Protocol

Example Protocol for the Culture of the TERA2.cl.SP12 Cell Line on Alvetex™ Scaffold in Well Plate and Well Insert Formats

1. Introduction

Alvetex Scaffold is available in several cell culture formats including 24 well plate (AVP006), 12 well plate (AVP002), 6 well insert (AVP004), 12 well insert (AVP005), and 24 well insert (AVP012).

24 well and 12 well plates are suitable for shorter term cultures and for applications where limited cell penetration into the scaffold is required. Well insert formats generally support longer term cultures and deeper cell penetration into the scaffold. They also provide for conveniently tailored media set ups (see the Alvetex Scaffold Quick Start protocol).

The availability of two different well insert formats enables choice on the basis of desired culture size and cell expenditure. 6 well inserts can be placed in conventional 6 well plates, while 12 well inserts can be placed in either 6 well plates or 12 well plates, depending on media requirements. Alternatively, both insert types can be housed in the dedicated Well Insert Holder in Deep Well Petri Dish (AVP015) to allow for increased media volumes and prolonged cell culture. Alvetex insert formats can also be used in the Alvetex Perfusion Plate (AVP011).

2. Methods

2.1. Preparation for 3D cell culture on Alvetex Scaffold

1. TERA2.cl.SP12 cells[1,2] were routinely maintained in T-75 flasks.

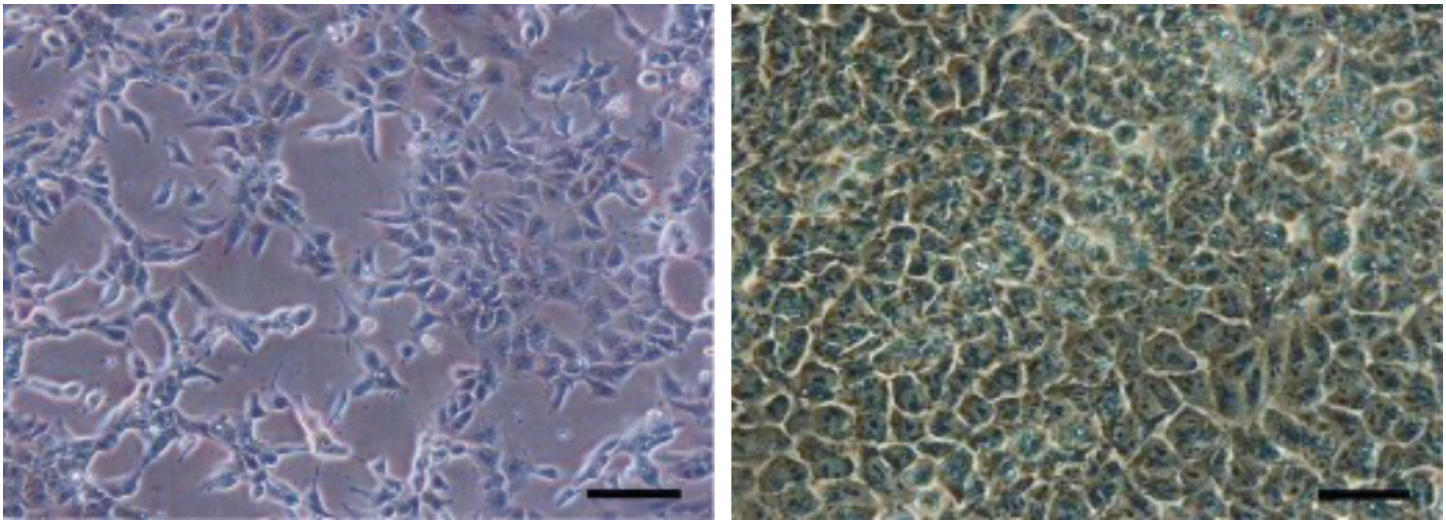

Figure 1. *Phase contrast micrographs of TERA2.cl.SP12 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 100 µm.*

2. Complete media consisted of: Dulbecco's 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 4×10^6 cells/mL for seeding.

