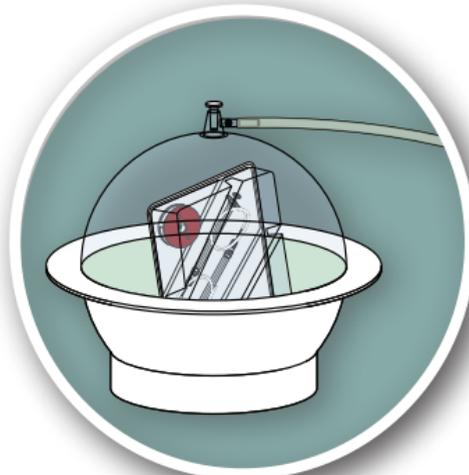




1. Equilibrate media

- » Place all cell media overnight in an incubator in a slightly open vial (for temperature equilibration and degassing).



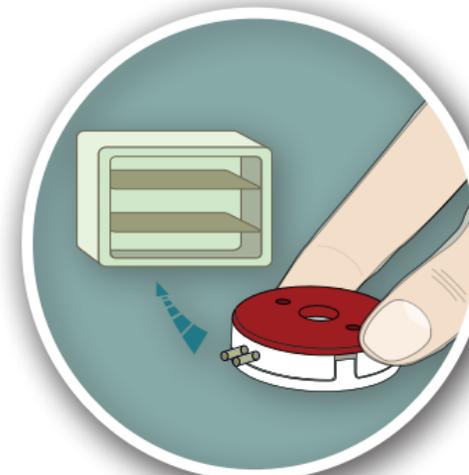
2. Prepare the experiment

- » Place the blister package containing CellDirector 3D in a vacuum chamber for 30 min.
- » Prepare your cell-matrix mixture to a final cell concentration of typically $0.5-2 \times 10^6$ cells/ml.



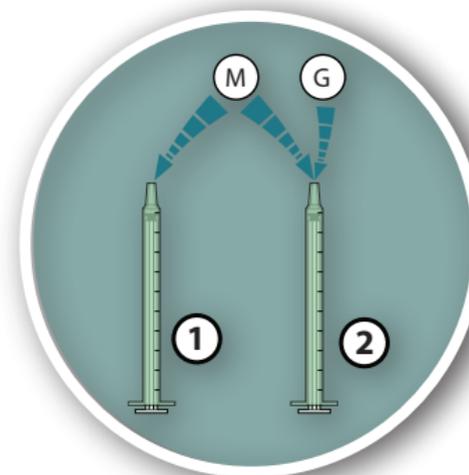
3. Load cell-matrix mixture

- » Load 8 μ l of the mixture by reverse pipetting using a 10 μ l or 20 μ l pipet.
- » Insert the pipet tip through the cross shaped slit and slowly inject the mixture.



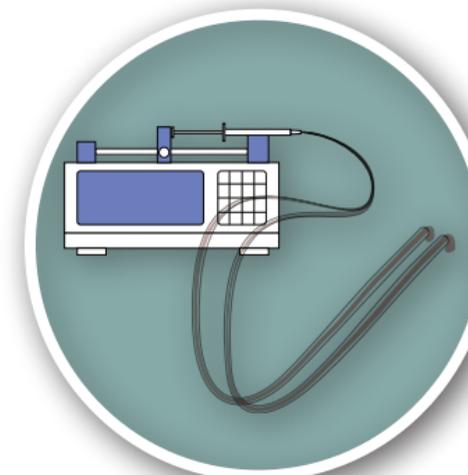
4. Matrix polymerisation

- » Place CellDirector 3D in a sterile petri dish and place in humidified incubator.
- » Let the matrix polymerise (10-60 min depending on matrix).



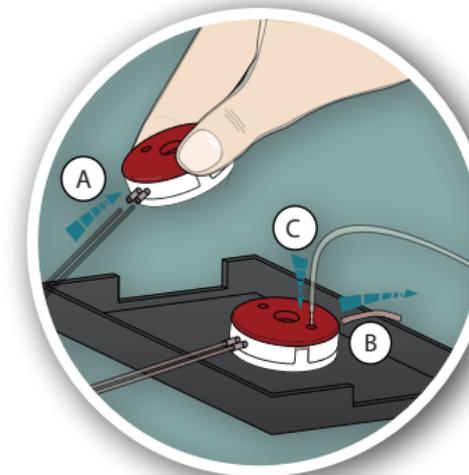
5. Syringes

- » Fill syringe (1) with cell media (M), and syringe (2) with cell media and gradient substance (G).
- » Connect the needles to the syringes and fill up the tubes.



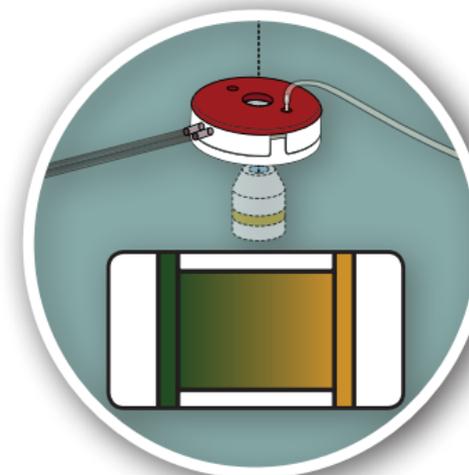
6. Syringe pump

- » Fit the syringes into the syringe pump (e.g. Fusion 100, REF 90-001).
- » Select the BD 1ml Plastic syringe in the syringe library (ID 4.78 mm).
- » Start pump – ensure the tubes contain no air bubbles and media is exiting tube ends.
- » Set flow rate to 2 μ l/min.



7. Start the experiment

- » Insert both tubes (A) until fully bottomed and resistance is noticeable.
- » Place CellDirector 3D under the microscope (CellDirector Holder, REF 70-001).
- » Remove the outlet tube by pulling it gently (B).
- » Connect the vacuum pump (C).
- » After 15 min, reduce flow rate to 0.5 μ l/min.



8. Image acquisition

- » Choose your magnification and the positions from where to collect images.
- » Collect images every 5 min (fast responding cells) up to every 30 min (slow responding cells).
- » Continue to collect images during the entire experiment (3 h up to 32 h).