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Short Communication

Fish Stem Cell Culture

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Abstract

Stem cells have the ability undergoes a self-renewal and differentiation. Medaka ES cells are excellent model system for differentiation. It provides properties for tissue repair, replacement and regeneration. This culture techniques are used to treat cancer, wound healing, drug screening, autoimmune diseases, cardiovascular and neurological disorders. Many cell lines are completely reported. Stem cell cultures have been studied in 3 vertebrate organisms. These are mouse, human and small laboratory fish called medaka. We will focus our review on fish stem cell culture, its applications and advancement.

Keywords: Stem cells, Medaka, Cell Culture

Introduction

This mainly focus on zebrafish and medaka [1]. The stem cell characterization include the pluripotency identification of fish homologous/paralogs of mammalian gene's [1].

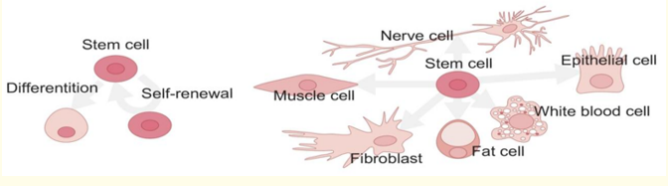

Figure 1: This culture requires high quality media. Some cell lines are immortal.

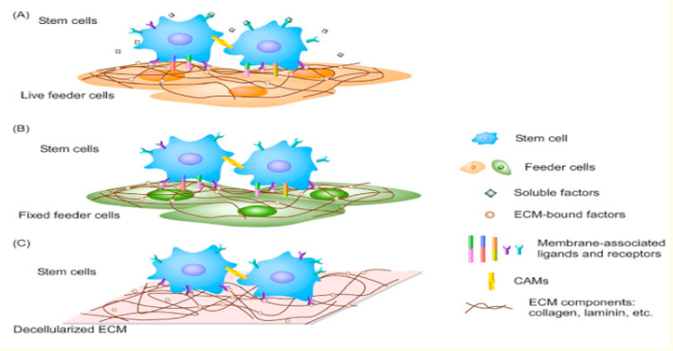

Figure 2

Methods

Major challenges in spontaneous differentiation are ES cell derivation [1]. Feeder layer techniques has developed [1,2].

Derivation is the main reason for developing 3 ES cell line in 1996.These cell lines are MES1 to MES3 [1]. ES are induced to undergoes differentiation into various cell types [1]. Medaka ES cells are excellent model system for differentiation [1]. Telomerase activity is used to markers for ES cell culture [1]. Differentiation is detected by RT PCR analysis. Single cell differentiation level can be detected by immunostaining [1].

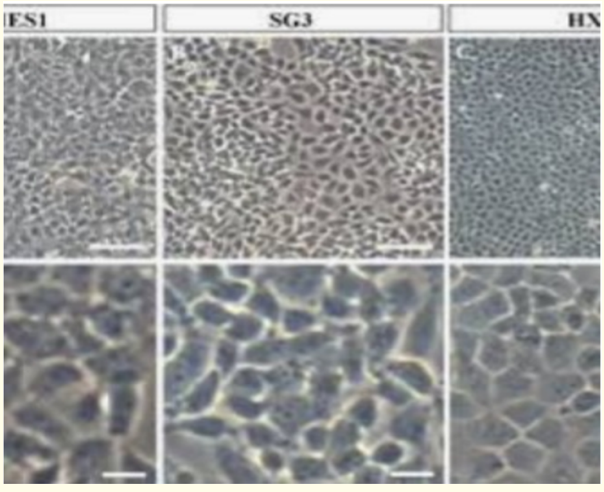

Figure 3

Examples of ES cell establishment in fish species are sea perch, sea bream, red sea bream. Stem cell culture are used in drug delivery or future therapeutic applications [1]. It provides properties for tissue repair, replacement and regeneration [3]. Stem cell transplants are used to replace the damaged cells and gene some patients a new immune system. 3D cell culture and CRISPR gene editing techniques may advance in stem cell research and treat disorders.

Types of stem cells

- Hemopoietic stem cell (HSCs)
- Mesenchymal stem cell (MSCs)
- Neural stem cell (NSCs)
- Embryonic stem cell (ESCs)
- Induced pluripotent stem cell (iPSCs)

Stem Cell Type	Description	Examples
Totipotent	Each cell has the capability to develop into a new individual	Cell from early embryos
Pluripotent	Cells can from any cell type	Some cells of the blastocyte
Multipotent	Cells can distinguish, but from different other tissues	Fetal tissue, adult stem cell and cord blood

Table 1

Primary germ layer consists of 3 parts. These are ectoderm, endoderm and mesoderm. Pluripotent adult stem cells are commonly random and occur in low amount. Goldfish and carp cell lines are used to study the tissue regeneration [5]. Feeder cells are composed of layer of cells. It can be unable to divide, which provides extra cellular secretion to help another cell to proliferate. It is a coculture of ESCs are fibroblast. Many cell lines are completely reported. Examples: Atlantic salmon and grass carp. Stem cell cultures have been studied in 3 vertebrate organisms. These are mouse, human and small laboratory fish called medaka [6].

