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Expt → Primary Cell Culture using Chick Embryo

Aim

To isolate and culture the chick embryo fibroblast under aseptic condition.

Principle:

Embryonic tissue of chick is used for the primary culture because cell viability is higher. Fibroblasts are cells widely used in cell culture, both for transient primary cell culture or permanent as transformed cell lines. Chick embryo fibroblasts (CEF) are useful cellular reagents in virology as they support the growth of many human and animal pathogenic viruses. Embryonic eggs are produced commercially through the process of incubation, which allows an egg (ova) develop outside of mother's body, thus making it available for outside intervation.

Trypsin is a digestive enzyme which breaks down proteins in the small intestine, secreted by the pancreas as trypsinogen. When added to cell culture, trypsin breaks down the proteins that make cells able to adhere to the vessel.

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Requirements:

- Eight day old embryonated eggs
- Sterile PBS
- MEM supplemented with 10% FCS
- Trypsin
- Culture flask - T-25
- Scissors
- Forceps
- Petriplates
- Beaker covered with cheese cloth.

Protocol:

1) An eight day old embryonated egg was surface cleaned with 70% ethanol.
2) The egg was cut open at top using a pair of scissors, & then with the help of forceps the embryo was removed and placed in the petriplate containing PBS.
3) The embryo was washed with the PBS and then transferred to another petriplate containing PBS.
4) The head, appendages and visceral organs were removed from the embryo.
5) Once it was cleaned, the remaining of the embryo were transferred to another petriplate containing PBS & minced thoroughly with a

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pair of bent scissors.

6) The suspension of minced tissue was poured into the beaker containing equal amount of trypsin solution (0.25% trypsin in PBS)

7) It was stirred at 37°C for 30 min using a magnetic stirrer.

8) The cell suspension was filtered through sterile cheese cloth and the filtrate was collected in a beaker.

9) The filtrate was centrifuged at 1200 rpm for 10 min.

10) The supernatant was discarded and the pellet was re-suspended in 5ml of PBS and centrifuged at 1200 rpm for 10 min. The resultant supernatant was discarded and the pellet was suspended in 1ml of medium.

11) After performing cell counting, the cells were seeded in a 25 cm^2 culture flask with the cell density of 2×10^5.

12) The seeded flasks were incubated at 37°C in a CO_2 incubator for 24 h.

Result :

Types of Contamination : Bacterial contamination, mold & virus contamination, Mycoplasma contamination, Yeast contamination.

- **Cell morphology**

Fibroblast cells are elongated cells while epithelial cells have cobblestone shape.
During fibroblast cells proliferation, we can also notice growth epithelial cells

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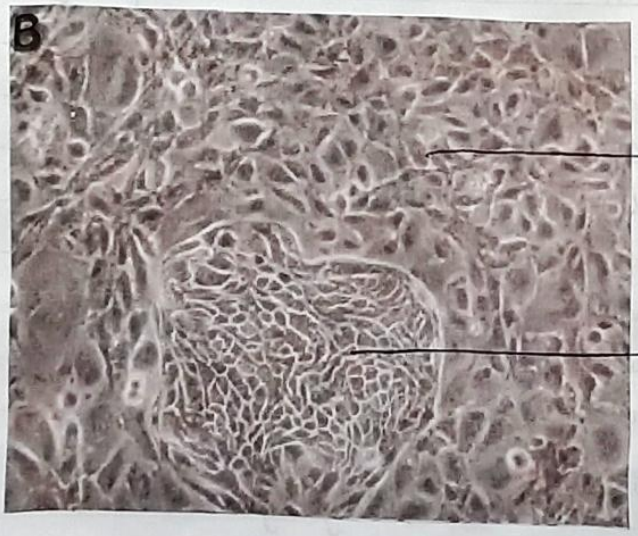
Primary cultures 1 week post-isolation

[A] Fibroblasts cells are elongated cells, while epithelial cells have a cobblestone shape.

[B] Epithelial cell nests embedded in a group of larger epithilial cells.



About Fibroblasts cells:

[1.] There will be few viable fibroblast cells after isolation from chick embryo.

[2.] Those cells will be seeded or cultured in tissue culture flask or plates to expand the population in CO_2 incubator for 24h - 48h.

[3.] During fibroblasts cell proliferation, we can also notice growth. epithelial cells are cobblestone shape whereas fibroblasts cells are spindle shape.

[4] The fibroblast cells are used for virus culture and vaccine production.

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Lab Exercises

Q1. What will be the result if we use 15 days embryonated egg for primary cell culture?

→ If a 15 day old embryonated egg was taken, it would be almost developed as an embryo takes around 3 weeks to completely develop into a chick.

Q2 Why fibroblast cells are multinucleate?

→ Multinucleate giant cells are important mediators of tissue remodeling and repair and are also responsible for removal of foreign material, bacteria, pathogens etc.

Discussion

- Different types of cell lines obtained in fibroblast.

→ F311, F253, FA147, FA150, FA121.

- Applications of fibroblasts → virus culture & vaccine production.

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