June 21, 2010
Anat Katsir, Seema Kara

# Vascular Graft Flow System
## Setup for Transfer into Incubator, Sterilization and Seeding Goretex

1.) Run system overnight outside of incubator.

2.) Add an additional chamber (two total).

3.) Add water to chambers outside of graft (~55mL).

4.) Place system in incubator with only one chamber.

5.) Sterilize system.

   a. In reservoir, place 70% Ethanol solution, run for 4 hours
   b. Then change reservoir, place PBS and run for 4 hours
   c. Then change reservoir again and run 10% Abam in PBS for 3 hours. Leave stagnant Abam in tubing overnight.
   d. The next day, change to PBS in reservoir and run for 3 hours. Refresh reservoir with fresh PBS run for another 3 hours

6.) Attach clotted goretex graft to chamber. Use only one chamber.

7.) Attach seeded Goretex Graft. Use only one chamber:

   e. UV Goretex both sides
   f. Leave it in 10% Abam at 37$^{o}$C overnight
   g. Pre coat with gelatin
   h. Wait 1 hr
   i. Wash in PBS (dip 1x)
   j. Then place coated goretex in 15mL tube with 250 x 10$^{6}$ cells/mL EAHY926.
   k. Every 15mins rotate tube
   l. Take out Goretex, place in flask with medium in 37$^{o}$C

8.) Run medium in system using only plastic tubing in chamber. After 1 hour refresh with fresh reservoir of medium.

9.) Cut off section of seeded Goretex to do BBZ staining. Use a second section for SEM imaging.

10.) Using the rest of the Goretex, attach to chamber and fill chamber with medium. Run for 30 mins and then fix.

11.) Create new Goretex seeded grafts and run separately for 1 hour, 3 hours and 5 hours and then fix.