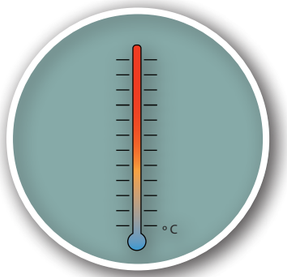
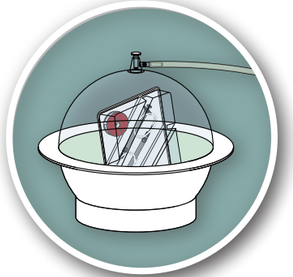


1. Equilibrate media

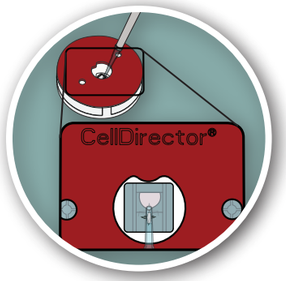
» Place cell media overnight in an incubator in a slightly open vial (for temperature equilibration and degassing).



2. Prepare the experiment

» Place the blister package containing CellDirector 3D in a vacuum chamber for 30 min.

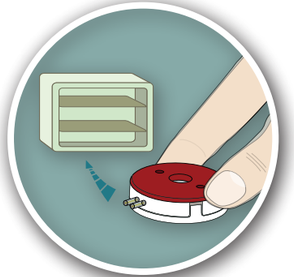
» Prepare your cell-matrix mixture to a final cell concentration of typically 0.5-2x10^6 cells/ml.



3. Load cell-matrix mixture

» Load 8 µl of the mixture by reverse pipetting using a 10 µl or 20 µl pipet.

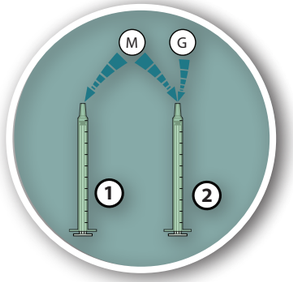
» Insert the pipet tip through the cross-shaped slit and slowly inject the mixture.



4. Matrix polymerisation

» Place CellDirector 3D in a sterile petri dish and place in a humidified incubator.

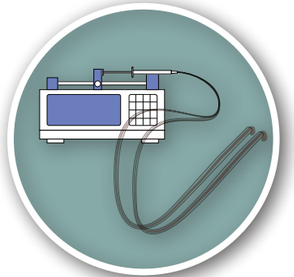
» Let the matrix polymerise (10-60 min depending on matrix).



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.



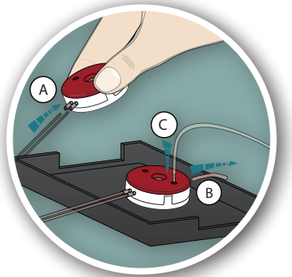
6. Syringe pump

» 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 µl/min.



7. Start the experiment

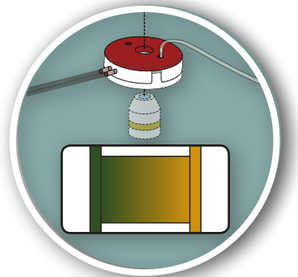
» Insert both tubes (A) until fully bottomed and resistance is noticeable.

» 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 µl/min.



8. Image aquisition

» 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).