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Review Article

Fish Cell Cultures - Uses and Prospects

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ABSTRACT

Fish cell cultures are employed in diverse research fields such as virology, physiology, toxicology, immunology, genetics, and pharmacology. These systems can be utilized for pathogen detection, confirmation, propagation, and characterization, especially of viruses. Cell cultures are also utilized in the case of intracellular bacteria, *Myxosporean*, or *Microsporidian* parasites. Fish cell cultures have gained more popularity in recent years and have prominent roles as model systems and in the large-scale production of biologicals. The recent swift growth observed in research employing cell cultures is definitely an outcome of the progress in this sector and also due to increasing ethical demands for reduction and replacement of animals used in research. *In vitro* fish cell cultures are excellent research models in simulating host animal *in vivo*. The diverse applications of fish cell cultures in various research fields are attributed to their versatility, cost-effectiveness, convenience in handling, and ease in genetic manipulation. For several infectious viral diseases, as therapeutic options are limited, early disease diagnosis and prophylactic measures are crucial for efficient fish health management. In this scenario, a better understanding of the viral pathogenesis and mechanisms utilizing *in vitro* cell lines are essential to facilitate disease management strategies such as vaccines and antiviral agents. Moreover, host preferences of pathogens, virus-host cell interactions, and virus localization can also be studied using cell cultures. Availability of host-specific or host-susceptible fish cell cultures is very limited, which is a major concern in this area. In near future, innovations in 3D cell culture, stem cells, and genome editing will further enhance the research prospects of fish cell cultures.

Keywords: Fish cell culture; *In vitro*; *In vivo*; Model systems; Virus

INTRODUCTION

Culture of cells in a controlled environment is referred to as cell culture. The versatility, cost-effectiveness, and high potential of cell cultures facilitate their use in diverse research fields [1-3]. In addition, cell cultures are utilized in the mass production of commercially relevant biologicals.

Fish cell cultures have gained more popularity in recent years due to progress in this sector and increasing ethical demands for the reduction, replacement, and refinement (the 3 R's) of animal use in research [4-6]. Different animal activist groups, environmental agencies, and cosmetic industries are also insistent in reducing animal testing and promote alternate *in vitro* models. In addition to avoiding the social and ethical concerns of animal use in research and several regulatory concerns, fish cell cultures offer multiple advantages such as easy dosing of drugs, reproducibility, rapid test results, and economical feasibility [7-11].

The acceptance of fish cell lines in various research disciplines is due to their ease of generation, maintenance, potential for genetic manipulation, quantification, characterization, and cryopreservation for future applications [12,13]. Fish-derived cells can be cultured in a wide range of temperatures and osmolarity conditions and are easily adjusted to bicarbonate buffered media [14-18]. In addition, the methodology for fish cell culture is more or less similar to that for terrestrial vertebrates. Also, fish cells can be maintained for longer periods of time due to lower metabolic rates [15,16].

Both primary (cells isolated directly from the host tissue) and established cell cultures (immortal) are used in research (Table 1). Primary cultures are physiologically closer to the host and thereby serve as an appropriate model [19]. In comparison, established cell lines are cancerous and might have lost normal host physiological properties [19-25]. A list of fish cell lines available from ATCC (American Type Culture Collection) and ECACC (The European Collection of Authenticated Cell Cultures) including the species of fish and tissue of origin is provided (Table 2). Cell cultures of marine fish and invertebrate origin (Tables 3 and 4) are also provided.

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Table 1: Characteristics of primary cell cultures and established cell lines [24,25,28,47,154-156].

