

# FISH CELL LINES: RECENT TRENDS AND ROLES IN BIOTECHNOLOGY

## Abstract

Fish cell lines provide an important biological tool for carrying out research in the field of biochemistry, physiology, cancer biology, immunology, genetics, pharmacology, virology, toxicology and transgenic. These model systems can be utilized for various vaccine development, pathogenic studies, regeneration research, stem cell research, propagation and characterization of viruses, parasitology studies and many more. In past decade, there are many fish cell lines developed from freshwater, marine as well as brackish water fishes from a broad range of tissues like ovary, gills, heart, liver, skin, brain, muscles, liver, fin bladder. The development, characterization, confirmation and applications of fish cell line are becoming most common nowadays in the field of fisheries, toxicology, aquatic sciences and applied biotechnology. The recent rapid growth in cell culture-based research is unquestionably a result of both the development of this field and the growing ethical pressure to replace and reduce the usage of animals in research. Excellent study models for simulating host animals in vivo are in vitro fish cell cultures. Fish cell cultures have a wide range of uses in research, which can be attributed to their adaptability, affordability, ease of handling, and ease of genetic manipulation. This chapter includes a list of novel cell lines and a scientific update. It also includes information on the significance of authentication, uses, maintenance, cross-contamination, and the effects of overpassaging cell lines. The chapter, in the authors' opinion, will serve as a current database for novice and experienced researchers working in the field of fish cell line in-vitro research.

**Keywords:** Fish Cell Lines, Cell Culture Techniques, Biotechnology, Vaccine Production.

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## I. INTRODUCTION

In vivo experiments are traditionally considered the gold standard for determining hazardous effects of components. On the other hand, due to ethical, commercial, and scientific considerations, companies are moving away from using animal models in safety testing. In Vitro testing can help understand adverse biological effects, but cross-species extrapolation remains. The 3Rs principles, replacing, reducing, and refining animals, aim to minimize in vivo testing and favour robust, predictive in vitro methodologies without compromising scientific safety tests (Maestri, 2021). In-vitro procedures are becoming more popular for economic, practical, and ethical reasons. Cell lines provide benefits such as avoiding contamination on living animals, requiring less upkeep, being cost-effective, non-invasive, using less chemicals, and producing less harmful waste. These approaches also minimise complicated interactions in organisms, resulting in findings with little variability (Kasi Elumalai, 2012; Nagpure et al., 2016; Schug et al., 2020).

For replacing or minimising the use of fish in toxicological testing, in vitro fish cell tests are recognised as an appropriate alternative to fish bioassays. Fish cells can be exposed to chemicals or water samples at temperatures corresponding to those the fish would experience in the wild. Additionally, it is much simpler to maintain fish cells alive and they are more resistant to standard culture conditions. In order to correlate in vitro cytotoxicity in fish cell lines with in vivo fish toxicity and show its broad applicability, a lot of research on hazardous compounds has been done. To overcome the barrier, Schirmer, (2006) proposed a number of methods for creating fish cell line-based toxicity studies, including choosing cell lines derived from tissues that reveal the specific mechanism of action of a given compound, enhancing cellular sensitivity by altering the culture environment to more closely resemble in-vivo exposure, and accounting for the chemical fraction available to the cells. According to reports, several experts are developing cutting-edge techniques to identify toxicity using different cell lines.

As soon as ecotoxicology was recognised as a legitimate subject of study, in vitro methods were used to address concerns about fish toxicity. Rachlin & Perlmutter (1968) carried out the first study on metal toxicity for fish utilising an in vitro experiment with fish cells. By the middle of the 1990s, fish cell systems had gained popularity as a tool for ecotoxicological study. In 1962, the first fish cell line, RTG-2, was developed utilising the ovaries of a cold-water species, the rainbow trout (Wolf and Quimby, 1962). Since then, there has been an increase in the development of fish cell lines from a range of tissues, including fish species from both tropical and temperate waters. In 1980, Wolf and Mann created the first thorough analysis of all fish cell and tissue cultures. In 1994, Fryer and Lannan published a list of all freshwater and marine fish cell lines from around the world. Later, Niels Bols' laboratory was successful in creating a variety of fish cell lines, including the RTL-W1 from liver and the RTgill-W1 from rainbow trout (*Oncorhynchus mykiss*) gills, which were used to detect distinct toxicant responses (Behrens et al., 2001; Bols & Dayeh, 2005). Moreover, fish cell lines were used to test the toxicity of complex environmental components such water effluents or sediment extracts as well as to determine whether chemicals had genotoxic or immunotoxic properties (Bols & Dayeh, 2005; Rehberger et al., 2018). Fish hepatocyte cell lines were previously chosen because of their crucial role in toxicokinetic and toxicodynamic processes, as well as xenobiotic biotransformation (Segner & Cravedi, 2001). Two fish cell lines, RTG 2 and PLHC1, were used to examine the harmful potential of fluoroacetate insecticide for the first time (Zurita et al., 2007). Later, for an in-

vitro investigation, a number of researchers looked into the toxicants on a fish muscle cell line called Wallago attu muscle (WAM) (Nagpure et al., 2016).

