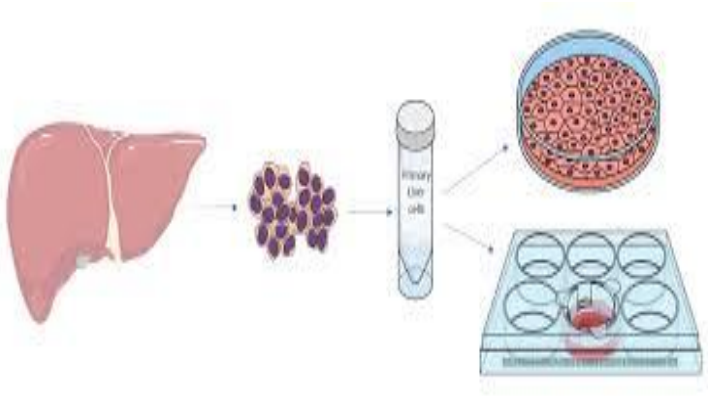
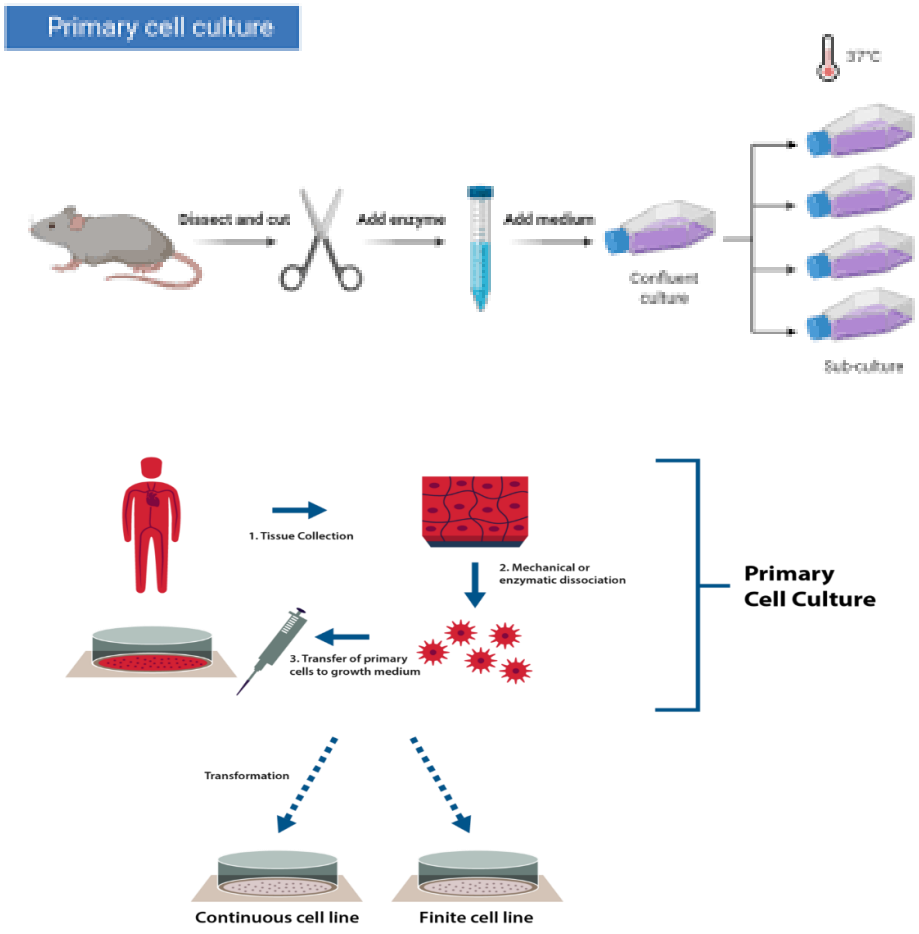
Animal tissue culture – Lab 3



Primary Culture

Primary cell culture:

- When cells are surgically removed from an organ and placed into a suitable culture environment, they will attach, divide and grow. This called primary culture
- This is the first culture (a freshly isolated cell culture) or a culture which is directly obtained from animal or human tissue.
- The primary culture aims to harvest the interested cells from the animal tissue.
- The cells from primary are typically slow growing, heterogeneous and carry all the features of the tissue of their origin.



primary culture can be obtained via 3 approaches including:

- **Mechanical disaggregation**:

- is used for processing soft tissue samples (e.g. brain, spleen, liver, soft tumors, etc.). In this technique soft tissue samples are carefully sliced into pieces. Cells are then collected by forcing the tissue fragments either through a syringe and needle or through a series of sieves of progressively smaller mesh size.
- Its act cheap method but may lead to lost some of sample.

