

## **Preparing Cells from Mouse Aortic Tissues**

(Jessica Wagenseil, 2005)

### Materials

1. Mice – anesthetized
2. Surgical tools- forceps, surgical scissors
3. 100x Penicilin/Streptomycin (P/S) solution
4. Collagenase Type I (CLS-1) – Worthington Biochemical Company, 158u/mg DW
5. Elastase (ESL)- Worthington Biochemical Company, 3.55u/mg Protein
6. Media with no serum: DMEM, NEAA, P/S, L-Glut
7. Media containing serum: DMEM, NEAA, P/S, L-Glut , 10% FBS
8. 22um sterile syringe filter
9. 20 mL syringe
10. 50 mL conical vials or 15 mL conical vial
11. Shaker at 37°C

### Methods

1. Dissect entire aorta and both carotid arteries and place in 35mm dish containing media with no serum.
2. Wash in 100x P/S.
3. Place dissected aortas on ice until all aortas are ready for digestion.
4. Dissolve collagenase (0.125 mg/mL) and elastase (0.125 mg/mL) in media containing no serum in a 50mL conical tube. Prepare 1 mL solution for each aorta.
5. Sterile filter media containing collagenase and elastase into a new 50 mL conical tube.
6. Add dissected aortas to 1 mL sterile media in the 50 mL conical (1 per mouse) using forceps.
7. Incubate the 50 mL conical on shaker at 37°C for 90 minutes.
8. Centrifuge at 400 x g for 5 min (Normal for splitting cells).
9. Resuspend in media containing serum and plate cells in 35 mm dishes (1 mouse per dish).

### **2<sup>nd</sup> Procedure: Smooth Muscle Cell Isolation from Rodent Aorta**

A collagenase (10 mg; Sigma type I) elastase (2.5 mg; Sigma type III) mixture in 20 ml medium without fetal calf serum is added to aortic tissue that is cut into small pieces. The digestion is carried out for 30-45 min with stirring at 37 degrees C. The resulting cell suspension is centrifuged at 400 x g for 5 min., and the cell pellet obtained is washed twice with DMEM containing 3.7 g/l sodium bicarbonate, 20% fetal bovine serum, and antibiotics. The cell pellet is then resuspended in fresh medium and seeded at  $5 \times 10^5$  cells/25 cm<sup>2</sup> tissue culture flask and maintained for in DMEM + FCS.

### Reference:

Oakes et al. (1982) Eur. J. Cell Biol. 27:34-46

Barone et al. (1985) Biochim. Biophys. ACTA 840:245-254