Protocol

Example Protocols for the Co-Culture of either the SW620 or the SW480 Cell Line with the 3T3 Cell Line on Alvetex™ Scaffold in 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).

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2. Methods

2.1. Preparation for 3D Cell Culture on Alvetex Scaffold

1. SW480, SW620 and 3T3 cells were routinely maintained in T-75 flasks

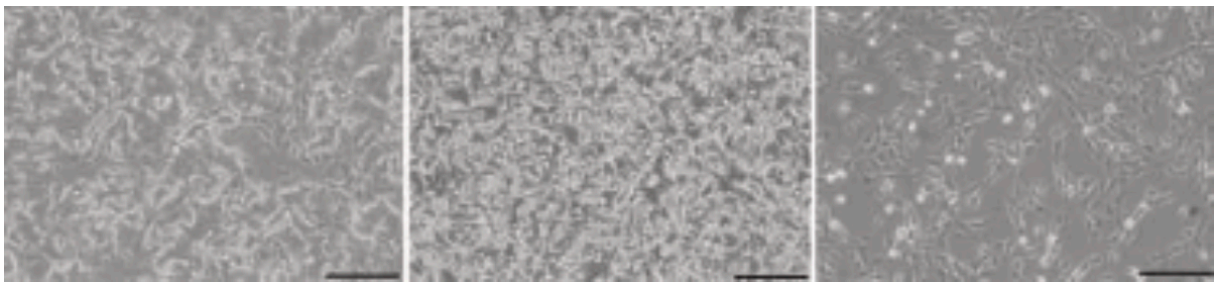

Figure 1. *Phase contrast micrographs of SW480 cells (left), SW620 cells (middle) and 3T3 cells (right) grown in conventional 2D culture plates. Images show cells at high confluency. Scale bars: 200 µm.*

2. For SW480 cells and SW620 cells, complete growth media consisted of: DMEM High glucose supplemented with 10 % v/v heat inactivated FBS, 2 µm L-glutamine and 100 U/mL Penicillin/Streptomycin.

3. For 3T3 cells, complete growth media consisted of: DMEM High glucose supplemented with 10 % v/v FBS, 2 µm L-glutamine and 100 U/mL Penicillin/Streptomycin.

4. 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.

5. Cells were re-suspended at a concentration of 1.0×10^7 cells/mL for seeding.

Note: 3T3 cells are seeded on Alvetex Scaffold 7 days prior to seeding SW480 cells (or SW620 cells).

2.2. 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. 50 µL of the 3T3 cell suspension was added in the centre of the Alvetex Scaffold disc, which was equivalent to 0.5×10^6 cells per well.

3. The plate was incubated for a minimum of 15 minutes at 37 °C with 5 % CO_2 to allow the cells to settle into the scaffold.

4. 3T3 media was added to each well to a total volume of 10 mL taking care not to dislodge cells from Alvetex Scaffold.

5. Plates were re-incubated and maintained for a further 7 days by complete 3T3 media exchange after every 2-3 days.

6. After 7 days, inserts were transferred to deep well Petri dish holders in deep well Petri dishes (AVP015) and dishes were filled from the outside of the insert with enough SW480/SW620 medium to allow the medium to rise inside the insert and cover the substrate, but not to groove the sides of the inserts, i.e. 50 mL ± 5 mL, as described for feeding above and below separately in the Alvetex Scaffold Quick Start protocol.

7. 100 µL of either the SW480 cell suspension or the SW620 cell suspension was added in droplets over the surface of the Alvetex Scaffold disc, which was equivalent to 1×10^6 cells per well.

8. The Petri dish was incubated overnight at 37 °C with 5 % CO_2 to allow the cells to settle on top of the scaffold.

9. The following morning SW480/SW620 media was added to each Petri dish to a total volume of 70 mL taking care not to dislodge cells from Alvetex Scaffold.

