Isolating endothelial cells from bovine aortas

Prep:

Sterilize scissors and forceps the night before by autoclaving.

The day of:

1. Prepare 50:50 Trypsin:Versene mix and incubate at 37C prior to harvesting cells.

2. In a 250 mm plate, being as sterile as possible, cut the aorta into 3-4 inch sections with sterilized scissors.

3. Cut length-wise to open up the tube and try to flatten into a sheet with sterilized scissors.

4. Using forceps to hold the aorta, rinse the endothelium with Media containing antibiotics. When doing this, tilt the plate so that the media runs off the endothelium and the plate.

5. Repeat step 4 three times.

6. Level plates and pipette onto the endothelium enough trypsin:versene to coat generously.

7. Incubate for 10 minutes, and then scrape off endothelium with cell scraper. Scrape once and then dip the scraper into complete DMEM with 10% FBS.
*Note: Be careful not to scrape too deep to avoid SMCs.

8. After scraping is done, spin down cells, resuspend in complete DMEM and plate onto two 10 cm tissue culture plates.

9. Monitor when cells start to attach and change media. This is help remove SMCs because it takes them longer to attach.