In a comprehensive review by Lakra & Swaminathan (2011), it was revealed that there were a total of 283 fish cell lines established worldwide. Bairoch, (2018) presented the most recent information regarding 517 fish cell lines in Cellulosaurus; a repository of data on cell lines. While comprising more than half of the vertebrate species, fish have seen relatively fewer cell lines established and characterized, especially when compared to mammals. However, the last decade has witnessed a notable acceleration in cell line research, particularly in India. During this period, various cell lines originating from organs of different fish species have been cultivated, including the development of the SICH cell line derived from the heart of *Catla catla*, cell lines RE and CB from the eye of *Labeo rohita* and brain of *Catla catla*, respectively (Ahmed et al., 2009). Moreover development of three cell lines RF, RH and RSB from heart, fin and swim bladder of *Labeo rohita*, respectively (Lakra & Swaminathan, 2011). cell lines from the fin tissue of *Tor tor*; two cell lines from fin and eye tissue of *Tor chelynooides* and fin tissue of *Scizothorax richardsonii* (Goswami et al., 2012; Goswami et al., 2014). These in vitro cell culture methods have shown to be crucial resources for research in toxicology, biotechnology, and cellular biology. (Goswami et al., 2012; Goswami et al., 2014; Taju et al., 2014). The cytotoxicity potential of more than 50 aquatic contaminants, including heavy metals, herbicides, and nanoparticles, has been effectively assessed using fish cell lines and the results reported from fish cell lines in vitro have exhibited good agreement with in vivo toxicity results.

Cell culture techniques were gradually improved with the help of the development of chemically defined cell culture media, such as Leibovitz -15 (L-15) and antibiotics, leading to the eventual production of cultured cells for the production of continuous cell lines. Cell cultures created from fish, shellfish, and seaweed can contribute significantly to the expansion of aquaculture in addition to being a crucial scientific tool like any other cell line. It is possible to manipulate the entire organism to increase its utility for aquaculture using the scientific information obtained through the cell culture technique. Their cell line may be valuable for revealing fundamental information about development, reproduction, and health. Because it allows for manipulation, their cell line may be utilized to produce biochemical products instead of the organisms (Gray, 1989). To fulfil the demands of the world's expanding population, cell-based aquaculture systems based on cell cultures may be a game changer in the production of seafood and other aqua foods across a variety of species (Rubio et al., 2019). An aqua food manufacturing technique based on fish cells rather than whole fish might help improve the conservation of aquatic ecosystems. For the safety of food produced using such animal cell culture technologies, the procedure must go by the FDA's regulatory framework and recommendations (FDA, 2018).

**Table 1: List of Fish Cell Line Repository**

Sr No		Website	Place	No of fish cell line
1.	American Type Culture Collection (ATCC), USA	<a href="http://www.atcc.org">http:// www.atcc.org</a>	USA	8
2.	Cell Bank Australia	<a href="http://www.cellbankaustralia.com">www. cellbankaustralia.com</a>	Australia	
3.	National Repository of Fish Cell Line (NRFC)	<a href="https://mail.nbfgr.res.in/nrfc/index.php">https://mail.nbfgr.res.in/nrfc/index.php</a>	Lucknow, India	50
4.	Aquatic animal health laboratory- Abdul Hakeem College	<a href="https://aahl.res.in/">https://aahl.res.in/</a>	Vellore, Tamilnadu India.	23

