Preparation for 3D cell culture on Alvetex® Scaffold

1. CHO-K1 cells (ATCC, CCL-61) were routinely maintained in T-75 flasks.

Figure 1. Phase contrast micrographs of CHO-K1 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 100 µm.

2. Complete medium consisted of: F-12K nutrient mixture (Kaighn’s modification; Gibco 21127022) supplemented with 10 % v/v FBS and 100 µg/ml Penicillin and 10 µg/ml Streptomycin. Note: Medium already contains L-glutamine.

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 appropriate volume of media for cell counting by Trypan Blue.

4. Cells were re-suspended at a concentration of 1x10⁶ cells / 150 µl for seeding per well.

5. Alvetex® Scaffold 6-well inserts fitted into 6-well plates were prepared for seeding by washing in 70 % ethanol and rinsing once with 10 ml of medium per well.

6. 150 µl of the cell suspension was added to the centre of the Alvetex® Scaffold disc, which was equivalent to 1x10⁶ cells per well.

7. The plate was incubated 30 minutes at 37 °C with 5 % CO₂ to allow the cells to settle into the scaffold.

8. 10 ml of medium was added to each well taking care not to dislodge cells from Alvetex® Scaffold.

9. Plates were re-incubated and maintained by complete medium exchange after every 2-3 days.
Figure 2. Brightfield micrographs showing the structure of CHO-K1 cells cultured for 3 days on 22 mm diameter Alvetex® Scaffold discs presented in 6-well inserts fitted into 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 CHO-K1 cells are shown. Each well was sampled in triplicate with 1/10 dilution. Mean values are shown. Cells were cultured for 7 days on 22 mm Alvetex® Scaffold discs presented in 6-well inserts fitted into 6-well plates.