Preparation for 3D cell culture on alvetex®

1. *TERA2.cl.SP12 cells[1,2] were routinely maintained inT75 flasks.*

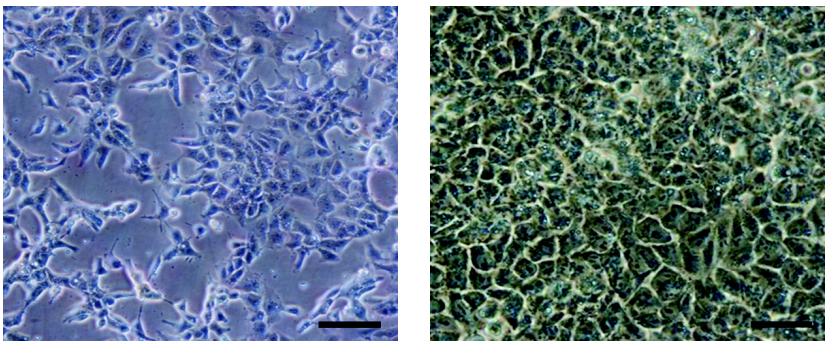
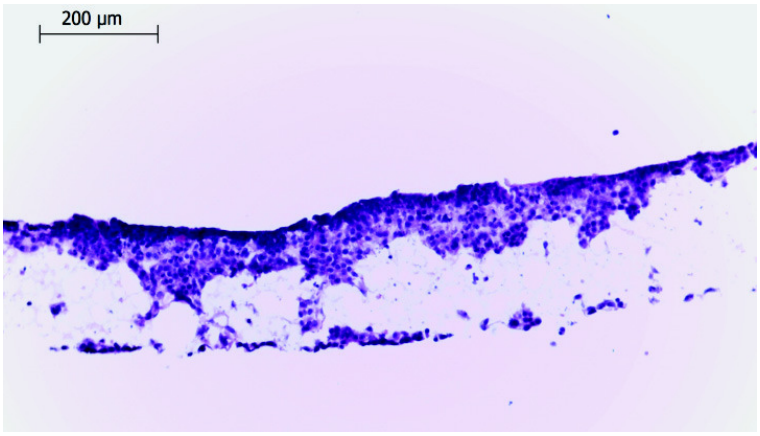


Figure 1. *Phase contrast micrographs of TERA2.cl.SP12 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 100 µm*

2. *Complete media consisted of: Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10% v/v heat-treated 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 $4x10^6$ 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 500,000 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.*

[1] *Przyborski S.A. (2001). Isolation of human embryonal carcinoma stem cells by immuno-magnetic sorting. Stem Cells,* **19***, 500-504.*

[2] *Stewart R., Christie V. & Przyborski S.A. (2003). Manipulation of human pluripotent embryonal carcinoma stem cells and the development of neural subtypes. Stem Cells,* **21***, 248-256.*



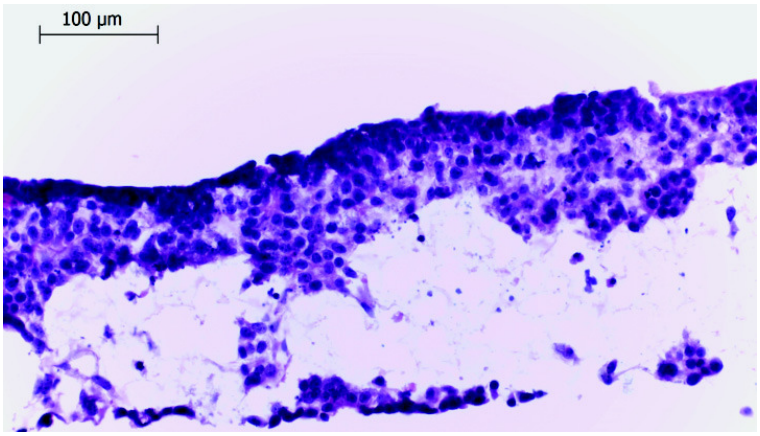


Figure 2. *Brightfield micrographs showing the structure of TERA2.cl.SP12 cells cultured for 7 days on 22 mm diameter alvetex® discs presented in 6-well inserts in 6-well plates. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.*

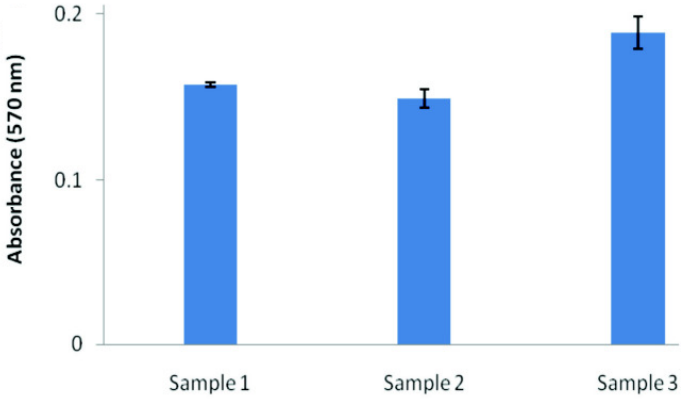


Figure 3. *Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of TERA2.cl.SP12 cells are shown (n=3, mean ± SD). Cells were cultured for 3 days on 22 mm alvetex® discs presented in the 6-well inserts in 6-well-plate format.*