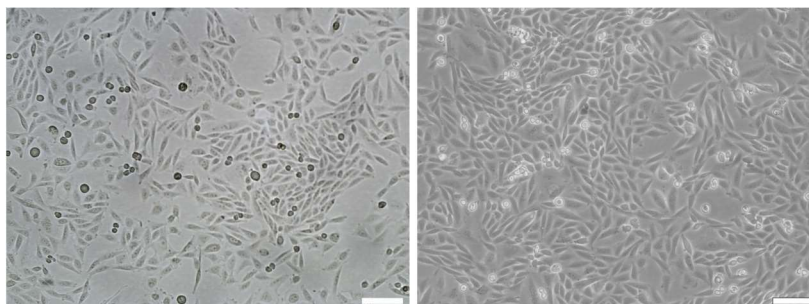


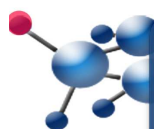
## Preparation for 3D cell culture on Alvetex® Scaffold

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  $\mu$ m.

2. Complete medium consisted of: F-12K nutrient mixture (Kaighn's modification; Gibco 21127022) supplemented with 10 % v/v FBS and 100  $\mu$ g/ml Penicillin and 10  $\mu$ g/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  $1 \times 10^6$  cells / 150  $\mu$ l 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  $\mu$ l of the cell suspension was added to the centre of the Alvetex® Scaffold disc, which was equivalent to  $1 \times 10^6$  cells per well.
7. The plate was incubated 30 minutes at 37 °C with 5 % CO<sub>2</sub> 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.



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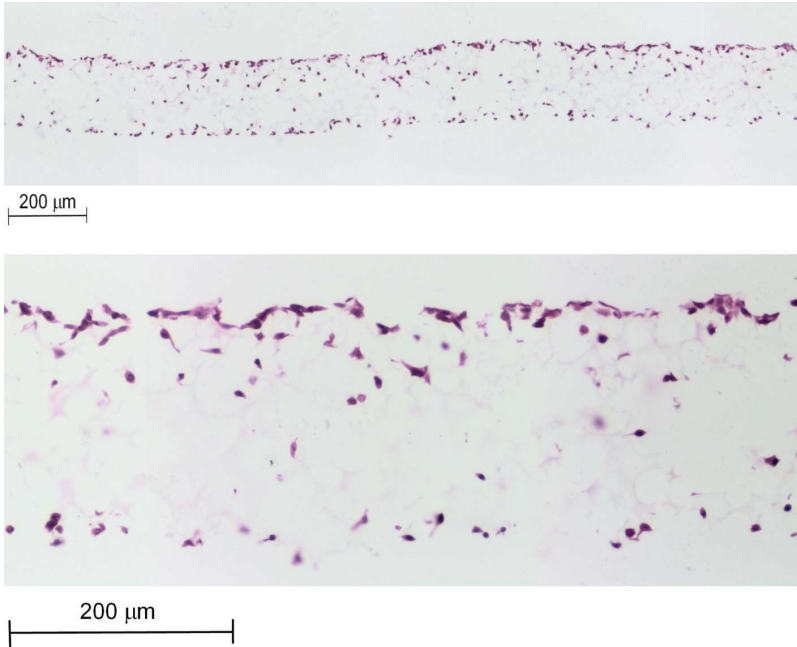
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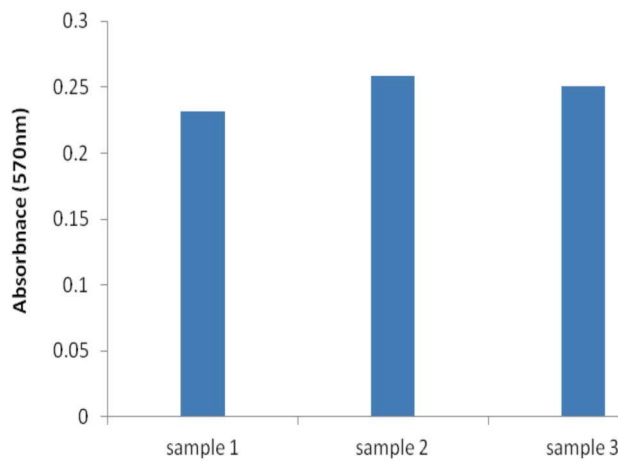
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**Example Protocol for the Culture of the CHO-K1 Cell Line on Alvetex® Scaffold (22 mm Disc in 6-Well Inserts Format, AMS.AVP004-32).**



**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.