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Development of cell culture systems from the striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878)

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Abstract
In this study we report the development of primary culture systems from the caudal fin, and heart, tissues of *Pangasianodon hypophthalmus* by explant outgrowth technique. The target tissue samples were aseptically excised from the healthy fingerlings of *P. hypophthalmus* and explanted and maintained in Leibovitz -15 medium supplemented with 15% Fetal Bovine Serum at an optimum temperature of 28 ^{0}C. Heterogenous population of cells comprising fibroblastic and epithelial like morphology were observed from all the three explants. Confluent monolayer of fin and heart cells were observed at different time period post seeding and its attachment to the flask. The established primary cultures from the explants were trypsini zed using trypsin in the subsequent passages.

Keywords: Striped catfish**,** explant, heart, caudal fin, l-15 medium.

Introduction

Cell lines have been used as an *in vitro* model for studying acute toxicity (Goswami *et al*. 2014) [1], gene expression studies (Misra *et al*. 1989) [2], drug development process (Collet *et al*. 2017) [3]. Number of primary cultures were initiated from different teleost species that eventually led to the development of many continuous cell lines. Primary cell culture systems from gill and heart tissues were initiated to develop into cell lines and used an *in vitro* tool for studying viral etiology (Shobana *et al*. 2009) [4]. *Pangasianodon hypophthalmus* is called as striped catfish or sutchi catfish introduced to India from Vietnam and its culture has been adopted widely in India for its growth and flavor. It is an non indigenous species which is well suited for its monoculture and polyculture practice in India along with Indian major carps. The culture practice of this species has not only been limited to India but also widely followed in countries like Bangladesh, Indonesia, Malaysia, China. The major drawback of this species is outbreak of disease due to intensified culture practice. Diseases that commonly affect *P. hypophthalmus* are epitheliocystis (Sood *et al*. 2018) [5, 7]. Channel catfish virus (Siti-Zara *et al*. 2014) [6]. Cell line derived from the fin of *P. hypophthalmus* is the only available cell line which was used to study the pathogenicity of Tilapia lake virus (Ti LV) (Soni *et al*. 2018) [7]. Hence the research gap in the development of cell lines from this species has to be filled up which aid in the study of virology and also in assessment of toxic effluents. In this context, we report the development of primary cell culture systems from the caudal fin, and heart tissue of *P. hypophthalmus* which could pave way for the establishment of continuous cell lines.

Materials and Methods

Experimental animal

Healthy fingerlings of *P. hypophthalmus* weighing 20-30 g were collected from the aquarium and they were maintained at Institutional Wet Laboratory complex, CIFE and conditioned in a FRP (Fiber Reinforced Plastic) tank holding sterile and well-aerated water.

Decontamination

The major concern in cell culture experiments is preparation of donor animal devoid of carrying any unwanted organisms and contaminants. Hence adequate care was taken to minimize the possible routes of contamination. The donor fish i.e *P. hypophthalmus* was starved for three days to reduce the possibility of contamination from feces and regurgitated

feed prior to the dissection. Donor fish (*P. hypophthalmus*) was then sacrificed by euthanization using ice for 5-10 min. The decontaminating solutions for this purpose included chlorine solution (500 ppm available chlorine), 70% ethanol, iodophore solution (0.5 w/v iodine).

Dissection

Target tissues such as caudal fin and heart tissues were dissected aseptically and washed with PBS containing 500 IU/ml penicillin, 500 µg/ml streptomycin and 2.5 mg/ml Fungizone. The tissues were minced thoroughly into small pieces of explants of sizes $1mm^3$ and the minced tissues were washed thrice with PBS containing antibiotics. These explants were then seeded into 25 cm^2 cell culture flasks. The adherence of explants was accomplished by addition of 0.2 ml of FBS, then the flasks were incubated at 28°C and allowed to attach to the surface of the flask overnight. After 18-24 hrs L-15 (Leibovitz*)* medium supplemented with 10% FBS was added gently. The medium was changed after an interval of 3-5 days.

Subculture and maintenance

Cells upon reaching 90%-95% confluency, the cells were trypsinized using TPVG solution (0.1% trypsin, 0.2% ethylenediaminetetraacetic acid, EDTA, and 2% glucose in 1× PBS). The detached cells were resuspended in 5mL of fresh growth medium (L-15 plus 20% FBS) and seeded in 25 cm^2 culture flasks. The cells were allowed to adhere to the culture flask for the confluent monolayer formation. The flasks were monitored regularly for its adherence and proliferation.

