Establishing 3D collagen cultures in device

1) To embed cells in collagen, it is necessary to lower the pH of the collagen to achieve polymerization by mixing the collagen with a setting solution. Prepare the setting solution by mixing 100 ml of 10X EBSS with 2.45 g of NaHCO3, 7.5 ml of 1 M NAOH and 42.5 ml of sterile distilled water. Further sterilize using a bottle top 0.22 micron filter unit attached to a sterile bottle.

2) Mix the rat tail collagen I (2.5- 3.5 mg/ml) (Corning Product #354236). with setting solution at a starting 4: 1 ratio. For 1 device, use 200-300 ul of collagen with 50-75 ul of setting solution in an eppendorf tube. Vortex briefly or pipet the sample up and down to mix the sample thoroughly. Note, volumes can be adjusted as desired.

3) Add collagen or setting solution at 5-10 ul increments and mix thoroughly until the phenol red dye in the mixture changes to a light pink to orange color, reflecting a neutral pH. A yellow color indicates acidic (low) pH while dark pink reflects basic or high pH. Keep the mixture, cold on ice as higher temperatures will accelerate polymerization.

4) Trypsinize cells. Check for de-ttachment using a microscope. Quench the trypsin by pipetting 9 ml of complete medium on the plate and transfer the cells to a sterile 15 ml conical tube.

5) Count cells. Mix 100,000 cells or more with collagen: setting solution. Swivel device closed. Add collagen:cell mixture to device. Incubate the devices in a test chamber 3.5 cm in diameter or larger at 37^{o}C for 10 minutes to polymerize.

6) Add at least 400 ul of media to device. Culture for 24 hours before treatments.

7) If test chamber is 3.5 cm in diameter, incubate test chamber containing at least 500,000 immune cells with 5> mls of media. If you choose a smaller volume in the test chamber, make sure opening of device is submerged in media.