General Matrigel Angiogenesis Assay

Each animal will have two Matrigel implants of 250ul each. One with Matrigel/heparin and the other with Matrigel/heparin/bFGF

Matrigel Preparation/Injection:
Materials needed –
1 vial Matrigel 10ml at ~15mg/ml stock, Low Endotoxin! Some batches have killed mice due to high endotoxin levels!
Prechilled Falcon tubes and 1ml pipet tips
Prechilled tuberculin syringes with removable needles
27G needles for injection
Avertin - 500ul/animal ~ 50ml
3CC needles for ip injections

Matrigel Formulations: Calculate 1ml needed for three injections at 250ul due to viscosity and mixing losses.

Matrigel-heparin - Total for both -/+ bFGF = 20 animals X 2 sites/animal = 40 total injections / 3 sites/ml needed = 15 ml total

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)
- Add 5 ml Serum-free DMEM pre-chilled on ice.
- Split into separate tubes
- Pre-mix 12ug bFGF with 7ul heparin (50ug/ul) and add to one tube (Note: this is essential the
- FGF is not effective without coupling to heparin!)
- Mix in Cold Room by Careful inversion. No Bubbles!!

Anesthetize animals with 300-500ul/animal prior to s.c. injection of Matrigel.

Load pre-chilled (ice) tuberculin syringes (remove needle while filling) place on 27G needle for injection.

Inject Matrigel/Heparin on left center of back and Matrigel/Heparin/bFGF on right center of back.

Typical maximal response is 7d post-injection

Matrigel Angiogenesis Harvest Protocol

Harvest will proceed with the following protocol:

Sac animals with CO2 asphyxiation dissect off skin to reveal Matrigel pellets.
Take gross pictures to record peripheral angiogenesis.
Implants are collected with the overlying skin and pinned out on styrofoam in Histoprep (10% formalin in PBS) for 2 hours and then washed in PBS 2X and stored in 70% ETOH at 4 C until processing for paraffin sections.

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 on styrofoam. Fix with 10% formalin in PBS for 15-30mins. Rinse with PBS and make sure to keep covered with PBS. Visualize gross vessel distribution with fluorescent dissection microscope. Bissect matrigel plug and skin with razor blade. Process half of the tissue by incubating 4hrs at 4oC 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.