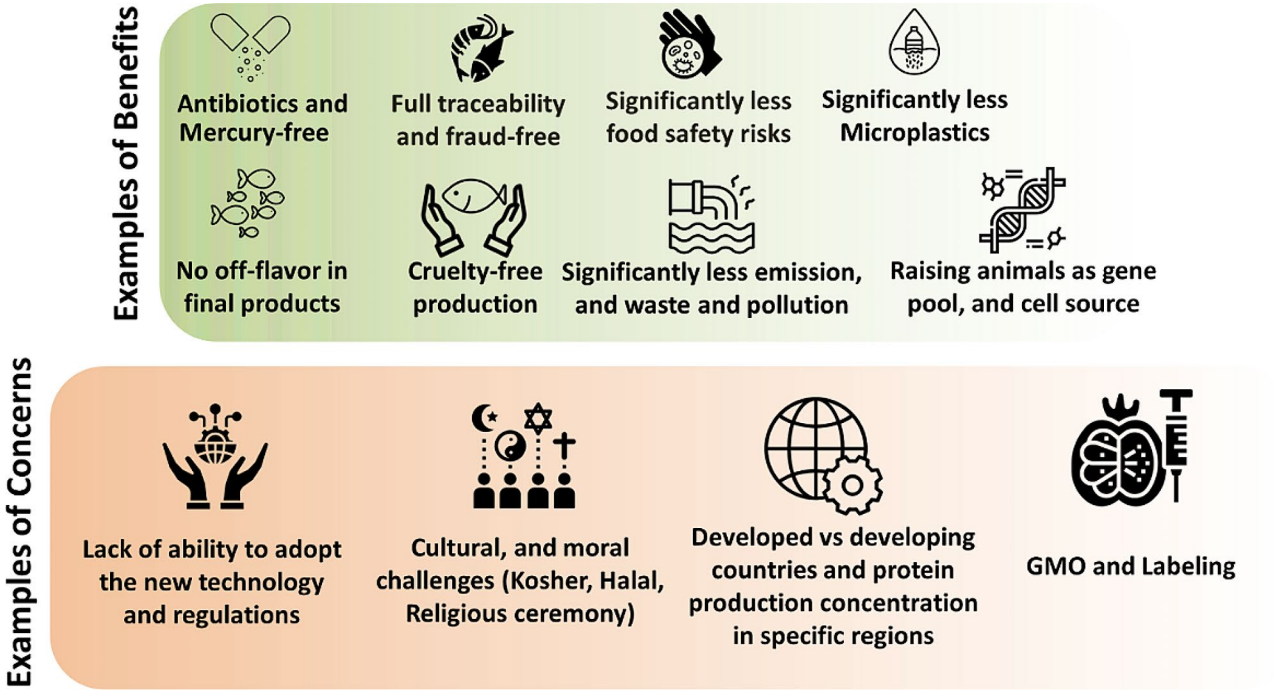

Fig. 5 Potential societal impacts of cell-cultivated seafood [86–88]

cell-cultivated meat and seafood production [89]. Cell-cultivated seafood is an important niche within the cell-cultivated protein sector [90] with industrial investment of $896 million for cell-cultivated meat and seafood [91] with many startups and established companies pursuing cell-cultivated seafoods in 2022. This includes companies in the US, Singapore; Europe; Canada; South Africa; Israel, South Korea, Hong Kong and India. The majority of companies are focused on business-to-consumer (B2C) and business-to business (B2B), with fewer companies in the B2B business model space [89]. Supply chain issues of cell-cultivated seafood will also need to be addressed as the market expands. The market potential for cell-cultivated seafood remains an unknown at the early stages, with price being one of the determinants. Costs are expected to decrease with cheaper ingredients and with scaling, but this has to be demonstrated in the coming years [92].

Transformative potential of cell-cultivated seafood

Cell-cultivated seafood as a technology offers a potentially transformative impact for foods of the future. This is based on the scientific tools now available, coupled with the features of the technology itself. For example, the potential to directly alter cell composition (e.g., fatty acid profiles) to provide healthier seafood products is compelling (see omega-3 example below). This impact can be further enhanced pending the acceptability of GM-based approaches, where seafood cells can be bioengineered to provide even further nutritional and perhaps even therapeutic benefits. Food safety can also be greatly enhanced, as shelf life, microbial community, tracking, and overall freshness can potentially be improved, along with a major reduction in antibiotic use [93, 94]. All of these potential benefits remain to be demonstrated as the field moves forward, but the underlying science to achieve such goals is already in place. In addition, improved food security, food access, novel foods and many other future outcomes can be anticipated.