Primary Cell Cultures	Established Cell Lines
Derived directly from host tissues/tumors. Can be initiated either as explants or from enzymatically dissociated cells. Closely represent host tissue, physiologically more similar to *in vivo* cells. No authentication required prior to use.	Cell transformation can be spontaneous or induced by viral oncogenes, chemicals and radiation. Can be developed from tumor tissues.
Finite life span, undergo senescence after a definite number of cell divisions. Usually 5-20 divisions.	Lost many properties of the parental cell tissue, less preferred as a biologically relevant option, authentication required before use.
Minimal adaptation to culture media, slow growth rate, high serum requirement, shows (susceptible to) contact inhibition.	Infinite lifespan, immortal as neoplastic cells, proliferate indefinitely given appropriate culture conditions.
Retention of cell identity, tissue-specific functions.	Completely adapted to culture, rapid growth rate, reduced serum dependence, absence of contact inhibition.
Normal number of chromosomes	Loss of cell specificity/ identity (due to high mutations and clonal selections).
Heterogeneous cell population, possibilities to study cells with varied donor characteristics.	Aneuploidy chromosome
Initiated from a broad range of tissues and fish spp. allowing to study species-specific responses. There appears to be a correlation between the maximum number of passages and aging.	Homogenous/clonal cell population, stop expressing tissue-specific genes.
Development is more difficult compared to established cell lines. Expensive to maintain.	Generated from cells with high mutations and clonal selections. Shows genotypic and phenotypic drift.
Cell density might be a limiting factor	Cells have overcome the Hay flick's limit. Shows phenotypic alterations from donor tissues/cells.
Potential to harbor resident pathogens	Less expensive, easy to handle and manipulate genetically; can be cryopreserved indefinitely.
Necessity to isolate cells for each experiment	Fast growth and provision of higher cell density
	Can harbor mycoplasma, usually free of microbes
	Reproducible and convenient source of cells

Table 2: List of cell lines developed from warmwater and cold-water fish species and currently available from American Type Culture Collection (ATCC) and the European Collection of Authenticated Cell Cultures (ECACC) [157-159]. This is not an exhaustive list.

Fish Type & Family	Species of Origin	Cell Line	Growth Mode
Warmwater fish			
Cyprinidae	*Danio rerio* (zebra fish)	ZF4	Adherent
	Danio rerio (zebra fish)	ZEM2S	Adherent
	Danio rerio (zebra fish)	AB.9	Adherent
	Danio rerio (zebra fish)	SJD.1	Adherent
	Pimephales promelas (fathead minnow)	FHM	Adherent
	Pimephales promelas (fathead minnow)	EPC	Adherent
	Cyprinus rubrofuscus (Koi carp)	KF1	Adherent
	Cyprinus carpio (Common carp)	CCB	Adherent
	Cyprinus carpio (Common carp)	CLC	Adherent
	Carassius auratus (Goldfish)	CAR	Adherent
Poeciliidae	*Poeciliopsis lucida* (live bearer)	PLHC-1	Adherent
Clariidae	*Clarias batrachus* (walking catfish)	G1B	Adherent
Ictaluridae	*Ictalurus punctatus* (channel catfish)	G14D	Suspension
	Ictalurus punctatus (channel catfish)	3B11	Suspension
	Ictalurus punctatus (channel catfish)	28S.3	Suspension
	Ictalurus punctatus (channel catfish)	42TA	Suspension
	Ictalurus punctatus (channel catfish)	1G8	Suspension
	Ictalurus punctatus (channel catfish)	CCO	Adherent
	Ictalurus nebulosus (brown bullhead)	BB	Adherent
Centrarchidae	*Lepomis macrochirus* (blue gill)	BF-2	Adherent

