TISSUE CULTURE IN PEARL OYSTER

S. DHARMARAJ AND C.P. SUJA

Central Marine Fisheries Research Institute,
Tuticorin Research Centre, Tuticorin - 628 002

INTRODUCTION

The first work on cell culture in marine molluscs started since 1960s. Many researchers attempted to improve the culture media composition by adding vertebrate sera as growth factor. In primary cultures, the tissue dissociation methods and medium composition were frequently complemented with homologous and heterologous substance. Inspite of such improvements, establishments of marine molluscan cell line or long-term primary cultures are rather limited. This is mainly because of some contaminant micro-organisms and protozoans and lack of data on molluscan cell physiology and biology. The chief difficulty for the cultivation of new medium components was related to available methods for cell viability analysis. In *in vitro* culture, cell survival appeared greatly variable according to its nature and degree of differentiation.

Works on marine invertebrate tissue culture has been initiated at the Tuticorin Research Centre of CMFRI, Tuticorin since 1995. A well established tissue culture laboratory has been set for the purpose of culturing mantle tissues from the pearl oyster. The laboratory consists of office-cum-record room, preparation room, dressing room and clean (operation) room. The rooms are arranged in different modules to avoid contamination from one room to another. A strict hygienic condition is kept up at all times. A pass box is constructed to store sterilised materials. It has one UV lamp on its top which keeps the materials in the chamber sterile. It has three doors; one in the preparation room, the second in the dressing room and the third in the clean room.

PREPARATION OF CULTURE MEDIUM

The culture media are developed based on the analysis of inorganic ion and free amino acids concentration of the body fluid of an animal. Vitamins and the other minor constituents of the medium were supplemented to the culture medium.

The medium Pf35 was developed exclusively for pearl oyster cell culture. The other culture media used for marine invertebrate tissue culture are Medium 199, L-15 and Ham's F-12. These culture media may be prepared as per the available formula or may be procured as ready made items from scientific companies.

Depuration of pearl oysters

The oysters to be taken for the experiment are utilised within 24 hr. of harvesting. They are kept in UV treated running seawater for a minimum period of 3 days. Everyday the oysters and the tank are cleaned with detergent powder.

Preparation of oysters

1. Depurated oysters immersed in 70% ethanol for 15 seconds.
2. The shell is allowed to dry and the oyster is cut into two halves by a sterile knife.
3. The mantle tissue is removed and the pallial organs are cut and discarded.
4. The mantle tissue is cut into 1mm explants with sterile scalpel.

Preparation of tissues

1. The cut pieces of the mantle (explants) are washed six times in 10 ml sterile seawater (SSW) or balanced salt solution (BSS) in petridishes.
2. Explants are treated in 10% ethanol for 15 seconds.
3. Washed four times in 10 ml SSW.
4. Explants are again treated four times in antibiotic solution (Table 1) each 30 minutes duration for 2 hr.
5. Three washings in 10 ml SSW; now the explants are ready for culture.

Table 1. ***Combination of antibiotics***

Gentamycin	125 μg/ml
Polymixin	100 μg/ml
Neomycin sulphate	100 μg/ml
Kanamycin sulphate	100 μg/ml
Mycostatin	200 μ/ml
Fungizone	5 μg/ml
Pencillin	200 μ/ml
Streptomycin	200 μg/kml

After Stephens and Hetrick

Organisation of explant cultures

Explants are inoculated in sterile petridishes and TD flasks. 3 ml of culture medium with 20% foetal calf serum (FCS) is added. The cultures are placed in CO_2 incubator at 30°C. Cells migrate from the explant after 24 hr. Fibroblast like cells are seen in the cultures from day 9 onwards (Fig. 1).