**Table 2: List of Fish Cell Lines Revisited at Aquatic Animal Health Laboratory- Abdul Hakeem College**

Sr. No	Name of the Fish	Organs used	Name of the cell line
1.	Asian sea bass ( <i>Lates calcarifer</i> )	Kidney	SISK
2.		Spleen	SISS
3.	Grouper ( <i>Epinephelus coioides</i> )	Eye	SIGE
4.		Kidney	GK
5.		Heart	GH
6.		Brain	GB
7.	Catla ( <i>Catla catla</i> )	Heart	SICH
8.		Brain	CB
9.		Gill	ICG
10.		Eye	SICE
11.	Rohu ( <i>Labeo rohita</i> )	Eye	RE
12.		Gill	LRG
13.	Pearl spot fish ( <i>Etroplus suratensis</i> )	Eye	IEE
14.		Gill	IEG
15.		Kidney	IEK
16.		Brain	IEB
17.	Catfish ( <i>Clarius batrachus</i> )	Fin	ICF
18.	Zebra fish ( <i>Danio rerio</i> )	Eye	DRE
19.		Fin	DRF
20.		Gill	DRG
21.	Snakehead fish ( <i>Channa striatus</i> )	Kidney	SHK
22.		Gill	SHG
23.		Heart	SHH

**Table 3: List of Fish Cell Lines Reposited at National Repository of Fish Cell Line at NBFGR, ICAR-National Bureau of Fish Genetic Resources, Lucknow**

Sr. No	Name of the Fish	Organs used	Name of the cell line	NRFC Accession
1.	<i>Amphiprion sebae</i>	Dorsal Fin	NRFC010	CFFN2
2.		Caudal Peduncle	NRFC013	CFCP1
3.	<i>Barilius bendelisis</i>	Fin	NRFC061	BBdF-1
4.	<i>Carassius auratus</i>	Caudal fin	NRFC058	FtGF
5.	<i>Catla catla</i>	Eye, Muscle	NRFC018	SICE
6.		Heart, Muscle	NRFC019	SICH
7.		Brain	NRFC020	CB
8.		Gills	NRFC021	ICG
9.		Thymus(Macrophage)	NRFC028	CTM
10.		Thymus(Epithelial)	NRFC029	CTE
11.		Blood (Lymphocyte)	NRFC034	CCM
12.	<i>Channa punctatus</i>	Gill	NRFC049	CPG
13.	<i>Channa striatus</i>	Kidney	NRFC045	CSK
14.		Gill	NRFC046	CSG
15.		Thymus macrophage	NRFC055	OST
16.	<i>Cirrhinus mrigala</i>	Peritoneal	NRFC056	CMP
17.	<i>Clarias dussumieri</i>	Fin	NRFC059	CIDu
18.	<i>Clarias magur</i>	Testes	NRFC062	CMgT-1
19.		Muscle	NRFC066	CMgM-1
20.		Barbel	NRFC067	CMgB-1
21.		Fin	NRFC022	ICF
22.	<i>Cyprinus carpio</i>	Fin	NRFC004	CCF
23.		Gill	NRFC064	CyCKG
24.	<i>Cyprinus carpio koi</i>	Fin	NRFC007	CCKF
25.	<i>Danio rerio</i>	Gill	NRFC053	DRG
26.		Retinal	NRFC054	DrRPE
27.		Muscle	NRFC069	ZFiM-1
28.		Fin	NRFC068	DDaF-1
29.	<i>Dascyllus trimaculatus</i>	Caudal Peduncle	NRFC024	DT1CPEx
30.		Fin	NRFC025	DT1F4Ex
31.		Caudal Peduncle	NRFC026	DT1CPTr
32.	<i>Epinephelus coioides</i>	Eye, Muscle	NRFC016	SIGE
33.		Kidney	NRFC017	IGK
34.	<i>Epinephelus malabaricus</i>	Gills	NRFC031	EM2GEx a
35.		Gills	NRFC032	EM3GEx
36.		Spleen	NRFC033	EM4SpEx
37.	<i>Epinephelus merra</i>	Spleen	NRFC038	HC2SPEX
38.	<i>Etroplus suratensis</i>	Eye	NRFC040	IEE
39.		Kidney	NRFC041	IEK
40.		Gill	NRFC042	IEG
41.		Brain	NRFC043	IEB
42.		Fin	NRFC065	PSF