Nutrition - Omega-3s and other inputs - Although fish are recognized as one of the best sources of nutritionally-important long-chain omega-3 fatty acids, the source of these compounds is actually the marine algae, bacteria, and protists. Fish consume these organisms either directly or indirectly via other fish or zooplankton, thereby bioaccumulating omega-3 fats in their tissues [95]. The fact that animal cells—including those of fish and aquatic invertebrates—are incapable of synthesizing omega-3 fats *de novo* means that producers of cell-cultivated seafood will need to acquire appropriate sources of omega-3 fatty acids as ingredients. These sources could include farming of microalgae [96], precision fermentation [97, 98], plant molecular farming [99, 100], or cell-free systems [101]. However, this latter strategy has not yet been explored for omega-3 production to our knowledge, and the former three strategies will still require substantial effort before they can be scaled to the levels that may be required to support the cell-cultivated seafood industry. Cellular engineering approaches could also provide an opportunity to engineer fish cells to synthesize long-chain omega-3 fatty acids. Codon-optimized transgene expression of omega-3 desaturase gene (*fat1*) of *C. elegans* in a fish cell culture and zebrafish model enhanced the conversion of n-6 PUFA to n-3 PUFA [102]. This study also illustrated that combined transgene expression of *fat-1* and *fat-2* enhanced the synthesis of n-3 PUFA [102]. In addition, cellular engineering may provide a potential solution to enhance the accumulation and stability of omega-3 fats. These approaches may include the use of exogenous reactive oxygen scavengers in the media to promote cell proliferation and suppress oxidation processes [101], as well as genetic modifications to over-express antioxidant genes, such as superoxide dismutase (SOD). Furthermore, cellular engineering approaches also enable the design of media compositions to promote the synthesis of omega-3 fats [103].

Other compounds with important impacts on nutrition and organoleptic properties of seafood are also ultimately derived from the diets of aquatic animals. This includes the carotenoid astaxanthin, which is responsible for the color of salmon and shrimp, as well as for protecting membrane lipids from oxidation [104]. As is the case with omega-3 fats, astaxanthin and other compounds that are diet-derived in conventional seafood will need to either be sourced as ingredients for addition to cell-cultivated seafood or synthesized by engineered cells. Notably, the U.S. government recently acknowledged the need to "bolster research into alternative feed ingredients for livestock and aquaculture, including plants, algae, or seaweeds, that can enhance or replace feed ingredients" [105]. Marine-derived feed ingredients such as omega-3s and astaxanthin may be a shared need across both conventional and alternative protein production platforms.

Roadmap and conclusions

Cell-cultivated seafood is in its infancy. There is growing research among academic labs, and a growing corporate effort mainly among startup companies worldwide to tackle the increasing consumer demand for seafood. In these early stages, the focus is on cell sources, media optimization and scaffolding, while with time these efforts will mature into scaling production for impact. With scale, pricing will be reduced and availability will increase. The vision is that this emerging approach to cell-cultivated seafoods will offer safer and healthier alternatives for consumers, while enhancing environmental sustainability goals (Fig. 6).

Fig. 6 Roadmap for the development of the cell-cultivated seafood industry. High-level research and commercial priorities are indicated based on the approximate timing with which they are likely to be high priorities for the field. MC: microcarrier, STB: stirred-tank bioreactor, HFB: hollow fiber bioreactor

For this growing industry to reach its potential, government support for research and commercialization efforts will be essential. A report by the UK Foreign, Commonwealth & Development Office and the ClimateWorks Foundation estimated that annual global public spending on R&D and commercialization—including that of plant-based proteins, precision fermentation, insects, and cell-cultivated meat—would need to increase to a total of US $10.1 billion to unlock the full benefits of alternative proteins [106]. Whereas terrestrial cell-cultivated meat benefits from a strong foundation of biomedical tissue engineering research, and a fairly detailed understanding of mammalian and avian cell biology generally, this is less true for cell-cultivated seafood. Therefore, basic research aimed at understanding piscine and invertebrate cell types, differentiation processes, and metabolic requirements is still needed. Public funding of such research will reduce duplication of effort and provide a strong foundation for commercial efforts, thereby benefiting the field as a whole, everyday consumers, and the planet. Universal in this evolution to grow cell-cultivated seafood as a major option for alternative food for consumers around

the world, safety, flavor and texture are paramount. Thus, regulations and methods to properly assess these new foods and to provide tracking will be a foundational need. In total, the potential for this emerging field to transform the seafood that we consume, while providing major benefits to sustainability, quality and food safety are expected to continue to drive the growth of this field. Both fishing and aquaculture already face major environmental challenges, and cell-cultivated seafood offers a new approach to address these issues, while also expanding our palates in ways never before possible [107, 108]. The future is exciting, but the path will need to be built upon a strong scientific foundation linked to consumer willingness to try these new foods and eventually to embrace them.

Acknowledgements
We thank the USDA (2021-69012-35978) for support. The authors express their sincere gratitude to Good Food Institute, USA for financial assistance and DG, Indian Council of Agricultural Research and Director, ICAR-Central Institute of Fisheries Education, Mumbai, for the support.

Author contributions
MG: wrote, reviewed and edited the review paperRO: wrote, reviewed and edited the review paperDK: wrote, reviewed and edited the review paperCB: wrote, reviewed and edited the review paperMP: wrote, reviewed and edited the review paperWL: wrote, reviewed and edited the review paper.

Funding
We thank the USDA (2021-69012-35978), the Good Food Institute, USA, and the DG, Indian Council of Agricultural Research and Director, ICAR-Central Institute of Fisheries Education, Mumbai, for support.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
not applicable.

Consent for publication
not applicable.

Competing interests
The authors declare no competing interests.

Received: 2 June 2024 / Accepted: 8 July 2024
Published online: 07 August 2024

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