

Preparation for 3D cell culture on alvetex®

1. 3T3 cells were routinely maintained in T-75 flasks.

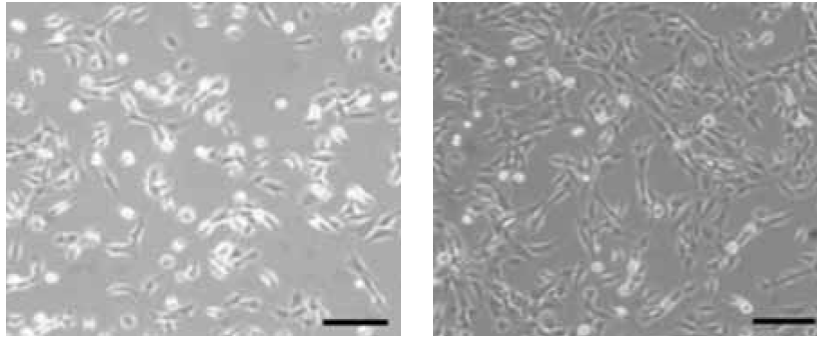
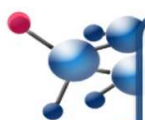


Figure 1. Phase contrast micrographs of 3T3 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 200 μm .

2. Complete media consisted of: Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10% v/v FBS, 2 mM L-glutamine and 100 U/ml Penicillin/Streptomycin.
3. Cells were harvested by trypsinisation and centrifuged for 5 minutes (1000 rpm). The supernatant was discarded and the cell pellet was re-suspended in an appropriate volume of media for cell counting by Trypan Blue.
4. Cells were re-suspended at a concentration of 8×10^6 cells/ml for seeding.
5. Alvetex® 6-well inserts were prepared for seeding by dipping in 70% ethanol and washing twice with 7 ml of media per well.
6. 125 μl of the cell suspension was added to the centre of the alvetex® disc, which was equivalent to 1×10^6 cells per well.
7. The plate was incubated 60 min at 37 °C with 5% CO₂ to allow the cells to settle into the scaffold.
8. 10 ml of media was added to each well taking care not to dislodge cells from alvetex®.
9. Plates were re-incubated and maintained by complete media exchange after every 2-3 days.



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Histology

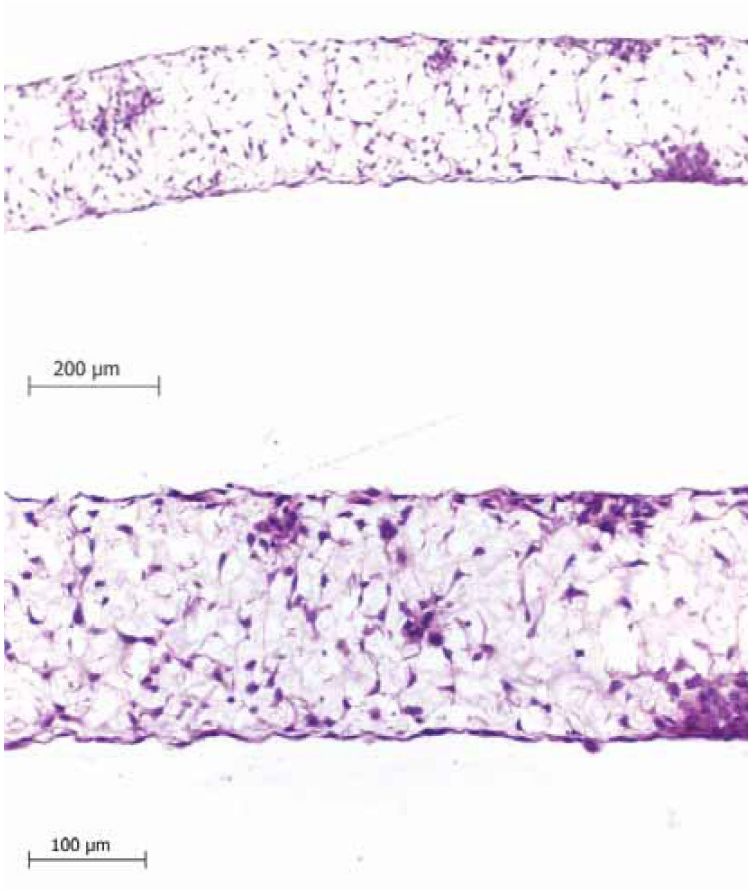


Figure 2. Brightfield micrographs showing the structure of 3T3 cells cultured for 7 days on 22 mm diameter alvetex® discs presented in 6-well inserts in 6-well plates. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.

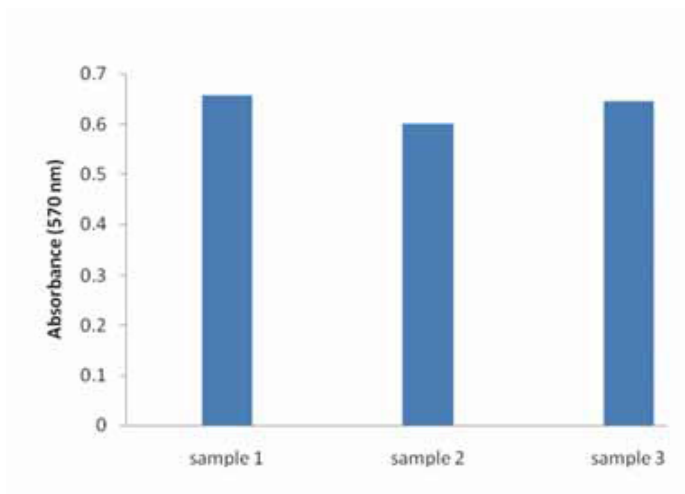


Figure 3. Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of 3T3 cells are shown. Each well was sampled in duplicate with mean value shown. Cells were cultured for 3 days on 22 mm alvetex® discs presented in the 6-well inserts in 6-well plates.