- **Enzymatic disaggregation**

- is commonly used to process tissue samples when a high recovery of cells is necessary. This technique uses enzymes, such as trypsin or collagenase, to digest pieces of tissue in order to release cells.
- Term trypsinization use when use trypsin to disaggregate tissue,
    - There are two techniques of trypsinization-warm trypsinization and cold trypsinization.

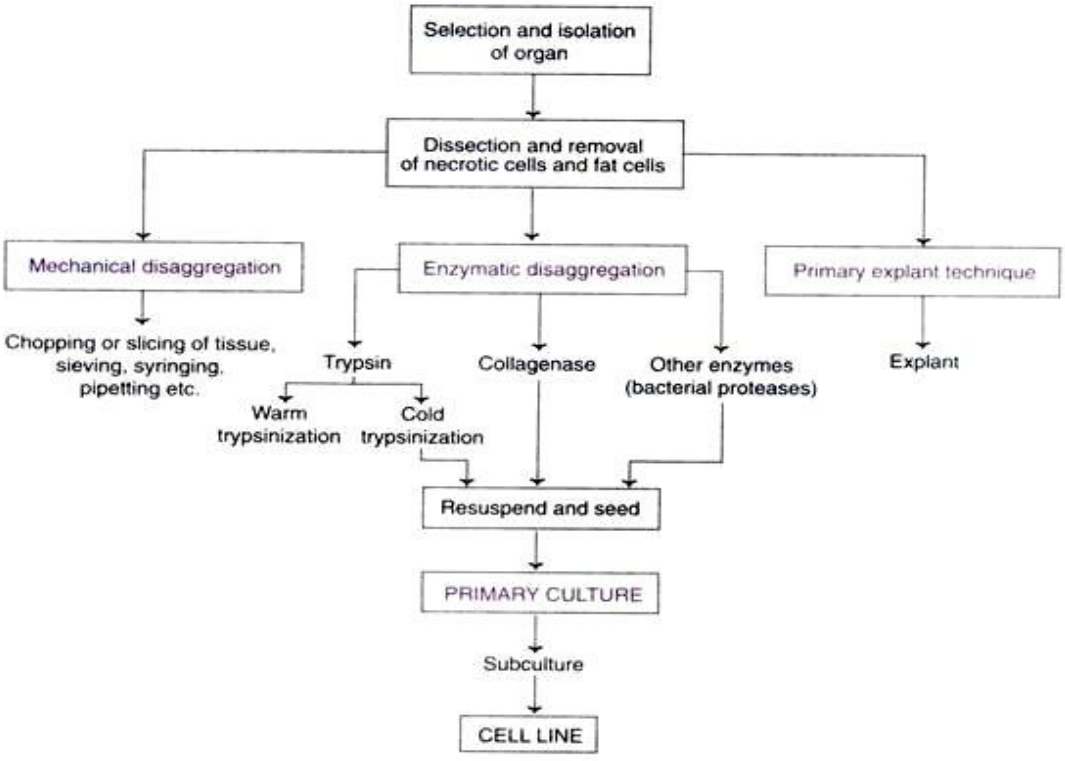


Fig. 36.1 : Different techniques used for primary culture.

