Preparation for 3D cell culture on alvetex®

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

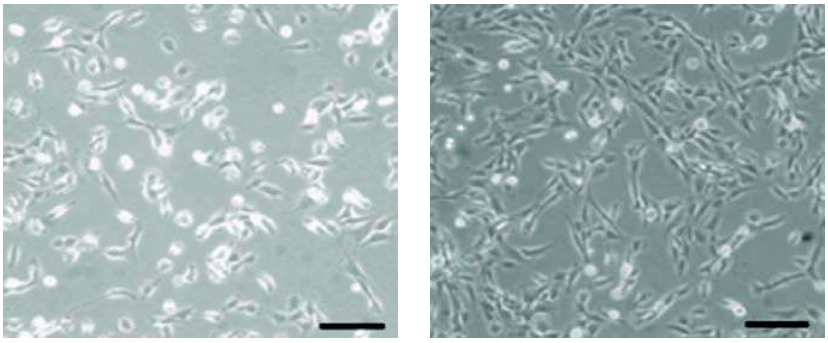

Figure 1. *Phase contrast micrographs of 3T3 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 200 µm.*

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 $8x10^6$ cells/ml for seeding.*
5. *Alvetex® 12-well plates were prepared for seeding with 70% ethanol (2 ml per well) and media washes (twice with 3 ml of media each) as described in the product information leaflet.*
6. *125 µl of the cell suspension was added to the centre of the alvetex® disc, which was equivalent to $1x10^6$ cells per well.*
7. *The plate was incubated 3 hours at 37 ˚C with 5% CO_2 to allow the cells to settle into the scaffold.*
8. *4 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 1-2 days.*

Histology

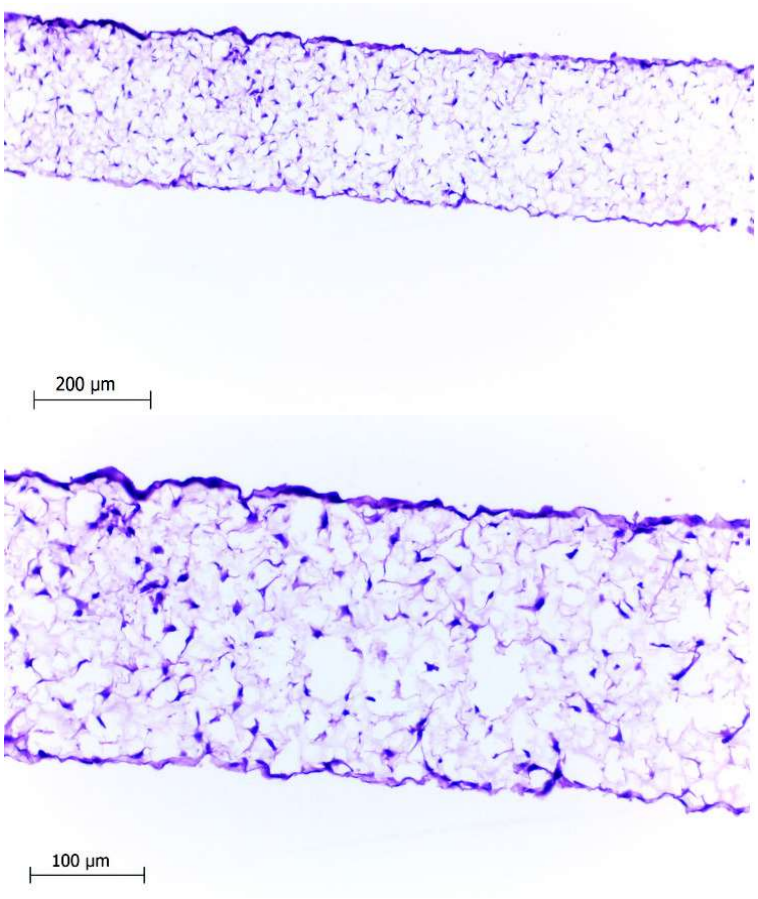

Figure 2. *Brightfield micrographs showing the structure of 3T3 cells cultured for 7 days on 22 mm diameter alvetex® discs presented in 12-well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.*

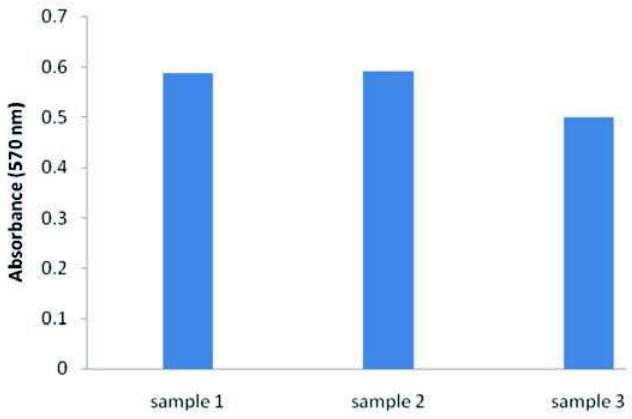

Figure 3. *Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of 3T3 cells are shown. Each well was sampled in duplicate with mean value shown. Cells were cultured for 3 days on 22 mm alvetex® discs presented in the 12-well plate format.*