43.	<i>Helostoma temminckii</i>	Fin	NRFC080	KGF
44.	<i>Horabagrus brachysoma</i>	Fin	NRFC008	HBF
45.	<i>Labeo calbasu</i>	Fin	NRFC063	LCF
46.	<i>Labeo rohita</i>	Eye	NRFC044	RE
47.		Fin	NRFC070	LROF-1
48.	<i>Lates calcarifer</i>	Kidney	NRFC014	SISK
49.		Spleen	NRFC015	SISS
50.	<i>Oncorhynchus mykiss</i>	Heart	NRFC075	RBT-H
51.	<i>Oreochromis niloticus</i>	Fin	NRFC077	OCrF-1
52.	<i>Oreochromis niloticus</i>	Liver	NRFC052	OnIL
53.		Heart	NRFC071	OnH
54.	<i>Pangasianodon hypophthalmus</i>	Fin	NRFC057	PHF
55.		Thymus	NRFC078	PHT
56.	<i>Paraneetroplus synspilus</i> X <i>Amphilophus citrinellus</i>	Brain	NRFC072	PFB
57.		Heart	NRFC073	PFH
58.		Spleen	NRFC074	PFS
59.	<i>Pomacentrus caeruleus</i>	Caudal Peduncle	NRFC035	PC1CpTr
60.		Fin	NRFC036	PC1F1Ex
61.		Liver	NRFC037	PC1L1Tr
62.	<i>Pristolepis fasciata</i>	Fin	NRFC039	CFF
63.	<i>Pseudetroplus maculatus</i>	Fin	NRFC076	OCF
64.	<i>Pterophyllum scalare</i>	Fin	NRFC051	AFF
65.	<i>Schizothorax richardsonii</i>	Eye	NRFC060	SREM-1
66.		Muscle	NRFC079	SRM-1
67.	<i>Tor tor</i>	Fin	NRFC003	TTCF
68.	<i>Trichopodus trichopterus</i>	Heart	NRFC081	TSGH
69.	<i>Wallago attu</i>	Gill	NRFC048	WAG

## II. APPLICATIONS OF FISH CELL CULTURES

Numerous fish cell lines derived from various fish species have been used in many areas of study, including toxicological studies, immunological research, genetic engineering, genetics, endocrine disorders, medical research and disease control, biotechnology, and radiation biology (Collet et al., 2018). Due to growing concerns about animal welfare, there is a greater need than ever to find alternatives to employing animals in research, and cell cultures may be the best option. Established cell lines typically originate from cancerous tumours (malignant tumours), spontaneous transformation, or oncogenic cells. Fish cell cultures are often employed as model systems, for in vitro research, and for the large-scale production of biologicals due to an increase in their utilisation in recent years. Several applications for fish cell cultures are listed below.

**1. Fish Cell Lines as Model Systems:** For a variety of in vitro studies in the biological sciences, fish cell lines have shown to be a great platform. It differs from mammalian cell lines in that they have more adaptable culture schedules, which makes it a useful tool for many in vitro fisheries investigations. Fish cell cultures provide great research models because they mimic the host species in vivo. However, it is simple to manipulate a cell's

genetic makeup in order to analyse how certain genes and/or proteins express themselves differently. Results consistency and repeatability are further benefits. For studying viral genetics and replication as well as for creating experimental vaccines for use in aquaculture, in vitro models have been employed. Studies on in vivo development were found to be supplemented by fish cell lines, demonstrating the importance of signalling pathways in the developmental processes (Bloch et al., 2015). The increased use of cell cultures as model systems has been beneficial for the study of basic cell and molecular biology processes, physiological science, cellular interactions, signalling pathways, expression profiling, apoptotic pathways, interactions with infectious agents, drug effects, metabolic effects of dietary components, and mutagenesis. They serve as crucial model systems for research in endocrinology, environmental biology, neuroscience, and embryology. Zebra fish cell lines are a potential in vitro model for studying illnesses and cellular processes due to their ease of manipulation and resemblance with functional genes involved in human diseases and cellular processes too (Heilmann et al., 2015; Rapanan et al., 2015). To investigate the topic of fish endocrinology, several cell lines originating from fish were employed (Chen et al., 2010; Higaki et al., 2013). An in vitro model for the synthesis of the growth hormone prolactin was created using organ cultures made from the pituitary glands of tilapia, eels, and trout (Baker & Ingleton, 1975).