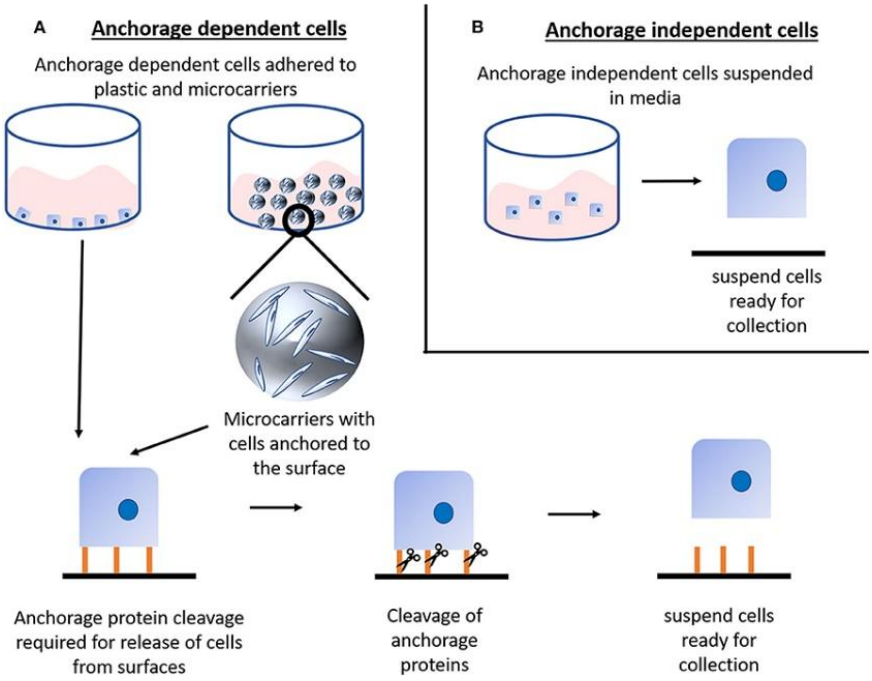
- **Discus the different between warm and cold trypsinization.**

➢ **Explant culture:** is a technique used for culturing tissue pieces themselves.

Types of cell culture systems.

• **Anchorage-dependent (Monolayer cultures or Adherent cells**): Cells shown to require the attachment for growth. They are usually derived from tissues of organs such as kidney.

• **Anchorage-independent or (Suspension Culture**): Cells which do not require attachment for growth. They are derived from cells of the blood system.



Practical parts

Mouse Embryo Fibroblasts:

Mouse embryos of age 13-15 days are needed for culture. To get these, Swiss mice were kept for mating and the gestation period was times by designating the day of finding the genital plug as the first day of development.

1. Sacrifice the pregnant mouse by cervical dislocation. Place the animal in supine

position.

2. Swab the abdomen with 70% ethanol and cut open along the midventral line.

3. Remove uterine horns and transfer into a beaker containing PBS. Transfer into flow hood immediately.

4. Transfer the uterine horns into Petri plates containing PBS inside the flow hood and cut open the uterine horns to remove embryos.

5. Wash the embryos with PBS and remove head, visceral organs and appendages.

6. Transfer the remains of the embryos to another Petri plate containing small amount of PBS and mince thoroughly with a pair of bent scissors.

7. Transfer the minced tissue to the trypsinization flask containing 40 ml of 0.25% trypsin in PBS.

8. Stir the contents at 37ºC for 30-60 mins.

9. At the end of the above period add 5ml of medium containing serum and stir the contents for 2 more minutes to inactivate the action of trypsin.

