MAEC ISOLATION (adapted from Core Lab Cardiology)

Materials

Matrigel (BD Biosciences, Fisher cat# 354234)(maybe less $ directly from BD)
DMEM (low glucose) with D-Valine (Specialty Media cat#: D-087-B)
ECGS
Heparin
Pen/Strep/Fungizone
Thymidine (Sigma cat # T9250)
Heat Inactivated FBS
Glutamine
Dissecting Microscope, sterile tools
Dispase (Fisher cat # 354235)
EGM-2 Endothelial Cell Medium supplemented with SingleQuots (Clonetics but the whole kit (media and supplement kit referred to as Bullet Kit can be purchased from Fisher cat# NC9525043) Supplements make medium 2% FBS we add 10% more.
Add also pen/strep (no Fungizone here)
100 mm Petri dishes, 6 well plates
PBS warmed

METHODS

•The night before MAEC isolation chill pipettes and 6 well plates in frig
•Thaw Matrigel on ice at 4° C overnight

Prepare Matrigel plates

On the day of isolatioin, remove a chilled plate and pipette from frig. Add 1 ml of Matrigel(no dilution) to one well of the 6 well plates (1 well/ aorta) on ice. Avoid bubbles. Spread Matrigel as evenly as possible by swirling plate. Place plate at room temp to solidify. Place in CO2 incubator.
Have ready: 100 mm Petri dish/aorta w 2ml PBS in center of plate in tissue culture hood.
100 mm Petri dish/aorta w 10 ml PBS at microscope

Prepare MAEC Media (Usually 10ml each harvest)

DMEM (low glucose) with D-valine (replacing L-valine with D-valine is thought to inhibit the outgrowth of fibroblasts)
ECGS (final concentration of 30ug/mL)
Heparin (final concentration of 50 ug/mL)
Pen/Strep Fungizone (1/100 dilution)
Thymidine (final concentration of 2.4ug/mL)
Heat Inactivated FBS (15%)
Glutamine (2mM)
Filter sterilize. ***Make fresh media every time. Use for one week.***

Created by Tom Chen, Professor Iruela-Arispe lab *February 21, 2006 7:08 PM*

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Dissection

Euthanize the mouse using Isoflurane Dip mouse in 70% EtOH

Expose the thoracic aorta. With aorta is still attached, carefully remove as much fat and connective tissue from a 1.5 cm segment as one can with the naked eye.

Remove and place in 100 mm dish with PBS.

Under a dissecting microscope further clean the aorta until all fat and adventitia is removed.

In a TC hood, transfer the aorta to a clean 100mm dish with 2ml PBS in center.

Cut the aorta into small pieces 1-2 mm (avg of 8-12 pieces/ aorta) using new scalpel.

Place the aortic ring segments close to each other on the Matrigel. ***Work Quickly. Dissection to Matrigel should take10-15 min.***

Initially add just enough media to keep pieces wet (100-200 ul). Adding too much media will float the pieces.

Incubate in CO2 incubator. At the end of the day, after the pieces had some time to adhere, add a little more media. The segments should be just covered.

Sterilize all surgical equipment between mice.

MAEC maintenance

Incubate plates for 3-5 days. Check media level and cell growth each day. Add media whenever necessary to keep segments just covered. One should be able to see endothelial cells migrate out of aorta and attach to Matrigel in 2-4 days.

After 3-5 days (growth varies from mouse to mouse), remove segments with sterile forceps. Add 2 ml of media and let cells grow out for another 2-3 days.

Passage the cells immediately if tubule formation starts. Avoid this if possible.

This all takes about a week.

Initial passaging of the MAEC (dispase treatment)

Gelatinize (0.1%)12.5 flasks (for @ least 30 minutes) Rinse with PBS

Remove media carefully, wash each plate 2 times with 2-3 mL of warmed PBS

Add 2ml of dispase and incubate plate for around 20 minutes (usually less). Check under the microscope to make sure majority of cells are off. (Incubation time varies)

Add 2 ml of D-Val media (1:1) (to inactivate dispase) and transfer to a 15 ml tube.

Gently pipette up and down to break up the clumps.

Wash plate with another 2 ml of media and add to tube.

Fill the tube up to about 8 mL with PBS (Another 2ml theoretically)

Spin @ 1000 rpm for 5 minutes @ room temperature.

Resuspend the pellet in 4 ml of EGM-2 media and plate in T12.5 flask.

After 1-2 hrs replace medium. When nearly confluent passage using Trypsin/EDTA and standard protocols