- 2. Research on Virology and Screening and Identification of Antiviral Agents:** As obligate intracellular parasites, viruses depend on the machinery of the host cell to replicate and propagate. Since cell cultures play so many different functions in virology, including the detection, identification, propagation, isolation, confirmation, and characterisation of viruses, they are referred to as "the gold standard" (Hsiung, 1984, Leland & Ginocchio, 2007). Animals can be effectively replaced by fish cell cultures, particularly in the area of virology (Kelly et al., 1978; Nicholson, 1989; Ott, 2004; Sommerset et al., 2005). Isolation of viruses depends on legal cell culture accessibility, potentially causing delays in characterization of host-specific fish viruses (Hanson et al. 2011). Cell cultures are essential for representing various cell types in viruses like cyprinid herpesviruses, salmonid herpesvirus, acipenserid herpesvirus, and walleye herpesvirus due to their specificity (Hedrick et al. 2000; Hanson et al. 2011). To develop effective pathogen-targeted management techniques, researchers must investigate fish viruses affecting aquaculture using species-specific cell cultures. However, limited host-specific cell lines hinder research on recently discovered, unknown viruses, and rely on generic cell lines (Bang 1960; Baron et al. 1996; Pandey 2013). Fish cell lines are used to screen antiviral compounds (Huang & Han, 2010; Krishnan et al., 2010), such as acyclovir, which is effective against human herpesvirus and cyprinid herpesvirus-3 in CCO cells. Exopolysaccharides from *Arthrospira platensis* inhibit KHV replication in CCB cells, while polyinosinic polycytidylic acid induces an antiviral state in CHSE-214 cells against IPNV. A compound that fights the Infectious Hematopoietic Necrosis Virus (IHNV) was discovered to prevent the fusion of the virus's membrane with that of the host cell (Balmer et al., 2017; Jensen et al., 2002; Reichert et al., 2017).
- 3. Fish Cell Line in Vaccine Production:** The prevalence of viral infections used to result in significant annual economic losses for the aquaculture industry worldwide. The production of vaccines is crucial for the aquaculture sector's efforts to reduce viral infections. The first health product to be used as a vaccine derived from piscine cell cultures is probably purified viruses (Bols, 1991). Numerous viral vaccines have been developed with enhanced delivery methods at competitive rates (Dolgin, 2019). Fish cell

lines have been examined for virus replication in the production of vaccines, with cell-culture-based technology being a reliable alternative. Live fish are required for testing potency, but cell lines like Madin Darby canine kidney (MDCK), Vero, chicken embryo fibroblasts (CEFs) can be used for the production of viral vaccine (Dhar et al., 2014). For iridovirus and NNV protection, several inactivated or attenuated fish viral vaccines have been created, some of which have been made available for purchase (Nakajima et al., 2002; Sato & Okamoto, 2010; Oh et al., 2016). Few cell lines exist for replicating betanodavirus, herpesvirus, and aquareovirus for vaccine production. Fish cell cultures have potential applications in recombinant, DNA/RNA particle vaccines. Rainbow trout pronephros cells could screen fish DNA vaccines (Biering et al., 2005). Grass carp reovirus (GCRV) was able to be neutralised by the anti-VP5 polyclonal antibody in an in vitro test using the grass carp cell line CIK (Ortega-Villaizán et al., 2012). This would be crucial for the creation of a vaccine to fight the virus in the current situation.

- 4. Fish Cell Lines in Research on Stem Cells:** Undifferentiated cells originating from growing embryos are called embryonic stem (ES) cells, and they are employed in research on aquatic biotechnology and biodiversity conservation (Thomson et al., 1998; Till & McCulloch, 1961; Hong et al., 1996). A reported achievement involves the establishment of a spermatogonial cell line from the testis of adult medaka fish. This cell line displayed the ability to undergo meiosis and facilitate the production of viable sperm through spermatogenesis. This accomplishment underscores the potential application of fish ES cell lines within the realm of biotechnology. Comprehensive investigations into fish embryonic stem cells have primarily focused on small model species like zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). This preference is attributed to the ease with which these species allow for the integration of embryological, genetic, and molecular analyses, thereby facilitating a holistic understanding of vertebrate development (Sun et al., 1995; Hong et al., 1996; Yi et al., 2009; Ciarlo & Zon, 2016). Fish ES cell lines are used as a vector for efficient transfer of foreign DNA into an organism's germ cells (Hong et al., 2004). Additional Salmonids have successfully produced offspring through the use of embryonic stem cell transplantation (Yoshizaki et al., 2010). For the goal of developing new treatments, the chemicals and exosomes generated during stem cell cultivation are collected. ES cell lines have been developed from zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) (Yi et al., 2009; Ciarlo & Zon, 2016). ES cell sources can also come from tumours. Epithelioma Epithelioma and hepatoma, respectively, are the sources of the Papulosum Cyprini (EPC) and rainbow trout liver (RTH-149) cells (Fijan, 1968; Lee et al., 1993). Blastula stage cells extracted from zebrafish embryos demonstrated the capability to express externally introduced genes through transfection methods commonly employed in mammalian cell cultures. This in vitro experimentation highlighted the prospect of modifying the genotype and phenotype of cultured cells (Collodi et al., 1992). Moreover, Cell lines originating from embryos have been successfully established in catfish, Nile tilapia, and various marine fish species. (Parameswaran et al., 2006; Chen et al., 2007; Holen et al., 2010; Lakra & Swaminathan, 2011; Fan et al., 2017; Vergès-Castillo et al., 2021).
- 5. Toxicology and Environmental Studies:** Fish cell cultures serve as a suitable substitute for animals and are frequently employed as in vitro models for environmental toxicology investigations, particularly cytotoxicity analyses. (Castaño et al., 1996; Fent, 2001; Rachlin & Perlmutter, 1968; Segner, 1998). The genotoxicity of drugs, metabolism, DNA binding, and method of action may all be assessed without incurring significant expenses