10. Filter the cell suspension through sterile cheese cloth and collect the filtrate into a 100 ml conical flask.

11. Centrifuge the filtrate at ~ 1000 rpm for 10mins.

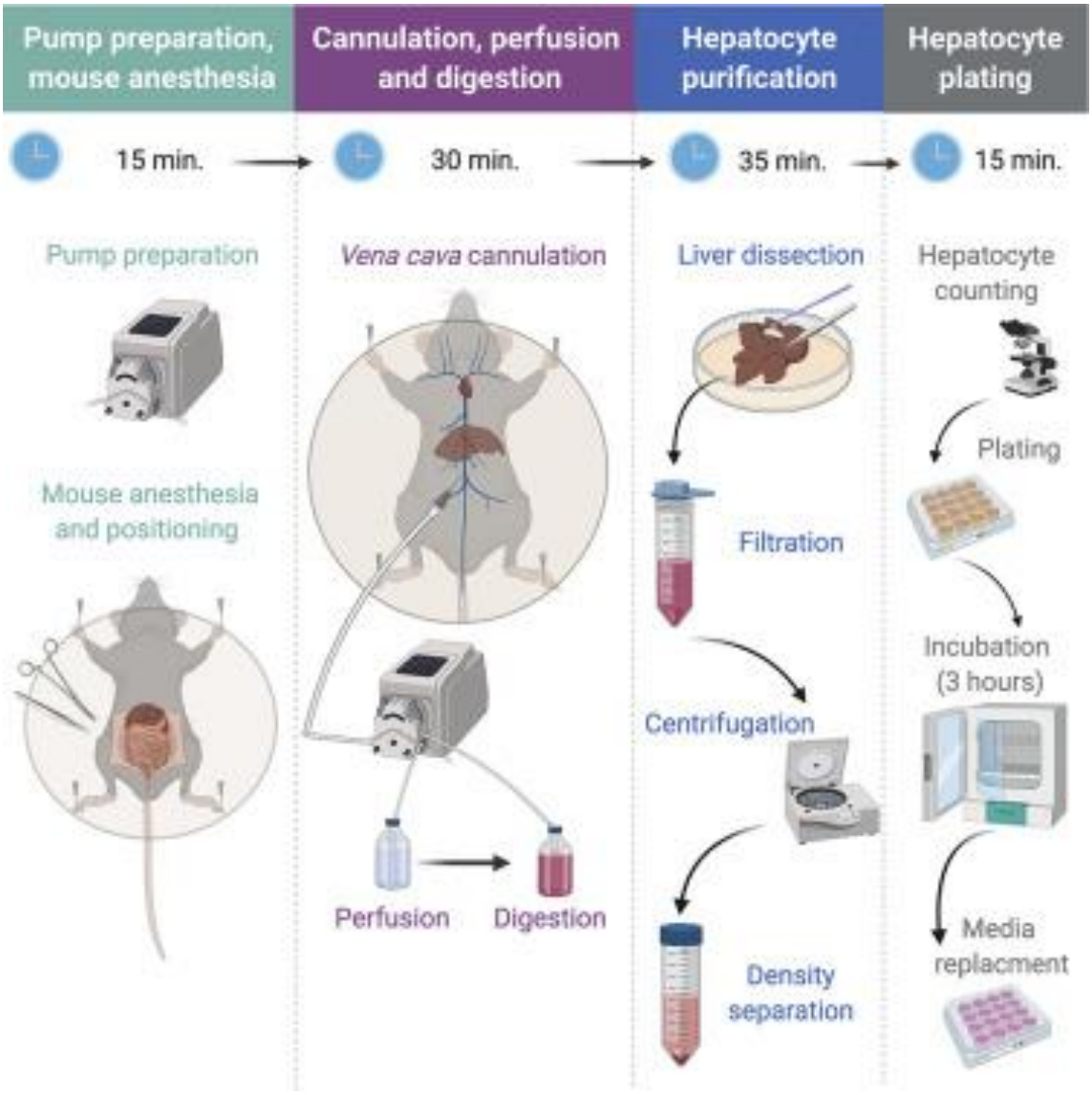
12. Pour out the supernatant and resuspend the pellet in 5 ml of medium.

Distribute equally to all the 120cm2 culture bottles and incubate at 37ºC.

Chick Embryo Fibroblasts:

The procedure for culturing CHF is the same as for MEF. Briefly

remove embryos from 8-10 day old embryos, break the shell with the help of forceps and transfer the embryo into Petri plates containing PBS and follow steps 5 to 12 of mouse embryo fibroblasts culture (see above). Although the chick embryo cells will grow in the same medium, they will grow better if 1% chicken serum is also added.


Pump preparation, mouse anesthesia
Cannulation, perfusion and digestion
Hepatocyte purification
Hepatocyte plating
15 min.
30 min.
35 min.
15 min.
Pump preparation
Mouse anesthesia and positioning
Vena cava cannulation
Perfusion
Digestion
Liver dissection
Filtration
Centrifugation
Density separation
Hepatocyte counting
Plating
Incubation (3 hours)
Media replacment