Results

The attachment of PHCF cells to the culture flask were observed after 18-24h post seeding and radiated outgrowth of cells around the explant were observed 120 h after attachment (Fig.1) A confluent monolayer of cells around the explant were noticed after 10-12 days post seeding. (Fig.2). The growth of the cells from the caudal fin explant was very slow indicating the regeneration power of the caudal fin cells were very low. The morphology of the cells were mixed populations comprising epithelial as well as fibroblastic-like shaped. The Confluent monolayer of cells were trypsinized using trypsin 0.25 % and 0.2 EDTA which results in dislodgement of cells and the detached cells were also found to be attached to the culture flask in the subsequent cultures.

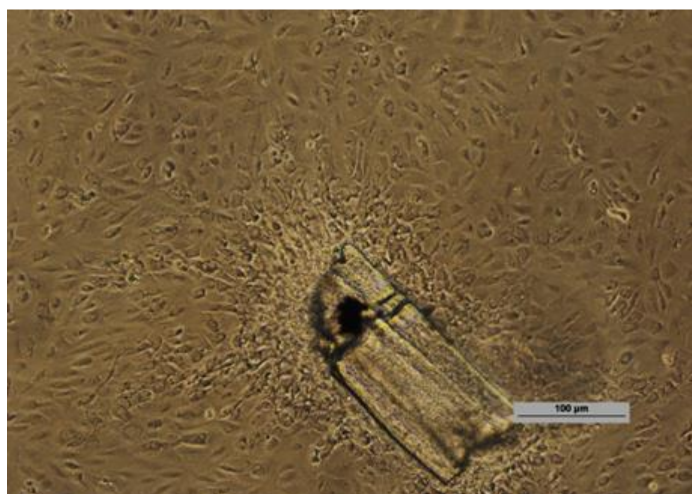

Fig 1: Phase contrast photomicrographs of PHCF cells along with the caudal fin explant of *P. hypophthalmus* (100X)

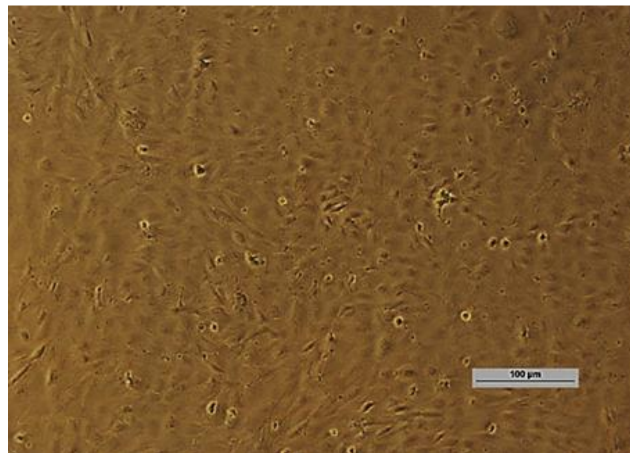

Fig 2: Phase contrast photomicrographs of confluent monolayer of PHCF cells (100X)

The primary cultures PHH initiated from the heart explant attached to the culture flask after 18-24h post seeding and radiated outgrowth of cells around the explants were observed 144 h after attachment (Fig.3) A confluent monolayer of cells around the explants were noticed after 15-20 days post seeding. (Fig.4) the morphology of the PHH cells comprised of mixed populations comprising epithelial as well as fibroblastic-like shaped. A confluent monolayer trypsinized using trypsin 0.25 % and 0.2 EDTA which results in dislodgement of cells and the detached cells were passaged subsequent cultures.

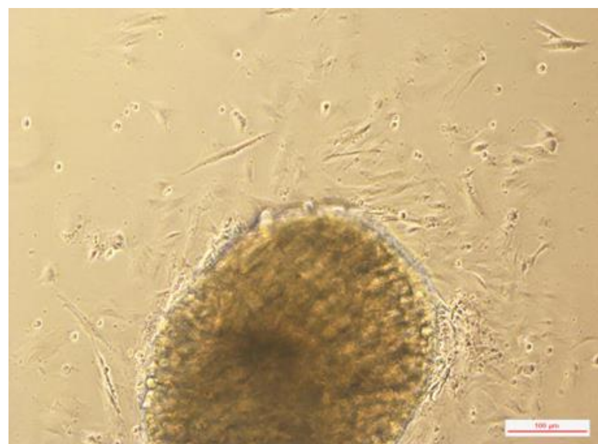

Fig 3: Phase contrast photomicrographs of PHH cells derived from the heart tissue of *P. hypophthalmus* after 7 days of explant preparation (100X)