5. Follow one of the below methods according to your choice of Alvetex Scaffold format.

2.2. 12 well Plate Format (AVP002)

1. Alvetex Scaffold 12 well plates were prepared for seeding with a 70 % ethanol wash (2 mL per well) and subsequent media washes (twice with 3 mL of media each).

2. 125 µL of the cell suspension was added to the centre of the Alvetex Scaffold disc, which was equivalent to 500 000 cells per well.

3. The plate was incubated 3 hours at 37 °C with 5% CO_2 to allow the cells to settle into the scaffold.

4. 4 mL of media was added to each well taking care not to dislodge cells from Alvetex Scaffold.

5. Plates were re-incubated and maintained by complete media exchange after every 1-2 days.

Note: This method can be applied to the use of Alvetex Scaffold in 24 well plate format (AVP006). Adjust cell seeding and media volumes according to the guidelines provided in the Alvetex Scaffold Quick Start protocol.

2.3. 6 well Insert Format (AVP004)

1. Alvetex Scaffold 6 well inserts in 6 well plate format were prepared for seeding by dipping in 70 % ethanol and washed twice with media (7 mL per well).

2. 125 µL of the cell suspension was added to the centre of the Alvetex Scaffold disc, which was equivalent 500,000 cells per well.

3. The plate was incubated for 3 hours at 37 °C with 5 % CO_2 to allow the cells to settle into the scaffold.

4. 10 mL of media was added to each well taking care not to dislodge cells from Alvetex Scaffold.

5. Plates were re-incubated and maintained by complete media exchange after every 2-3 days.

Note: This method can be applied to the use of Alvetex Scaffold in 12 well insert format (AVP005). Adjust cell seeding and media volumes according to the guidelines provided in the Alvetex Scaffold Quick Start protocol.

3. Example Data

3.1. 12 well plate format (AVP002)

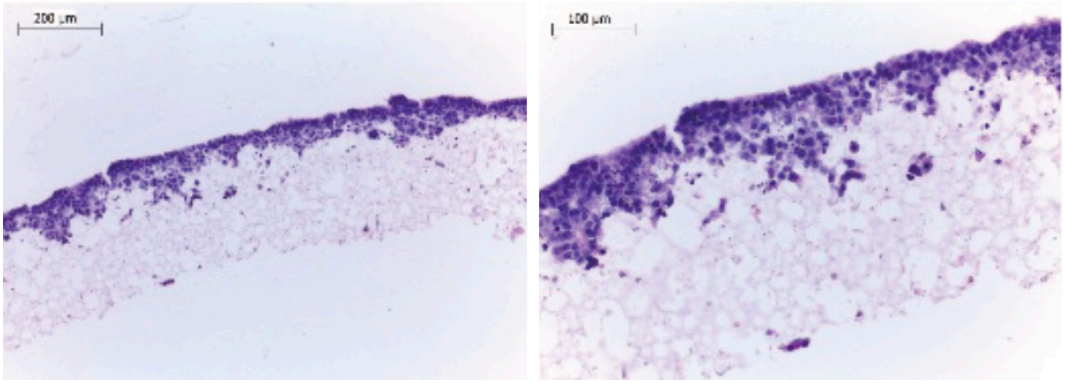

Figure 2. *Brightfield micrographs showing the structure of TERA2.cl.SP12 cells cultured for 7 days on 22 mm diameter Alvetex Scaffold discs presented in the 12 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.*

