**Cell Biology and Physiology Lab**

**Establishing a Primary Cell Line**

Establishing a Primary Cell Line Using Chick Embryos

* Isolation of the embryos
  + Materials:
    - PBS with high concentration of antibiotics
    - 70% ethanol
    - Small beaker or egg cup to hold egg
    - Forceps (sterilized), beaker of alcohol to dip them in
    - Sterile petri dishes
    - Eggs, 10th day of incubation, humid incubator to incubate the eggs
    - Dissection tray or large petri dish (that has been sterilized)
  + Procedure:
    1. Incubate the eggs at 38.5C, turn daily
    2. Swab the egg with alcohol and place in egg cup
    3. The rest should be done in the hood
       - However, it can be done on lab bench if space is issue
    4. Crack the top of the egg and peel off the shell to the top of the air sac with forceps
    5. Using another pair of sterile forceps, peel off the shell membrane to reveal the chorioallantoic membrane
    6. Gently pour contents into sterile dish
* Isolating Chick embryo organ rudiments
  + Materials:
    - Dissecting scope
    - PBS with antibiotics
    - Media with high concentration of antibiotics
    - Trypsin
    - Petri dishes
    - Sterile scalpels
    - Sterile forceps
    - Pipettes
    - 50ml centrifuge tubes
    - Culture flasks or petri dishes
  + Procedure:

1. There are a couple of ways to do this. Choose what organ/tissue you would like to dissect out
2. Extract that organ or tissue using scalpel and forceps, place in 50ml centrifuge tube
3. Add PBS to rinse organ, discard PBS
4. Add 3-5ml warm trypsin and incubate for 20min at 37C
5. Pipette media up and down to disperse the tissue, allow large pieces to settle to bottom
6. Collect supernatant (this now has your cells you want) CAREFULLY in new 50ml centrifuge tube (Label this tube CELLS)
7. In tube with tissue, repeat again steps 3-5 (trying to collect more cells)
8. CELLS tube – centrifuge 1200rpm for 5 minutes
9. Resuspend pellet in culture media
10. Transfer cell solution to sterile petri dish or flask and incubate
11. Change medium as required and observe for growth

 

 