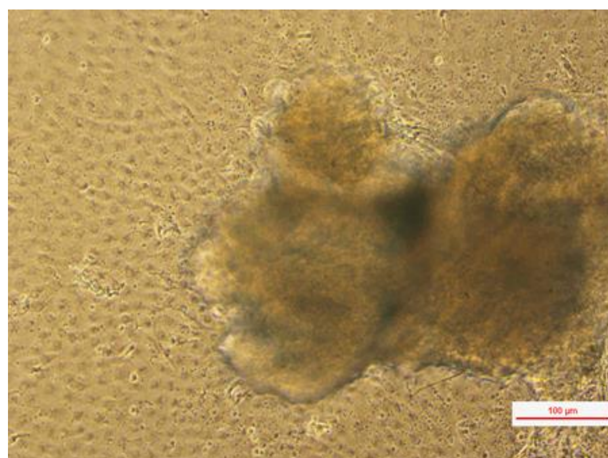

Fig 4: Phase contrast photomicrographs of PHH cells confluent monolayer observed around the explant after 15 days (100X)

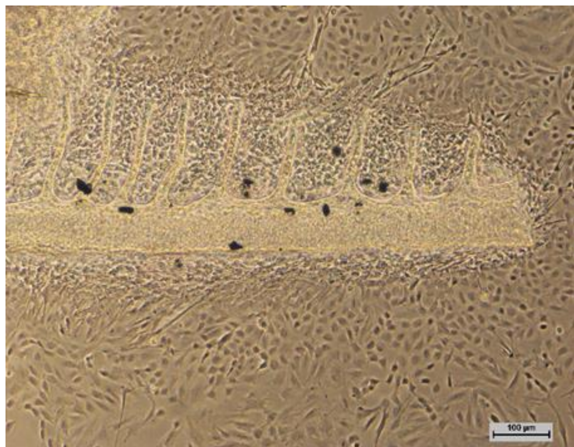

Fig 5: Phase contrast photomicrographs of PHG cells radiated from the explant (100X)

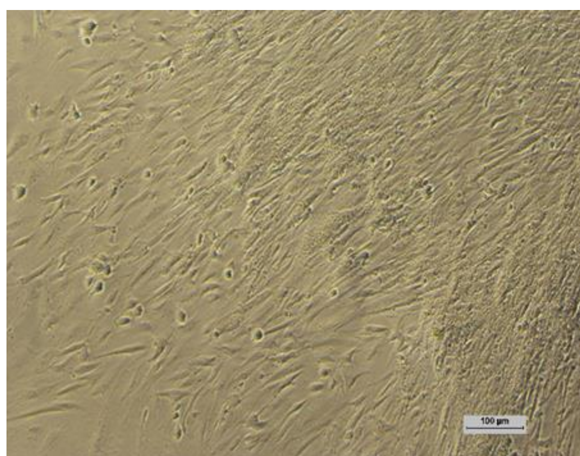

Fig 6: Phase contrast photomicrographs of PHG Confluent monolayer around the explant observed after 7 days. (100X)

Discussion

In the present study the development of primary cultures PHCF and PHH cells from the caudal fin and heart *of P. hypophthalmus* respectively were achieved by the explant technique. The explant technique is the most suitable technique for the proliferation of the cells, and better attachment to the culture flask (Nanda *et al*. 2014) [8]. The advantage of explant technique is, it has no enzymatic activity and also non-toxic compared to the enzymatic method (Fernandes *et al*. 1995) [9]. Many cell lines have been developed by using explant technique from different fishes such as *Clarias gariepinus* (Kumar & Singh, 2000), *Lates calcarifer* (Parameswaran *et al*. 2006) [11, 16], *Puntius denisonii* (Lakra *et al*. 2011) [12]. and etc.

Most commonly used parameters for growth study of any cell line are temperature and FBS concentration. Fish cell line has the ability to grow in temperature ranging from 24^0C to 32^0C (Tong *et al*. 1997, Lakra *et al*. 2006) [13, 14]. Taju *et al*. 2013 [15] developed a gill cell line by explant technique and maintained at an optimum temperature of 28^0 C and supplemented with 15% FBS. In the present study the optimum temperature and FBS concentration for the initiation of primary cultures were 28 ^{0}C and 15 % respectively which is in accordance with the previous study by Taju *et al.* 2013 [15].

Hameed *et al*. 2006 [16] reported the presence of both epithelial and fibroblast-like morphology in the initial stage of primary cultures of SISK cell line, but their morphology turned into only epithelial type after 20 number of passages. [16]. In the present study, the morphology of the primary explants of caudal fin and heart tissues comprised of mixed population i.e both epithelial and fibroblastic-like. Such heterogenous population of cells in the primary cultures were already reported by many researchers and the morphology of the cells predominates into single type either epithelial or fibroblastic as the culture progress (Swaminathan *et al.*2010) [17].

Conclusion

The present study reports the establishment of primary cultures from the caudal fin and heart tissues of *P. hypophthalmus* by explant outgrowth method. The caudal fin and heart tissues are easy to isolate compared to other visceral body parts. Hence this study provide scope for the development of cell lines from the fin and heart tissues of *P. hypophthalmus*. In the other hand the established primary cultures would also be beneficial in assessing the toxic effluents of aquatic pollutants.

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