Moronidae	*Morone chrysops* (White bass)	WBE	Adherent
Cichlidae	*Oreochromis mossambicus* (Tilapia)	OmB	Adherent
Channidae	*Channa striatus* (Striped snakehead)	E11	Adherent
	Channa striatus (Striped snakehead)	SSN-1	Adherent
Tetraodontidae	*Fugu niphobles* (Grass puffer fish)	Fugu fry	Adherent
Cold-water Fish			
Salmonidae	*Oncorhynchus mykiss* (Rainbow trout)	RTgill-W1	Adherent
	Oncorhynchus mykiss (Rainbow trout)	RTH-149	Adherent
	Oncorhynchus mykiss (Rainbow trout)	RTG-2	Adherent
	Oncorhynchus mykiss (Rainbow trout)	SOB-15	Adherent
	Oncorhynchus mykiss (Rainbow trout)	RTG-P1	Adherent
	Salmo salar (Atlantic salmon)	ASK	Adherent
	Oncorhynchus kisutch (Coho salmon)	CSE-119	Adherent
	Oncorhynchus tshawytscha (Chinook salmon)	CHH-1	Adherent
	Oncorynchus nerka (Sockeye salmon)	SSE-5	Adherent
	Oncorhynchus tshawytscha (Chinook salmon)	CHSE-214	Adherent
	Oncorhynchus mykiss (Rainbow trout)	STE-137	Adherent
	Oncorhynchus mykiss (Rainbow trout)	TPS	Adherent
	Oncorhynchus keta (Chum salmon)	CHH	Adherent
Esocidae	*Esox lucius* (Northern pike)	PG	Adherent

Table 3: Cell lines of marine fish origin [116,140,142,143]. The cell lines (SAF, SaBE-1c) are currently available from the cell culture repositories.

Family	Species of Origin	Cell Line	Growth Mode
Gadidae	*Melanogrammus aeglefinius* (Haddock)	HEW	Adherent
Sparidae	*Pagrus major* (Red sea bream)	SBES1	Adherent
	Sparus aurata (Gilthead seabream)	SAF	Adherent
	Sparus aurata (Gilthead seabream)	SaBE-1c	Adherent
Paralichthyidae	*Paralichthys olivaceus* (Olive flounder)	OFEC-17FEN	Adherent
Scophthalmidae	*Scophthalmus maximus* (Turbot)	TEC	Adherent
Lateolabracidae	*Lateolabrax japonicus* (Japanese sea bass)	LJES1	Adherent
Pomacentridae	*Amphiprion ocellaris* (Ocellaris clownfish)	OCF	Adherent
Gadidae	*Gadus morhua* (Atlantic cod)	GML-5	Adherent

Table 4: Cell lines originated from aquatic invertebrates. These are not currently available from the cell culture repositories.

Family	Species of Origin	Cell Culture	Growth Mode
Nephropidae	*Homarus americanus* (American lobster)	Olfactory sensory neurons (Primary culture)	Adherent
Palinuridae	*Panulirus argus* (Caribbean spiny lobster)	Hemocytes (Primary culture)	Adherent
Ostreidae	*Crassostrea gigas* (Pacific oyster)	Heart tissue (primary culture)	Adherent
Planorbidae	*Biomphalaria glabrata* (freshwater snail)	Bge	Adherent
Penaeidae	*Penaeus monodon* (tiger shrimp)	PmLyO-Sf9	Adherent
	P. monodon (tiger shrimp)	PMO	Adherent
Portunidae	*Scylla serrata* (giant mud crab)	Testicular tissue (# no designation)	Adherent

Applications of fish cell cultures

Rainbow Trout Gonad (RTG2) was the very first fish cell line to be developed and used for virus studies [26]. Subsequently, several fish cell lines were established from different fish species and employed in diverse research fields including immunology, toxicology, genetic engineering, genetic regulation, gene expression studies, endocrinology, biomedical research, disease control, biotechnology, biomedical research, and radiation biology [9,26-35]. With the growing concerns for animal welfare, there is more than ever pressure to find alternatives for animal use in research, and cell cultures could be the perfect substitute. Some of the applications of fish cell cultures are given below.

