**Modified Matrigel Assay**

Lee, Sunyoung
2-17-06

## A. Materials required:

Animal
Matrigel, BD Biosciences (cat # 356234), need to be thawed at 4°C
Low Endotoxin! Some batches have killed mice due to high endotoxin levels!
Sphingosine-1-P, Avanti Polar Lipids (Cat # 860492P)
Fatty acid free BSA, Sigma (cat # A8806-5G)
Your choice of growth factor (VEGF or FGF)
Prechilled Falcon tubes and 1ml pipet tips
Prechilled tuberculin syringes with removable needles
18G needles for injection
Avertin

Each animal will have two Matrigel implants of 250ul each.

## B. Matrigel Preparation/Injection:

1. Matrigel Formulations: Calculate 1ml needed for three injections at 250ul due to viscosity and mixing losses. For 20 animal injection: 20 animals X 2 sites/animal = 40 total injections /3 sites/ml needed = 15 ml total Prepare 0.5mg/ml FA-free BSA/Serum-free DMEM
2. Prepare 2mM Sphingosine-1-P in MeOH: stock
3. Prepare 0.5mg/ml Fatty acid-free BSA in serum-free DMEM
4. Prepare Sphingosine-1-P (final conc. 1μM) in “3”
5. To make 15ml total Matrigel at 10mg/ml (Note: do not go below 7mg/ml final concentration!), transfer 10 ml Matrigel Stock thawed at 4°C into pre-chilled 50ml Falcon tube on ice with 1ml cold blue tip and pipetman (do not use serological excess loss will occur)
6. Add 5 ml of pre-chilled “4” to “5”
7. Split into separate 1ml eppendorf tubes
8. Pre-mix 12ug bFGF with 7ul heparin (50ug/ul) and add to each tube (Note: this is essential. FGF is not effective without coupling to heparin! VEGF (use at 4μg/ml) does not require heparin, though). Mix by Careful inversion. No Bubbles!!
9. Anesthetize animals with 300-500ul/animal prior to s.c. injection of Matrigel
10. Load pre-chilled (ice) tuberculin syringes (remove needle while filling) place on 18G needle for injection
11. Inject Matrigel on left and right centers of back. Typical maximal response is 7d post-injection

**C. Matrigel Angiogenesis Harvest Protocol:**

1. Sac animals with CO2, dissect off skin to reveal Matrigel pellets.
2. Take gross pictures to record peripheral angiogenesis.
3. Implants are collected (with the overlying skin and pinned out), fixed in 4% paraformaldehyde in PBS O/N and then washed in PBS 2X and stored in 70% ETOH at 4 C until processing for paraffin sections.
4. Alternative for fluorescent imaging and confocal analysis. IV inject 200ul FITC-Dextran (1x106mw) in PBS (30mg/ml). Wait 10-15mins. CO2 euthanize animal. Dissect skin/implant to expose matrigel plug and pin out. Fix for 15-30mins. Rinse with PBS and make sure to keep covered with PBS. Visualize gross vessel distribution with fluorescent dissection microscope. Dissect matrigel plug and skin with razor blade. Process half of the tissue by incubating 4hrs at 4°C with PBS:0.3M sucrose. Remove sucrose with Kimwipe and embed as cross-section into OCT. Cryosection and hydrate with PBS, coverslip with PBS:Glycerol and analyze with fluorescent or confocal fluorescent microscopy. Secondary staining with red fluorescent detection to evaluate other cell type or protein markers. The other half is processed for paraffin sections.