Mouses cell culture

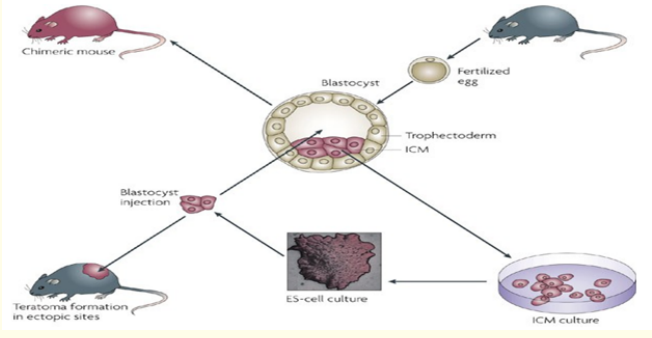

Figure 4: Mouse Cell Culture.

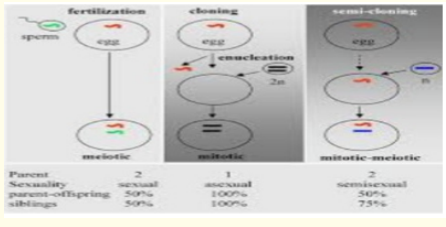

Figure 5: Reproductive Technologies.

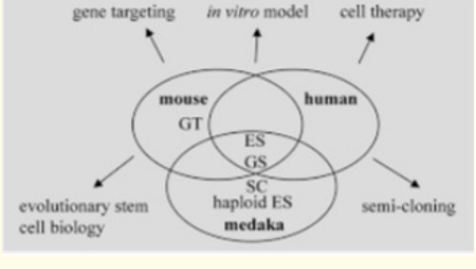

Figure 6: Stem cell technology in human & model organisms.

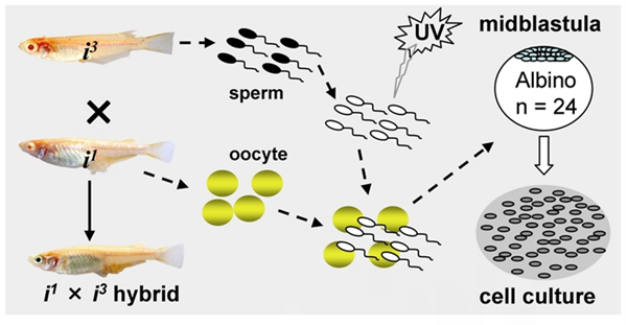

Figure 7: Procedure for medaka haploid es cell derivation.

Stem cell: Following fertilization the developing embryo contains a group of cells known as the inner cell mass that we eventually go on to form all tissues of the body however at this early stage of development these cells have not yet committed to become a specific type of cell is said to be in an undifferentiated state if the cells of the inner cell mass are isolated that they can grow infinitely in a plastic fish and maintain this undifferentiated state these cells are known as ES cells. Developing embryo ES cells retain the ability to form any type of cell in the adult body.

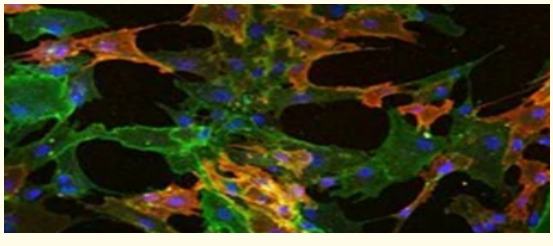

Figure 8: Studying the signals required to control how ES cells develop into specific cell types.

Protocol for es cell culture in fish

- Collection of the fertilised eggs
- Blastula stage embryos are observed under a microscope and embryos are immediately transferred to 35 mm small petri dish.
- A batch of 25 to 30 embryos are first disinfected with 70% ethanol and then wash five times with sterile phosphate buffer saline in the Petri dish.
- The inner cell mass is harvested by tearing the Chiron with fine forceps, and the chorion and eggshells are removed.
- Single cells are planted through gentle pipetting, and cells are transferred to a new gelatine coated cell culture flask under ES cell conditioned medium
- Maintenance of cells and characterization

Subculture

- Take 2 ml of trypsin = EDTA, 5 ml of buffered saline solution, 4 ml of trypsin neutralizing solution.
- Rinse the flask with 5 ml of BSS and remove the BSS from the flask
- Then 2ml of trypsin/EDTA solution is added to the individually T25 flask. Permit the trypsinization to continue until nearly 90% of the cells have been detached from the surface. Hit the flask on the palm of the hand to detach the detached cells. If only a few cells detach, wait 30 seconds and repeat this
- After cells have been detached, neutralizes the trypsin in the flask with 4 ml of trypsin neutralizing solution.
- Transfer the detached cells to a strike 15ml centrifuge tube and rinse the flask with a final 2ml of BSS. Add this rinse to the centrifuge tube.

Conclusion

The important role of stem cell is produced new skin tissue, collagen production. It also promotes hair growth. Stem cells are important for living animals for many purposes. Scientists will be able to find these how stem cells transplants replace the damaged cells [1].

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