Preparation for 3D cell culture on alvetex®:

1. HepG2 cells were routinely maintained in T-75 flasks.

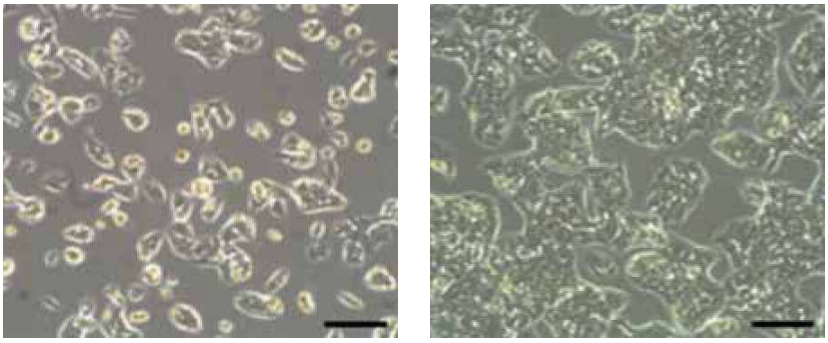
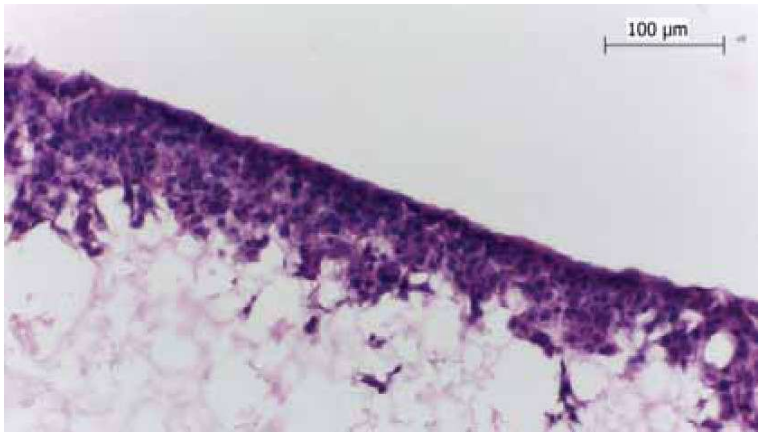


Figure 1. *Phase contrast micrographs of HepG2 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 100 µm.*

2. Complete media consisted of: MEM media (Gibco 21090) 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 $1.6x10^7$ cells/ml for seeding.
5. Alvetex® 6-well inserts were prepared for seeding by dipping in 70% ethanol and washed 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 $2x10^6$ cells per well.
7. The plate was incubated 30 min at 37 ˚C with 5% CO_2 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.



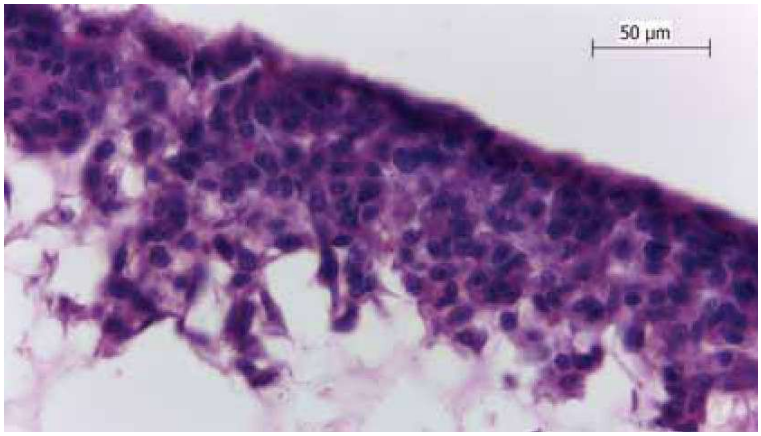


Figure 2. *Brightfield micrographs showing the structure of HepG2 cells cultured for 7 days on 22 mm diameter alvetex® discs presented in the 6-well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.*

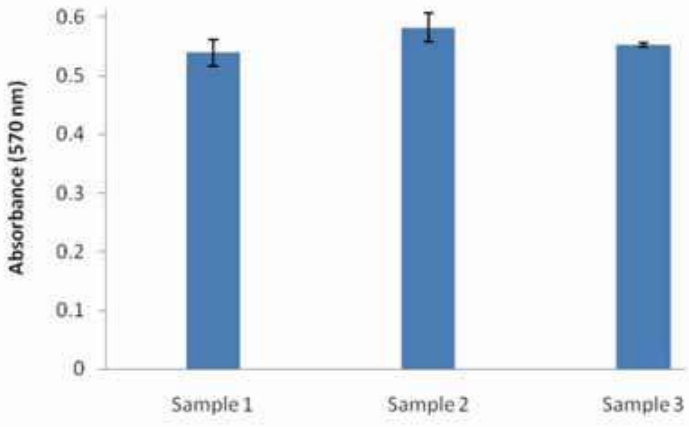


Figure 3. *Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of HepG2 cells are shown (n=3, mean ± SD). Cells were cultured for 3 days on 22 mm alvetex® discs presented in the 6-well inserts in 6-well plate format.*