or having variable results (Behrens et al., 2001; Klingelfus et al., 2019; Rehberger et al., 2018). To investigate the xenobiotic efflux activity of human medications, fish hepatoma cell lines were discovered to be helpful (Caminada et al., 2008). Fish cell lines were used to evaluate the cytotoxicity of chromium (Taju et al., 2017), Polycyclic Aromatic Hydrocarbons (PAH) (Behrens et al., 2001), aflatoxins and agrochemicals (Salunke et al., 2022) using different toxicological techniques including comet assays, neutral red dye uptake method, proliferation markers etc. Additionally, fish cell lines can detect cellular DNA damage caused by toxic materials and chemicals using comet assays, which are sensitive and reliable (Klingelfus et al., 2019). The RTG-2, RTgill-W1, and RTL-W1 cell lines derived from rainbow trout (*Oncorhynchus mykiss*), along with ZFL and ZF4 originating from zebrafish (*Danio rerio*), are frequently employed in comet assays (Žegura & Filipič, 2019) These assays aid in evaluating DNA damage prompted by environmental genotoxic substances, underlining the cells' promise as potential environmental biomarkers (Kienzler et al., 2013). Fish cell lines play a crucial role in deciphering the mechanisms and comparative toxicity of environmental samples. In vitro assays also have the ability to reveal the mechanism of action of the tested chemical because in vivo testing usually focus on final results rather than the actual mechanism of action. Given the strong correlations seen between in vitro and in vivo outcomes, cell lines have become a popular substitute for whole live fish. These cell lines have demonstrated to be useful and affordable instruments for quickly assessing the toxicity of pollutants in vitro (Behrens et al., 2001).

- 6. Genetic Engineering and Genome Editing:** Fish cell lines offer a unique advantage as they can be genetically manipulated, making them valuable tools for knockout studies aimed at observing the effects of specific gene deactivation. Notably, the CRISPR-Cas9 system has been developed for genetic modification in various fish somatic cell lines (Dehler et al., 2016; Ma et al., 2018). In the context of Chinook salmon, a genetically modified embryo cell line capable of expressing geneticin and hygromycin resistance was generated using knockout technology by Liu et al., (2018). Further advancements in gene editing have been achieved in medaka embryonic cell lines, as demonstrated by Gratacap et al., (2020). This success paved the way for developing gene editing protocols using the gRNA-Cas9 ribonucleoprotein complex in the CHSE-214 cell line through lentivirus transduction, offering the potential for disease resistance manipulation in salmonid species (Chang et al., 2013). Moreover, successful genome editing using RNA-guided Cas9 nuclease in zebrafish embryos was reported by Hwang et al., (2013).

Similar to this, plasmids-producing cytokines like Interleukin-6 and macrophage colony-stimulating factor (MSCF) were transfected into viable trout head kidney cell lines. In addition, IL-2, IL-6, and macrophage colony-stimulating factor (MCSF) were designed to express in RTG-2 stable cell lines and rainbow trout head kidney cell lines (Corripio-Miyar et al., 2012). The efficiency of the anti-apoptotic protein Bcl-xL was tested through genetic alteration in the context of the liver cell line GL-av from the greasy grouper *Epinephelus tauvina* (Chen et al., 2006).

Beyond gene editing, fish cell lines also have applications in in vitro ploidy manipulation. Zhou et al., (2016) successfully induced polyploidization in crucian carp using a chemical compound, leading to the development of an autotetraploid cell line. In summary, fish cell lines serve as a versatile platform for genetic studies, enabling gene

editing, manipulation of disease resistance, cytokine expression, and even in vitro ploidy manipulation, thus contributing significantly to the advancement of fish biology research.