Model systems: Since *in vitro* cell cultures mimic the host animal *in vivo*, fish cell cultures act as excellent research models. Also, these are not subjected to interference from environmental disturbances to which animals are sensitive. On the other hand, genetic manipulations of the cells can be easily achieved to study differential expression of genes and or proteins. Consistency and reproducibility of results are added advantages. Cell cultures

have been increasingly used as model systems to study basic cell biology, physiology, cellular communications, signaling pathways, expression profiling, apoptosis, interactions between cells and pathogenic agents, effects of drugs, metabolic effects of nutritional elements, and mutagenesis. They are also important model systems in embryology, neurobiology, endocrinology, and environmental biology. Consequently, cultured cells are vital for the identification of specific molecules and/or mechanisms used in initial pathogen-host cell interactions. For example, the macrophage cells from tilapia gill were used to investigate the attachment of pathogens during infection [36]. Ease of manipulation and homology with functional genes engaged in human diseases make zebra fish cell lines, a potential *in vitro* model to study diseases as well as cellular processes [37-40]. Many fish-derived cell lines were used to explore the field of fish endocrinology [29,41,42].

Virology: Being obligate intracellular parasites, viruses require host cell machinery for replication and propagation. Cell cultures are considered 'the gold standard' due to their diverse roles in virology such as detection, identification, propagation, isolation, confirmation, and characterization of viruses [43-45]. Due to the relevance of cells in virology, the OIE (Office International des Epizooties) protocols require cell cultures, in viral disease diagnosis and confirmation. Fish cell cultures can function as an effective replacement for animals, especially in the field of virology [46-49]. Cell cultures can be reliable sources of viruses when compared to the uncertainties associated with obtaining viruses from infected animals for research purposes [50,51].

Susceptible cell lines are essential to determine the detailed etiology of viruses as evidenced in the case of Infectious Pancreatic Necrosis (IPN) and Infectious Hematopoietic Necrosis (IHN) viruses [46,49]. For the emerging fish viruses, the infectious cycle, mode of infection, pathogenicity, potential host range, and viral replication inhibition strategies need to be determined for establishing comprehensive management approaches. Since treatment options are limited for many viral diseases, early disease diagnosis and proactive management measures are key for successful fish health management.

Many fish cell lines have been established for the detection and isolation of fish viruses and are valuable for studying species-specific responses to viral infection at the cellular level. Cell cultures are an integral part of verifying River's postulates to establish the causative agent of a disease as a virus [52]. Replication and propagation of virus in the host cells result in Cytopathic Effects (CPEs) (Figure 1). Cell cultures are also increasingly utilized to determine cellular translocation and localization of viral proteins during acute and chronic infections. For example, the Viral Hemorrhagic Septicemia Virus (VHSV) was detected in Fat-Head Minnow (FHM) cell line by using RNA probes targeting viral transcripts.

Cell lines of aquatic invertebrate origin are scarce (Table 4). Some of the finfish cell lines are used to study viruses isolated from molluscs and crustaceans. For example, Akoya virus infecting pearl oysters was cultured in Eel Kidney (EK-1) and EPC cell lines [53]. Bluegill Fry (BF2) cell lines were used to propagate a reo-like virus isolated from juvenile American oyster, *Crassostrea virginica* [54].

Virus isolation relies on the availability of permissible cell cultures. In this context, suitable fish cell cultures for the propagation of viruses and disease diagnosis are very limited, which is a major concern in this area. Host and tissue-specificity of viruses necessitate the development of cell cultures from appropriate hosts and from different tissues to represent diverse cell types (epithelial cells, fibroblast cells, etc.). For example, ictalurid herpesviruses, cyprinid herpesviruses (koi herpesvirus, goldfish hematopoietic necrosis virus, and carp pox virus), salmonid herpesvirus, acipenserid herpesvirus, and walleye herpesvirus are highly host-specific and most of them are refractory to nonspecific cell cultures [55-57]. Species-specific cell cultures are relevant to study the evolving and

