Example Protocol for the Culture of the CHO-K1 Cell Line on Alvetex® Scaffold (22 mm Disc in 12-Well Plate Format, AMS.AVP002).

Preparation for 3D cell culture on Alvetex®Scaffold

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

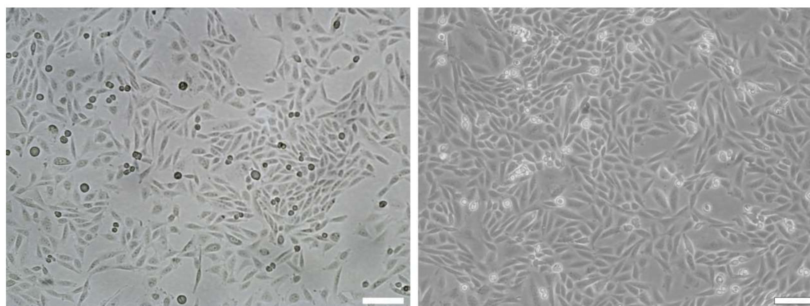

Figure 1. *Phase contrast micrographs of CHO-K1 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 100 µm.*

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 $1x10^6$ cells / 150 µl for seeding per well.
5. Alvetex®Scaffold 12-well discs were prepared for seeding by washing in 70 % ethanol and rinsing once with 4 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 $1x10^6$ cells per well.
7. The plate was incubated 30 minutes at 37 °C with 5 % CO_2 to allow the cells to settle into the scaffold.
8. 4 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 1-2 days.

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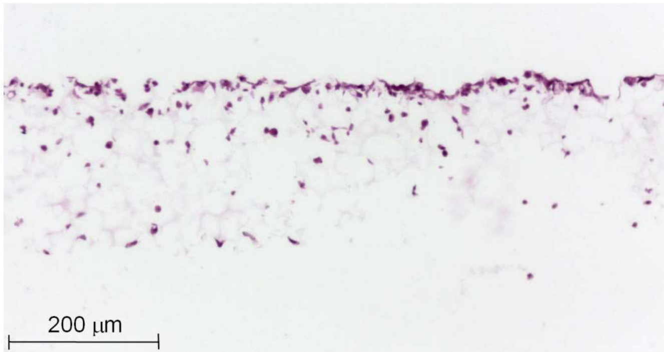

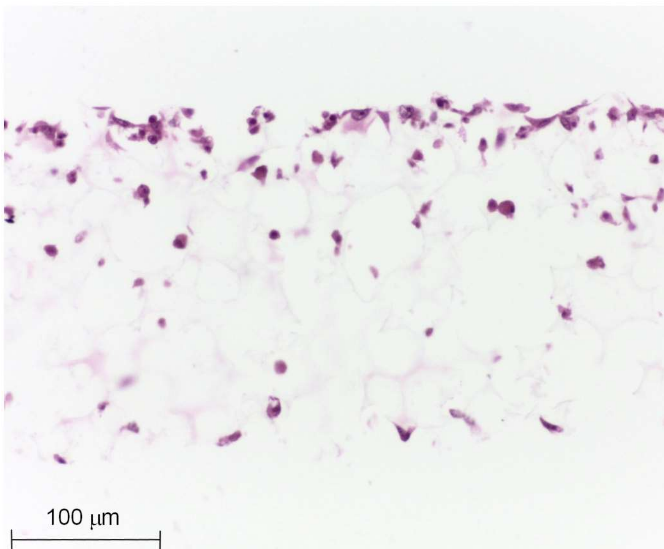

Figure 2. *Brightfield micrographs showing the structure of CHO-K1 cells cultured for 3 days on 22 mm diameter Alvetex® Scaffold discs presented in 12-well plates. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with Haematoxylin and Eosin.*

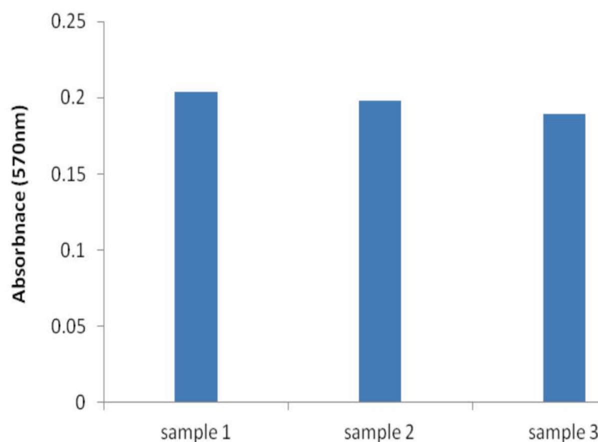

Figure 3. *Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of CHO-K1 cells are shown. Each well was sampled in triplicate. Mean values are shown. Cells were cultured for 3 days on 22 mm Alvetex® Scaffold discs presented in 12-well plates.*

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