10. Petri dishes were re-incubated and maintained by complete SW480/SW620 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. 6 well insert format (AVP004)

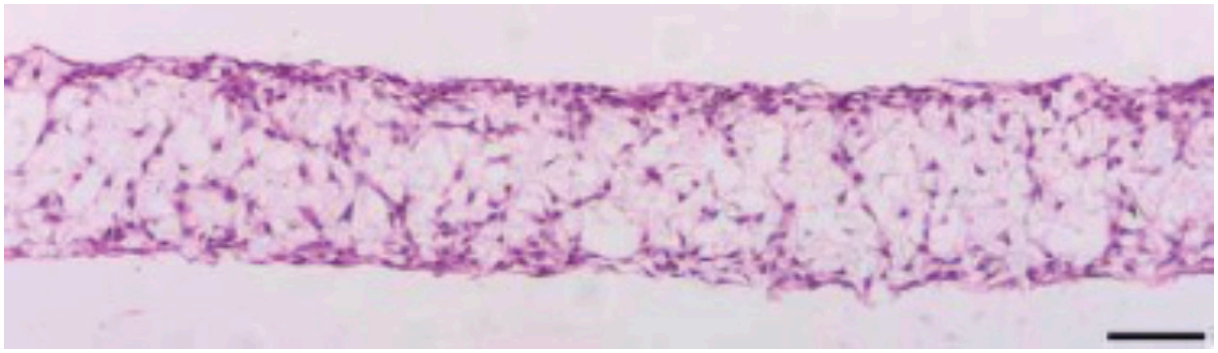

Figure 2. *Brightfield micrograph showing the structure of 3T3 cells cultured for 7 days on 22 mm diameter Alvetex Scaffold discs presented in 6 well insert in 6 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bar: 100 µm.*

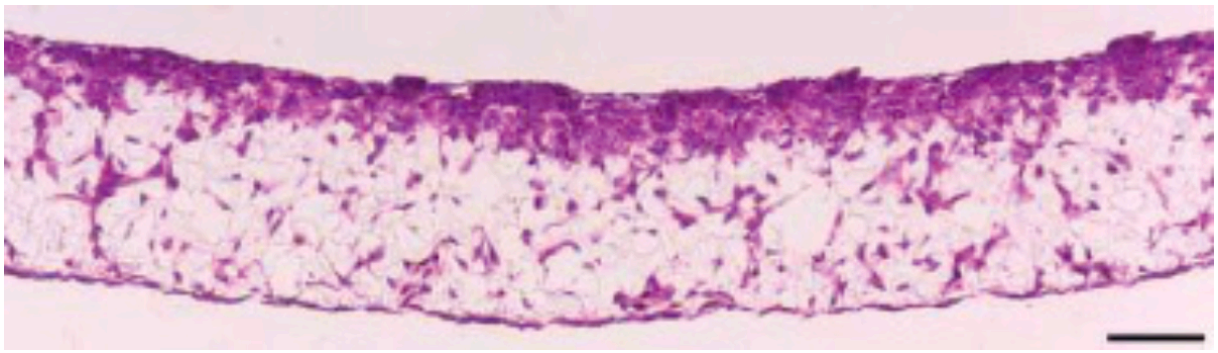

Figure 3. *Brightfield micrograph showing the structure of SW480 cells co-cultured for 7 days with established 3T3 cells on 22mm diameter Alvetex Scaffold discs presented in 6 well insert in 6 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bar: 100 µm.*

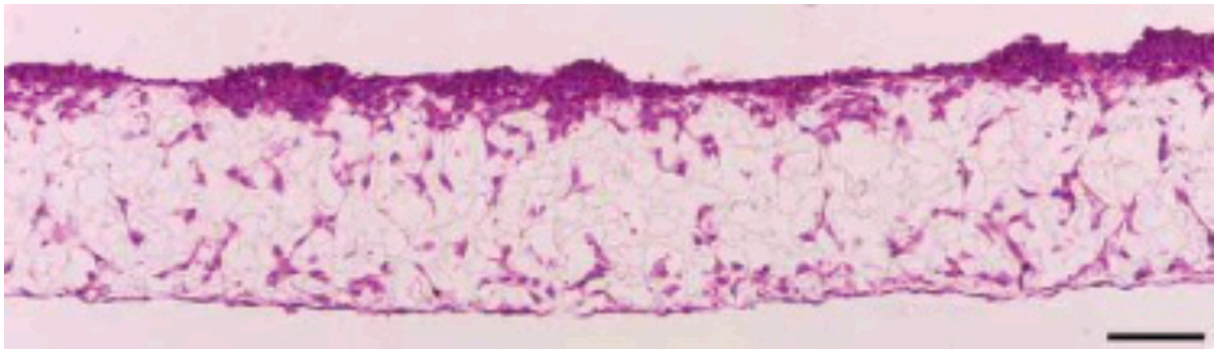

Figure 4. *Brightfield micrograph showing the structure of SW620 cells co-cultured for 7 days with established 3T3 cells on 22 mm diameter Alvetex Scaffold discs presented in 6 well insert in 6 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bar: 100 µm.*