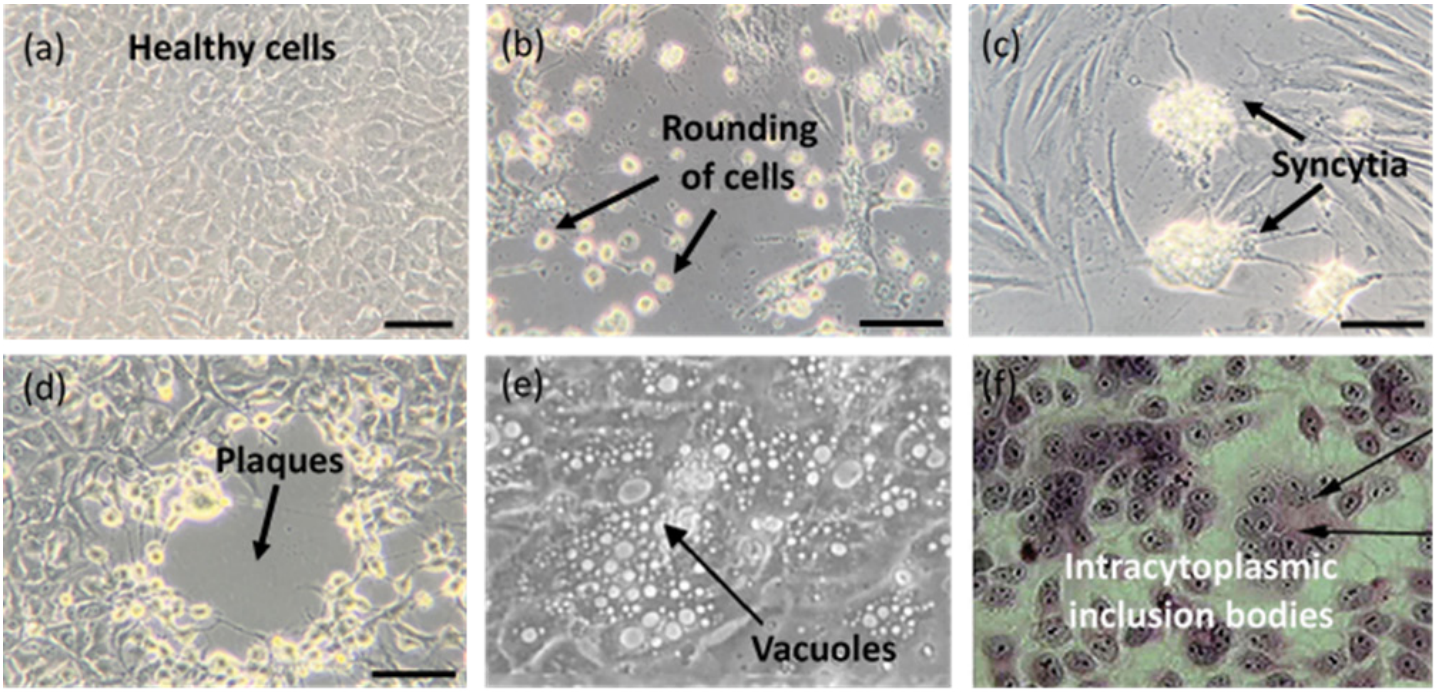

Figure 1: Cytopathic Effects (CPEs) displayed by viruses in susceptible host cell lines. (a) healthy catfish cell line, (b-d) cells infected with catfish viruses displaying CPEs such as rounding of cells, syncytia, plaques, and destruction of cell monolayer, (e) vacuoles in Vero cells caused by vaccinia virus, and (f) intracytoplasmic inclusion bodies in carp cells infected with koi herpesvirus (scale bar-10 μm) [43,44,57,152,153].

infectious fish viruses that affect the aquaculture industry. The unavailability of suitable fish cell cultures hinders investigations on newly emerging unknown viruses [18,58,59].