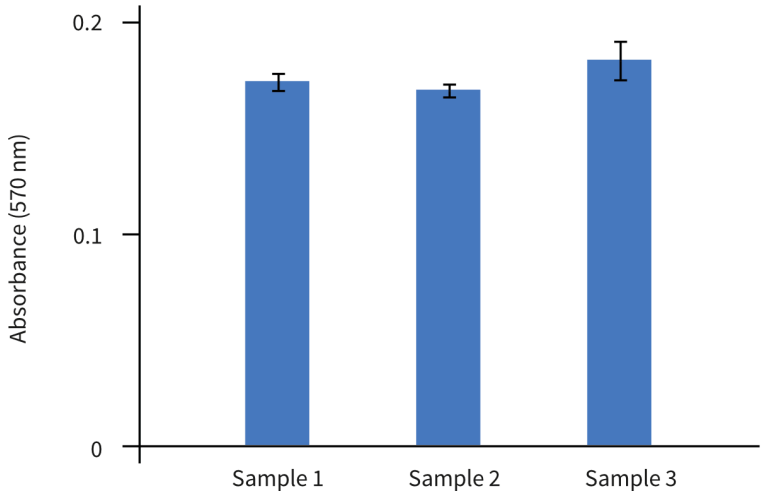

Figure 3. *Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of TERA2.cl.SP12 cells are shown (n = 3, mean ± SD). Cells were cultured for 3 days on 22 mm Alvetex Scaffold discs presented in the 12 well plate format.*

3.2. 6 well insert format (AVP004)

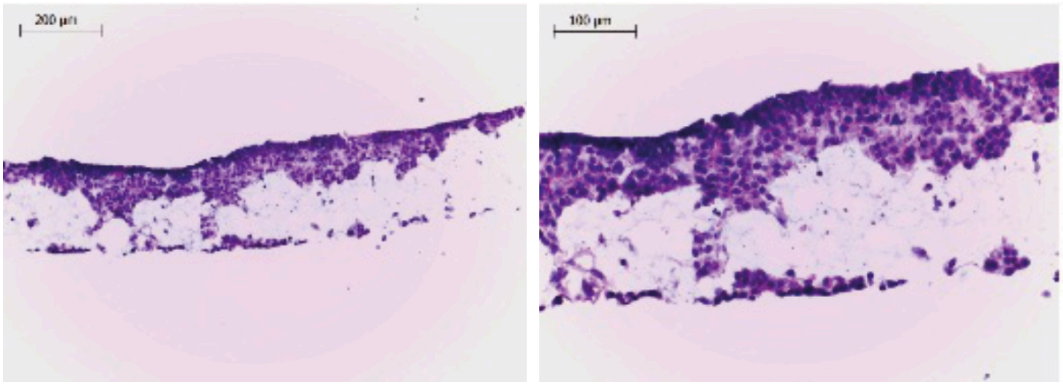

Figure 4. *Brightfield micrographs showing the structure of TERA2.cl.SP12 cells cultured for 7 days on 22 mm diameter Alvetex Scaffold discs presented in 6 well inserts in 6 well plates. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.*

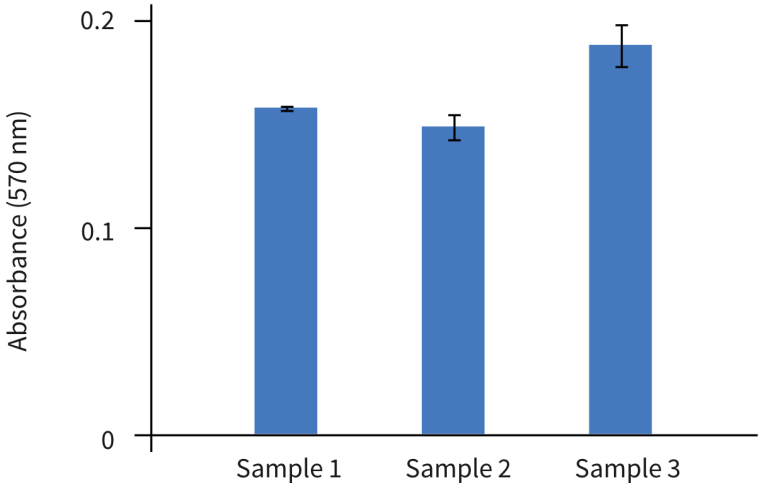

Figure 5. *Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of TERA2.cl.SP12 cells are shown (n = 3, mean ± SD). Cells were cultured for 3 days on 22 mm Alvetex Scaffold discs presented in the 6 well inserts in 6 well plate format.*

4. References

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

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