Bovine Coronary Artery EC and SMC Primary Culture Protocol 1

1. Autoclave all instruments prior to removal of the coronary artery. Include a razor blade. Coat the 25 cm^2 flask with 1 ml of a 2% gelatin solution. The gelatin will need to be pre-warmed at 37 degrees C to liquefy it (After 30 minutes pour off the excess). Also pre-warm the holding and the feed media prior to removing the coronary artery.

2. Always wear gloves. When removing the coronary remember to be very gentle and not to pull on the artery. Keep it moist with the holding solution.

3. After removing the artery (you want the crown and the descending) place into a sterile 50 ml conical tube with 25 ml holding solution

4. Remove the coronary from the holding solution and place in a 150 cm dish with new holding solution. Remove all fat from and connective tissue from the artery. When clean it should be almost pinkish-white. Be careful not to nick the artery

5. At this point weigh out 125 mg collagenase A and dissolve in 50 ml of RPMI, also weigh out 1mg/ml of BSA and mix in with the RPMI-collagenase mixture. This needs to be sterile filtered through a 0.2 micron syringe filter. Use a 25 ml syringe. Sterile filter into a 50 ml conical tube.

6. Pour off the excess holding solution and drain the coronary. Shoot the collagenase solution through the coronary so the vessel is full. Cover and place in the incubator for 30 minutes. (37 degrees)

7. After 30 minutes pour the collagenase form inside the vessel into a clean 50 ml conical tube. Rub 5 times all the way down. Rinse with 6-10 ml of rinse solution (wash solution). Repeat once.

8. Spin for 3 minutes at ¾ speed.

9. After removing the collagenase, resuspend the pellet in 5 ml of EC feed media. Take that 5 ml of cells and put into a precoated 25 cm flask

10. To make SMC's take the coronary artery and cut it into 1cm x 1 cm pieces and place into a 60 mm dish lumen side down. Then add 4 ml of SMC feed media gently so as not to disturb the tissue.

11. Note that the amount of collagenase A is determined for Lot # GMB. Any other lot number will have to be determined.

Culturing Procedure for Bovine Coronary Arteries (Protocol 2)
(6 Hearts)

Before starting dissection, make the solutions up!

A. Wash Solution: (2x – 100 ml) RPMI + 2 ml Ab (Antiobiotic/Antinycotic) + 300ul Gentamycin. Filter before using any antibiotics.

B. Collagenase Solution: (1x – 50 ml) RMPI set aside for later use (incubate this). When enzymatic solution is needed, weigh out collagenase and add to pre-filtered M199 media for .15% solution. Mix well, and let set for a few minutes before using. NOTE: It has been found that 0.2 g of 188U/mg collagenase works extremely well. You may need to adjust this if the collagenase U/mg differs. (160 mg/per 50ml RMPI) (not same collagenase).

C. ***Different Collagenase solution*** 35 ml RPMI + 35mg collagenase + 35 ml BSA. Filter and set aside for later use. When enzymatic solution is needed, add 80 mg collagenase A to 35 ml pre-filtered RPMI media. Mix well and let stand 3 minutes before using

D. Holding Solution: (2x – 100 ml) 5% FBS + 2ml Glutamine + 300ul Heparin + RPMI qs + 1ml Ab + 300 ul Gentamycin.

E. ***Different Holding Solution*** 100 ml (take 25 ml into each 50 ml tube –do 2 times, for outside use) 5% FBS (5ml) + RPMI (95 ml) + 2ml Ab + 300 ul nystatin + 300 ul Gentamycin…Filter before adding antibiotics

F. EC Plating/Feed Media: 78 ml RPMI + 25 % (15ml) for EC with Novacell + 1 % Glutamine (1ml) + 1 Ab *Filter before adding antibiotics*

G. SMC Plating/Feed Media: (100ml)
M199 (89 ml) + 10% FBS (10ml) + 1% Glutamine + 1ml Ab + 300 ul Nystatin + 300 ul Gentamycin + 100 ul Tylosin *filter before adding antibiotics

*Filter first using a .45 micron filter and then by using a .22 micron filter.
*Filter using a .22 micron filter; one filter may be used for solutions: A, B, C, D, and E if filtered in order from lowest [serum] to highest [serum].

Hearts: Remove circumflex and LAD (left anterior descending) avoiding tears and punctures. Place vessels into 50ml centrifuge tubes containing 22-25 ml holding media. (one tube per one hearts vessel)

PREP: On a clean sterile surface, lay out vessels one at a time and trim off fat and connective tissue. (Make sure instruments are sterilized in autoclave). Store vessels in 50 ml centrifuge tubes containing 22-25 ml holding media until

until enzymatic step. When all vessels are clean, place vessels in a sterile petri dish containing sterile guaze soaked in wash media. Continue process, keeping vessels moist. (Cut vessels in convenient size)

Prepare 2 syringes (12 ml) with 18 gauge needles and platic tubing on tips. One will be used for wash solution and one for collagenase solutiuon.

Fill Lumens of vessels with collagenase solution; incubate in covered petridish for 20-30 minutes.

After incubation, empty contents of each vessel (by squeezing) into a 50ml centrifuge tube containing 7.5 – 10 ml plating media/50ml tube. (One tube per vessel contents from one heart. Rinse 2x with wash media, massaging each vessel 10x per rinse. (Retain vessels in holding solution for smooth muscle cell extraction.

Centrifuge 4 minutes at ¾ speed. While cells are spinning, calculate the number of flasks needed: use ratio of 3 hears/ 25 cm^2 flask. If not tissue culture treated, warm gelatin (1% solution), coat the bottom of each flask, and label. 1 heart = T25 flask which has been coated with 1% gelatin.

When cells are finished spinning, aspirate off supernatant and resuspend pellet in calculated amount of plating media; (2.5 ml plating media/50 ml tube) Combine suspensions, then add calculated amount of volume of plating media to result in a total of 5ml/25cm2 flask. Plate 5 mls of cell suspension to each 25 cm2 flask (with vent cap) ***Remember*** to aspirate off gelatin before applying cell suspension. Cap flask loosely and allow adequate O^2, incubate at 37º in 5% CO^2.

After initial culturing of BCA cells, wash and feed cells 24-48 hours after initial culture and geed only about very other day thereafter until cells are confluent. As cells reach confluency you may need to feed daily as the cells use up their media at a much faster rate

Smooth muscle cells:

While endothelial cells are finished spinning, prepare smooth muscle cell extraction. Remove vessels from holding solution and cut vessels to flat square sizes. Place vessel pieces, lumen side down, onto 15mm petri dishes (1 petri dish/1 hearts vessel(s)). *Outer surface of vessels are shinier than lumen surface.

Add 5ml plating media/petri dish. Incubare at 37ºC in 5% CO^2