Attenuated viral vaccines can be developed by repeatedly passing the wild-type virus through susceptible fish cell cultures, which has proven to weaken the virus [60]. Virus attenuation has been achieved in the case of koi herpesvirus vaccine in koi fin cell culture [61] and Channel Catfish Virus (CCV) vaccine in *Clarias batrachus* kidney cell line [60]. Cyprinid Herpesvirus (CyHV3) was attenuated by serial transfer (20 passages) of the virus in Koi fin (KF-2) cells and found to be very effective against CyHV3 infections [61-63]. Attenuation using cell culture systems also avoids undesired recombination, complementation, and reversion to a pathogenic virus as evidenced in previously published studies.

Research on antivirals

Fish cell lines are routinely used for screening antiviral compounds [64,65]. Hao K, et al. [66] reported the efficacy of acyclovir, a common antiviral to treat human herpesvirus infection, against channel catfish virus infection in CCO cells. Acyclovir was also found to exert effective antiviral activity against cyprinid herpesvirus-3 (CyHV-3) infection in Common Carp Brain (CCB) and Koi Fin cells (KF-1) [67]. Exopolysaccharides isolated from the algae *Arthrospira platensis* inhibited KHV replication in CCB cell lines [68]. Similarly, polyinosinic polycytidylic acid (poly I:C) was reported to induce an antiviral state in CHSE-214 cell line against IPNV [69]. Balmer BF, et al. [70] studied the efficacy of a compound against Infectious Hematopoietic Necrosis Virus (IHNV) using EPC cell lines, which was found to hinder viral entry by inhibiting virus-host cell membrane fusion.

Toxicology

Being relevant representatives for the aquatic environment, fish cell cultures function as apt alternative for animals and are extensively used as *in vitro* models for environmental toxicology studies especially cytotoxicity analysis [8,30,71-75]. In addition to avoiding high costs and variability of results; the genotoxicity of chemicals, metabolism, DNA binding, and mode of action can be evaluated [73,76-79]. Fish hepatoma cell lines were found useful to test the xenobiotic efflux activity of human drugs [80]. Fish cell lines were used to evaluate the cytotoxicity of chromium, Polycyclic Aromatic Hydrocarbons (PAH), and aflatoxins [76,77,81,82] using comet assays and or neutral red dye uptake method [80,83-85].

Fish cell cultures were found sensitive to several bacterial or fungal toxins/extracellular products [86-88]. The EPC cell line was found to be a suitable substrate for the study of intracellular antigens and virulence factors produced by *Renibacterium salmoninarum* [89].

Drug screening and development

Cell-based assays have become an inevitable part of the pharmaceutical industry for high throughput screening of potential compounds and to test the cytotoxicity of candidate drugs. Other related applications include dose optimization, drug delivery, drug safety, pharmacology, cellular targeting, pharmaceutical analysis, and quality assurance [31,90]. Fish cell cultures can potentially play an important role in the research and development of drugs aimed to benefit fish and also to identify therapeutic targets such as receptors.

Production of biologicals

Cell cultures allow for the large-scale production of vaccines, interferons, blood clotting factors, monoclonal antibodies (mABs), interleukins, lymphokines, insulin, growth factors, hormones, viruses, enzymes, and anticancer agents [11,73,91-97]. Fish cell lines are less expensive and thus more economical for the mass production of biologicals compared to mammalian cell cultures [98]. Fish cell cultures can act as miniature factories to express substantial quantities of commercially important proteins after being infected with genetically engineered baculoviruses. More than 90% of the mABs are produced using *in vitro* methods due to the ease of culture and less economic consideration compared with the use of animals. Human cell lines are used to produce numerous FDA-approved therapeutic proteins [99]. Similar efforts could be ventured using fish cell cultures.