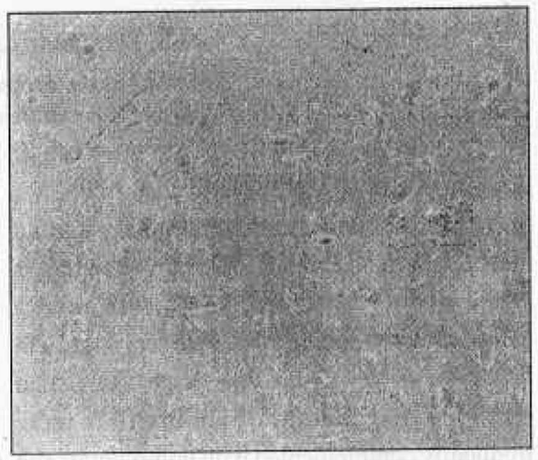

Fig. 1. ***Mass of cells dissociating from the explant of pearl oyster mantle tissue.***

Organisation of Cell Culture

Trypsinisation

The mantle tissues are subjected to trypsinisation. Cut pieces of tissues are transferred to trypsinisation flask containing 30 ml of Marine Mollusc Calcium Magnesium Free Phosphate Buffer Solution (MM CMF PBS) with 0.05% trypsin. A teflon stirrer is placed in the flask for proper dissociation of cells from the tissues. Stirring is done for 10-15 minutes at 1200 rpm. The cell suspension was first filtered through 150μ and 60μ sieve. The filtrate is centrifuged at 4°C for 5 minutes at 800 rpm and the supernatant solution is removed gently without disturbing the precipitate. A drop of medium is added to the precipitate and mixed well. The mixture containing free cells is distributed to culture dishes (Fig. 2). 3 ml medium is added to each flask and incubated in CO_2 incubator at 30°C.

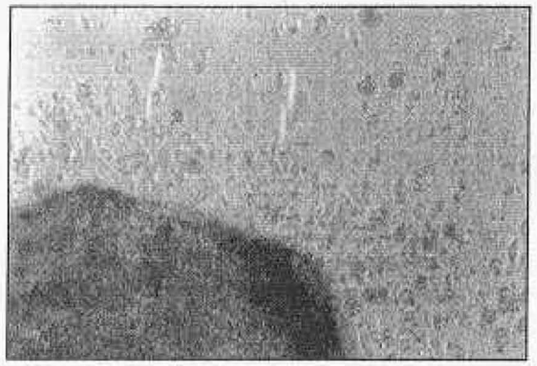

Fig. 2. ***Free cells moved away from the explant.***

If there is any contamination in the culture, the medium is removed and the cells are washed with SSW. After ensuring that no organism is there, the cells are removed from the flask and inoculated into fresh flasks or cell well.

Cell well culture

The cell well is also called as microplate. The microplate with 24 wells and 96 wells is available. It is disposable after use. It is provided with a lid. The cell well is used to culture single cell for cloning purposes. 3 or 4 drops of medium is added to each well. The cell well is placed in CO_2 incubator.

Medium change

Medium change is done on alternative days. The periodicity of medium change is decided on the basis of the condition of cells. Half the medium is changed for the first and second time and the whole medium is changed subsequently. At times cell suspension is centrifuged and fresh inoculation is done. In some of the established cell lines, the cell will be active and hence the entire medium is changed. When the cells are weak in a cell line, only half of the medium change is done. Frequent medium change is needed to establish cell line.

Cell counting

Cell counting is carried out with haemocytometer. It is very essential to fix the optimum cell density in each type of culture vessels. Variation in cell density may affect the growth of cells. The rate of proliferative cells in a cell population is calculated by counting the number of colonies formed by the cells.

Application of Tissue Culture Techniques

There is an increasing trend in the case of tissue culture in various fields of biological research. Tissue culture techniques are being developed in marine invertebrate animals only in the recent years. Valuable information is being gathered on the aspects of cell structure, cell division, cytogenetics, cell physiology and cell viability. Tissue culture techniques are used in studying the structural and functional aspects of cells, tissues, organs, etc. by *in vitro* studies. The study on the effect of chemicals and radio elements on normal tissues and cancer cells are being taken up in tissue culture. Study on pathological organisms in culture techniques has led to curing of several diseases and production of vaccines. Careful studies in tissue culture would be useful in transplantation of tissues/cells among members of a species or from species to species.