- 7. Cancer Research:** Cell cultures offer a valuable platform for exploring the fundamental distinctions between healthy and malignant cells. Various factors like radiation, chemicals, and viruses have the potential to transform normal cells into cancerous ones, thus offering insights into the mechanisms and origins of cancer. Moreover, cancer cells cultivated in these cultures serve as a vital testing ground for identifying specific drugs capable of selectively targeting cancer cells. This approach has significantly enriched the study of cancer biology (Mehta et al., 2012). The area of cell culture research has played a pivotal role in delving into several aspects of cancer. It has enabled the investigation of malignancy growth, cell death induction, DNA methylation, histone modifications, the expression of tumor suppressor genes, and the influence of diverse carcinogenic agents (Mehta et al., 2012). Mechanisms underlying the activation of procarcinogens, as well as the dynamics of genetic material breakdown and repair, have been extensively explored using cell lines such as fathead minnow cells (FHM), goldfish erythrocyte-derived cell lines, and the goldfish fibroblast cell line RBCF-1 (Grist et al., 1986; Hightower & Renfro, 1988). Furthermore, primary cell cultures of rainbow trout have been harnessed to examine the impact of aflatoxin B on cancer (Bailey et al., 1982).

In the field of cancer research, fish cell lines have emerged as invaluable tools for unravelling the intricate mechanisms related to procarcinogen activation, molecular damage, and DNA repair processes (Grist et al., 1986). In essence, cell culture-based studies have revolutionized cancer research by enabling the detailed exploration of cancer cell behaviour, responses to carcinogenic agents, and potential therapeutic interventions.

- 8. Three-Dimensional Cell Cultures:** 3D cell models, interacting with their environment in all dimensions, provide more reliable data, closely resembling in vivo conditions. This study aimed to create a new in vitro infection model using a reproducible 3D spheroid cell culture system, potentially reducing the need for animal testing in fish disease research. The efficiency of 3D spheroids of rainbow trout cell lines, RTG-2 and RTS-11, in propagating *Saprolegnia parasitica* spores was successfully tested, simulating in vivo infection (Desoize et al., 1998). 3D cell cultures, as per Faber et al., (2021), allow for the examination of complex physiological processes in vitro. 3D cell systems, an advanced cell culture technique, better simulate in vivo conditions than traditional 2D culture. Studies indicate that essential receptors and signaling molecules are reduced or lost in 2D cell cultures (Hayward et al. 1995; Novaro et al. 2003; Pickl and Ries 2008; Yang et al. 2000). However, the utilization of 3D cell culture enables cells to proliferate and engage with their surroundings in a manner that mirrors the three-dimensional aspects of their natural environment. This technique was employed to develop 3D spheroids of rainbow trout cell lines, namely RTG-2 and RTS-11. These cellular constructs proved to be highly effective for cultivating *Saprolegnia parasitica* spores, successfully emulating a real-life infection scenario (Faber et al. 2021).

Adding extracellular matrix (ECM) components to cultures significantly improves cell morphology, differentiation, adhesion, polarity and gene expression in 3D setups (Kenny et al. 2007; Pampaloni et al. 2007; Yamada and Cukierman 2007; Sung et al. 2013; Xu et al. 2013), resembling the natural ECM's structure and chemistry (Wolf et al. 2009). 3D cell cultures in spheroids, simulating physiological conditions (Desoize et al.

1998), better replicate in vivo tumor behavior (Hirschhaeuser et al. 2010; Shield et al. 2009; Weiswald et al. 2015). Spheroids are created by favoring cell-cell adhesion over matrix adhesion (Santini et al. 1999) and can interact with various biomaterials (Hsiao et al. 2009; Loessner et al. 2010; Ong et al. 2010). 3D cultures emulate complex physiological processes, useful for drug screening, discovery, and cancer biology (Tsuruga et al. 2008; Ballester et al. 2019). Primary cells, like hepatocytes, thrive in 3D collagen gels for extended periods (Schippers et al. 1997), enhancing accuracy and reliability. Developing fish primary cell 3D cultures, especially for drug screening, accelerates targeted treatment discovery, crucial for advancing drug development and biomedical research.

### **III. FISH CELL CULTURES: PROS AND CONS**

Fish cell culture systems offer a defined, adjustable environment, cost-efficiency, ease of use, and an infinite source of homogenous cells, avoiding animal use in research. However, cell lines can mutate and drift genotypically and phenotypically during serial culture. Subpopulations often form in frequently used cell lines, especially those stored in cell banks for a long time. Rapidly growing clones within a population can cause phenotypic changes over time (Bahia et al. 2002; Burdall et al. 2003). Bioinformatic assessment of proteomic characteristics revealed a diminished presence of mitochondria in Hepa1-6 cell lines, suggesting notable shifts in metabolic pathways in comparison to primary hepatocytes (Pan et al. 2009). This poses a significant issue when considering the validity of using these cell lines as reliable models.