Genome editing

Cell lines are amenable for genetic modifications. Hence, fish cell cultures are used in knockout studies, where certain genes are inactivated and their effects are traced. The first gene editing using CRISPR-cas9 system in fish somatic cell lines [100] was followed by several such studies [101]. Chinook salmon embryo (CHSE-214) cell line capable of expressing geneticin and hygromycin resistance was generated by knockout technology. Liu Q, et al. [102] reported successful gene editing using gRNA-Cas9 Ribonucleoprotein (RNP) Complex in medaka embryonic cell lines. Gratacap RL, et al. [103] developed protocols for successful CRISPR gene editing in CHSE-214 cell line using lentivirus transduction which could be used to manipulate disease resistance in salmonid species. Chang N, et al. [104] and Hwang WY, et al. [105] successfully carried out genome editing with RNA-guided Cas9 nuclease in zebrafish embryos. Fish cells can be fused with one another and with mammalian cells [106,107]. For example, microcells have been prepared from goldfish RBCF-1 and fused with human cells [108].

Embryonic stem cells

Embryonic Stem (ES) cells are pluripotent (ability to differentiate into any cell type) and used in biodiversity conservation and biotechnology studies [109]. Extensive studies in fish ES have been done in small model fishes, such as zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) due to the convenience in combining embryological, genetic and molecular analysis of vertebrate development [109-112]. Fish ES cell lines are used as a vector for the efficient transfer of foreign DNA into the germ cells of an organism. Hong Y, et al. [113] developed a spermatogonial cell line from the testis of adult medaka fish which produced viable sperm via spermiogenesis. With the hybrid catfish (♀ channel catfish × ♂ blue catfish) production, the blue catfish are sacrificed for sperm collection. Development of a blue catfish spermatogonial cell line could be of potential benefit to the industry. Embryonic germ cell transplantation was successfully used for surrogate production in salmonids [114]. Embryonic cell lines have been established from catfish, Nile tilapia and several marine fish species [115-120].

Cancer research

Normal cells can be transformed into cancer cells using radiation, chemicals, and viruses to study the mechanism and functions of

various carcinogenic chemicals, induction of cellular apoptosis, DNA methylation, histone modifications, tumor suppressor gene expressions, etc. [121]. Fish cell lines are used in cancer biology to study the mechanism of activation of procarcinogens, molecular damage, and DNA repair activity [122]. Fathead Minnow Cells (FHM), goldfish erythrophoromas, and goldfish fibroblast cell lines were used to study the mechanism and activation of procarcinogens and subsequently the damage and repair of genetic materials [29,122].

Parasitology

Several fish cell cultures were used to study the development and pathogenesis of parasites [123-126]. EPC cell line supported the attachment and transformation of various stages of a fish ectoparasite, *Ichthyophthirius multifiliis* [127]. Buchmann K, et al. [128] studied the non-specific response of EPC to encapsulate and degrade the fish parasite *Gyrodactylus derjavini*. Primary cell cultures derived from salmonid fish allowed investigation of the phagocytic activity of microsporidian parasite *Loma salmonae* [129]. Primary cultures of rainbow trout kidney were used to study the comparative development of two microsporidians infecting AIDS patients and salmonid fish [130].

Regenerative therapy

Cell culture systems can produce functional cells or tissue analogues on a large-scale that can be used as replacement tissue or organs [131]. Reconstitution of skin following severe burns is considered the most successful application of cell-based regenerative therapy. In this regard, fish cell cultures are experimentally utilized for producing artificial skin to treat patients with burns and ulcers.

Three-dimensional cell cultures

Since cells in 3D systems interact with their surroundings in all three dimensions; these models are physiologically similar to *in vivo* conditions and provide more reliable data. The 3D spheroids of rainbow trout (*Oncorhynchus mykiss*) cell lines, RTG-2 and RTS-11 were successfully developed and tested for their efficiency to propagate *Saprolegnia parasitica* spores that resembled *in vivo* infection [132,133]. The 3D cell cultures raise the possibility for the study of complex physiological processes *in vitro*.

