**Culture Experiment PDGFBR Mediated Differentiation**

**Rationale**:

**Cells**: Sya

**Media**: DF10, SFM +0.2% Serum

**Protocol**:

 Plate mouse aortic SMC (1.2 x 104 density)

Day 0 Change (at 70% confluency) media to SFM + 0.2% Serum w/ or w/o chol

Day 3 72h – Wash 2x with PBS then add PDGF BB or DD

Day 5 24h later – Wash 2x with PBS then mRNA extraction with Trizol

 Keep conditioned media in -80

**PCR**:

1. SMC marker genes + 18s or B2M (SMaA, MHC, 22a, calponin)
2. MSC markers (sca1, CD105)
3. Macrophage markers (mac2)

4. MF markers (periostin, coll 1, 3, 4, 8, 15)

5. Inflammatory markers: IL1b, Mcp1, CCL20

**Notes**:

Plate cells on 100mm dishes (10 ml of media)

Plate SMCs on 6 well plates in triplicate for each treatment.

 

vehicle Cholesterol then DD – day 3

DD (30ng/mL) -day 3 Cholesterol AND DD – day 0