### **IV. FUTURE PROSPECTS**

The absence of suitable fish cell cultures presents a barrier to isolating pathogens specific to species and tissues. Additionally, established cell lines are not accessible through repositories, and the potential of using fish as a cell line source remains unexplored. To establish cell lines as consistent research tools, it's essential to employ standardized media, reagents, tools, quality-control protocols, thorough characterization, and proper documentation during their development. Utilising 3D cell culture technologies to maintain organoids obtained from the snake venom gland for an extended period of time in vitro can be beneficial for producing antivenom and other therapies (Puschhof et al. 2021). Sharks, sting rays, silurid catfish, stonefish, and rabbitfish are just a few of the fish clades known to generate highly toxic poisons (Pandey and Upadhyay 2020). Fish toxins are physiologically important substances that can affect the body in a variety of ways, such as enzymatic, antimicrobial, cytotoxic, hemolytic, cardiovascular, neuromuscular, and potentially carcinogenic ways (Ortiz et al. 2015). Fish toxins are therefore useful for a variety of pharmacological, medicinal, and pesticidal uses (Church and Hodgson 2002; Pandey and Upadhyay 2020). To generate fish venom for use in biomedical research, employ the aforementioned technique.

For the development of new drugs and investigations of biological mechanisms, luciferase-labeled reporter cell lines are employed. Through the use of bioluminescence imaging, these cutting-edge models offer a relatively easy, reliable, and extremely sensitive way to quantify biological processes and evaluate medication efficacy in live animal models. Toxicologists require primary cells' strong biological relevance and cell lines' ability to proliferate for use in regular predictive experiments. Finding cells with a high level of

biological relevance and then creating or getting enough cells to conduct the experiment without adding cell variability are two of the main difficulties that many scientists face while constructing a cell-based assay. The genetically altered hTERT-immortalized primary cells display the growth traits of a continuous cell line while retaining the physiology of a primary cell.

Since using incorrectly identified or cross-contaminated cell lines might render experimental results invalid, authenticating cell lines need to be a step in the cell culture procedure. Because of improper handling and a disregard for tissue culture best practises, cross-contamination of cell lines has persisted. These drawbacks raise concerns about their applicability in biological research since they may lead to illogical, inconsistent, and unreplicable results or encourage unneeded further investigation. Before starting a multitude of research, cell lines must be well characterised in order to be utilised as models in a relevant way. DNA fingerprinting employing multi-locus probes, short tandem repeat (STR) profiling, karyotyping, isoenzyme typing, and HLA typing may all be used to identify and characterise cell cultures (Masters et al. 2001). Multiple screening techniques may be employed to detect contamination. Mycoplasma infection can negatively impact the health of cells in culture for an extended period of time without being visible to the naked eye. The study will be more effective and productive if an appropriate cell model is used. It is important to take into account certain factors while choosing the medium and reagents for the cultivation of stem cells or primary cells. Cryo-containers and proliferation tests are tools that can help maintain the health of cells.

## V. CONCLUSION

Cell culture systems have emerged as the cornerstone of diverse fields within the life sciences, supplanting the need for animals in numerous tests and assays. Their untapped potential in stem cell research and targeted therapy holds promise. Ongoing efforts by scientists aim to enhance cell lines in terms of growth, product synthesis, energy metabolism, and glycosylation attributes. Fish cell cultures have showcased their efficacy as ethical alternatives for biological research, underscoring the need for increased representation in repositories. It is imperative to establish a variety of fish cell lines from various tissues, organs, and species to facilitate disease diagnosis and the exploration of species- and tissue-specific responses. Fish cell line development for therapeutic protein expression remains empirical, with improvements in selection procedures and genetic engineering. Primary cells are increasingly needed in various applications, including drug discovery. Combining primary cells with 3D cell culture technologies is essential for improved research, as 3D cell culture systems with primary cells show promise in biomedical research.

Acknowledging and authenticating cell lines is crucial for accurate research efforts. Fish cell cultures are considered standard research agents and require proper care and quality control measures. They can aid in aquaculture production, early disease diagnosis, and efficient management strategies against infectious pathogens. Developing cell cultures from economically relevant fish species can aid in virus disease diagnosis, vaccine development, and antiviral agent identification. Despite the diversity of fish species, the fish cell culture field remains unexplored. Future research will require improvised 3D cultures with physiological relevance and adopt animal cell culture guidelines to achieve these goals.

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