Cell-based fish

Cell culture systems can function as an innovative way of animal-free production. Considering the adaptation of fish cell culture to *in vitro* growth conditions in terms of tolerance to hypoxia, high buffering capacity, and low-temperature, an advanced approach towards the sustainability of global fishery resources is the production of cell and tissue culture-based seafood through bioreactor culture [134-136]. Benjaminson MA, et al. [137] used tissue engineering for the *in vitro* culture of skeletal muscle of goldfish that resembled the fillet from a fibroblast fish cell line to use in space travel.

Other uses

A recent study by Morin G, et al. [138] revealed the nutritional-research capabilities of fish cell lines. Another study by Lescat L, et al. [139] used fibroblast cell line from medaka fish (*Oryzias latipes*) to demonstrate that chaperone-mediated autophagy (CMA) pathway involving lysosomal proteolysis exists in fish, which was thought to be present only in mammals and birds. This study was a breakthrough in fish metabolism and provided insight into the evolutionary relationship of vertebrates including fish, mammals, and birds. The potential utility of fish cell lines for transgenic and genetic manipulation studies was identified from the fluorescent signals produced, when transfected with pEGFP vector DNA [86,140-144].

Toxins produced by fish species such as chimeras, sharks, sting-rays, silurid catfish, and surgeonfish, stone-fish, and rabbitfish exhibit enzymatic, antimicrobial, cytotoxic, hemolytic, cardiovascular, neuromuscular, and anti-cancerous properties and have pharmacological and therapeutic applications [145-147]. Maintenance of venom gland organoids via 3D technology can be used to produce venom for use in biomedical research [148].

While the applications of cell cultures are numerous, one must be mindful of the disadvantages as well. Cell lines are prone to genotypic and phenotypic drift [149]. Another concern is misidentification or cell line cross-contamination [150]. Apart from these, several biological pathways cannot be represented by cell line, which limits their use in certain research areas. Primary cells and cell lines could show variability in drug dose, thus the data acquired through cell lines need to be adjusted or cannot easily be replicated in an *in vivo* model. Additionally, primary cell cultures have the potential to harbor resident pathogens [28]. In research involving fish cell cultures (in virology and toxicology), a common practice observed is to use non-specific cells [29,30,47] unlike in mammalian biology studies. Utilizing fish cell lines with specific functions (originated from specific tissue type) will greatly advance fundamental knowledge in the respective fields [151-159].

CONCLUSION

Fish cell culture systems have the advantages of defined, but pliable physiochemical environment, cost-effectiveness, convenience, and infinite source of cells exhibiting a high degree of homogeneity. Though fish cell cultures have proven to be a successful biological alternative to the use of animals in research, fish as a source of cell lines remains unexplored. Despite the huge diversity in fish species, there is still a scarcity of host-specific fish cell lines in the aquaculture research which is concerning. Considering the wide diversity of fish, there is untapped potential for the development of cell cultures from various fish species and tissues, allowing the study of species-specific as well as tissue-specific responses of cells towards different etiologic agents.

Since it will be beneficial for researchers to have the high biological relevance of primary cells and the proliferative capacity of cell lines, attempts are made to combine primary cells with 3D cell culture. More research progress in this direction with fish cell cultures will be appreciated. As properly standardized *in vitro* assays can provide relevant data, cell cultures should be given proper care and quality control measures including the use of standardized media and other commercially available laboratory reagents.

In near future, innovations in 3D cell culture and CRISPR-Cas9 genome editing will further enhance the research prospects of fish cell culture systems. So far, fish cell lines have been an under-utilized research resource. In the coming years, the fish cell area will see diverse applications in molecular biology especially in gene editing, production of recombinant proteins, and regenerative therapies. Its enormous potential in the fields of stem cells has hardly started to be realized. Across the world, scientists are trying to improve cell lines for enhanced growth, product synthesis, energy metabolism, etc. employing genomic and proteomic approaches. Undoubtedly,

cell cultures are likely to be the key technology for the foreseeable future.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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