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Cartilage and muscle

We will try to isolate some intact cells from the epiphyses of long bones or sternum of chicks age 12 day or 10 day. Cartilage cells are embedded in an extracellular matrix containing cadherens, CAMs and collagen and glycoseaminoglycans. It can be dispersed using Ca++,Mg++ free saline (CMF-PBS) containing collagenase types 1 and 2, and 0.25%trypsin, EDTA pH 7.6, releasing the cells. This is best done in chick phosphate-buffered saline. We will not use sterile procedures, so we will add penicillin and streptomycin to our media for growing the cells. We will also try muscle at the same time.

Before you start. Place dissection tools in 70% alcohol. Wash off your desk top and dissection microscope stage. Place a few paper towels where you will work, have three new petri dishes, two culture dishes and four new centrifuge tubes ready.

1. Swab the eggshell with 70% alcohol after candling to be sure an embryo is present. Open the egg. Just tap the round end with scissors, cut away the shell so that the embryo can be seen in the shell.
2. Then remove the embryo to a sterile petri dish and then cut off the limbs and tear off the skin with forceps. Remove breast muscle by cutting along sternum and tearing off with forceps. Separate the muscle into one petri dish and the skeleton into another.
3. Cut them into small pieces with a clean scalpel. Place all the pieces of cartilage into a micro-centrifuge tube and all the muscle into another. Add enough trypsin solution to cover- probably 0.5 ml. Vortex. Place in water bath or incubator at 37 degrees for 15 min for cartilage and 1/2 hr for muscle. Remove tube and vortex every ten minutes during digestion. For all centrifugations use 3 min at highest speed on microfuge.

For cartilage after trypsin, centrifuge tube for 3 min, decant and discard supernate. Add CMF-PBS to wash off trypsin. Vortex. Spin, discard supernate. Add collagenase-pour into tube with cells to cover them, Vortex. Place back at 37 degrees for 40 min, vortex every 10 min

4. After digestion is complete, let any pieces settle, spin down the cells at full speed

3 min and if you can see a pellet, decant off and discard the supernate.

5. Wash cells in the bottom of the tube in .5 ml CMF-PBS without enzyme, Vortex and spin down again. Decant fluid. Disperse the cells by adding to them 0.5 ml culture medium and Place into a labeled culture flask containing 5.5 ml culture medium with a tight fitting lid..
6. Place into incubator and leave until next lab. Make sure the tube is lying on the side with the removable side down so we can fix the cells later and stain them

with antibody.

Materials needed FOR CHICK TISSUE CULTURE

- Dissection tools: scalpel, scissors, forceps, fine forceps
- 70% alcohol to sterilize microscope and tools and egg
- sterile petri dishes 4/group
- micro centrifuge tubes- 2 per group with 0.5 ml 0.25% trypsin isolation medium
- microcentrifuge
- collagenase solution- 0.5 ml per sample of cartilage in sterile 15 ml cent tube
- CMF-DMEM for wash, 3 different times, so 1.5 ml/group, pour into sterile tubes.

Ca++,Mg++ free saline- DMEM with added NaHCo3, 50 uM ascorbic acid, P&S, 10% fetal calf serum, 0.02% EDTA pH 7.6 for isolate muscle and cartilage-0.25%trypsin, or containing collagenase - 0.5 ml per sample of muscle or cartilage modified F12 medium containing 3.6 mg/ml of collagenase (1.8 mg each types I and II) types 1 and 2, for releasing the cartilage cells. We will not use sterile procedures, so we will add penicillin and streptomycin to our media for growing the cells. We will also try muscle at the same time.

CMF-PBS for wash- 100 ml autoclave

- 0.806g NaCl, 0.020g KCl, 1 ml 1MPO4 buffer pH 7.5 (or .115 g Na2HPO4 and .020g of KH2PO4)
- 2004 used isolation medium for washes, dissolved enzymes in isolation medium

Myoblast culture:

MEM Earle's salts 100ml
Horse serum 12.5 ml
Penicilin&Streptomycin
58 mg l-glutamine
.3mg phenol red

vortex stirrer
microfuge
70% alcohol

- 37 degree incubator
- culture medium- 5.5 ml/ culture
- culture flasks

Each pair of partners: all sterile tubes

1. 2 microtubes with 0.5 ml trypsin
2. 2 microtubes with 0.5 ml collagenase
3. DMEM for wash after enzymes -3 ml in 15 ml cent tube
4. cent tube with 1 ml culture for muscle
5. cent tube with 1 ml culture for cartilage
6. 2 culture dishes containing 5.5 ml culture medium for separately muscle or cartilage