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Example Protocol for the Culture of the SW620 Cell Line on Alvetex™ Scaffold in Well Insert and Well Plate Formats

Introduction

Alvetex Scaffold is currently available in four different cell culture formats: 24 well plate (AVP006), 12 well plate (AVP002), 6 well insert (AVP004), and 12 well insert (AVP005). 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 cellpenetration into the scaffold. They also provide for conveniently tailored media set ups (see Quick Start Protocol). 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. The availability of two different Alvetex Scaffold sizes enables choice on the basis of desired culture size and cell expenditure.

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Methods

Preparation for 3D Cell Culture on Alvetex Scaffold

1. SW620 cells were routinely maintained in T-75 flasks

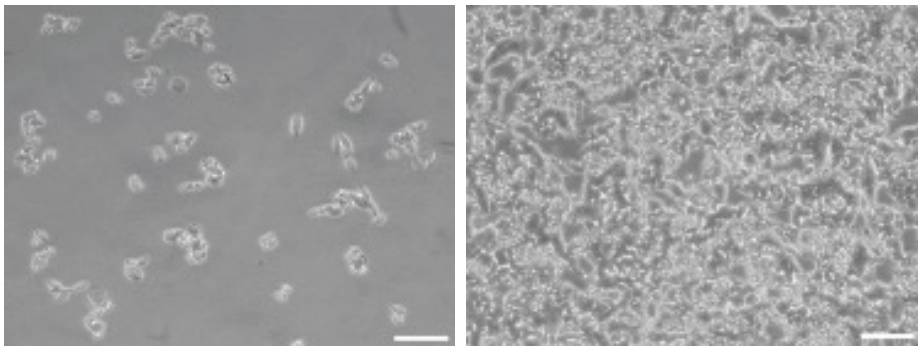

Figure 1. Phase contrast micrograph of SW620 cells grown in conventional 2D culture plates. Image shows cells at low (left) and high (right) confluency. Scale bars: 100 µm.

2. 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. 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 1.0×10^7 cells/mL for seeding.
5. Follow one of the following methods according to your choice of Alvetex Scaffold format.

24 well Plate Format, AVP006

1. Alvetex Scaffold 24 well plates were prepared for seeding with a 70 % ethanol wash (2 mL per well) and subsequent media washes (twice with 2 ml of media each).
2. 50 µL of the cell suspension was added to the centre of the Alvetex Scaffold disc, which was equivalent to 0.5×10^6 cells per well.
3. The plate was incubated for at least 1 hour at 37 °C with 5 % CO_2 to allow the cells to settle into the scaffold.
4. 2 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 in12 well plate format, AVP002. Adjust cell seeding and media volumes according to the guidelines provided in the Quick Start Protocol.

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 followed by media washes (twice, with 7 ml per well).
2. Wells were filled from the outside of the insert with enough medium to allow it to rise inside the insert and cover the substrate, but not to go over the sides of the inserts, i.e. 7 mL ± 1 mL, as described for feeding above and below separately in the Quick Start Protocol.
3. 100 µL of the cell suspension was added in droplets over the surface of the Alvetex Scaffold disc, which was equivalent to 1×10^6 cells per well.
4. The plate was incubated overnight at 37 °C with 5 % CO_2 to allow the cells to settle into the scaffold.
5. Media was added to each well to a total volume of 10 mL the following morning taking care not to dislodge cells from Alvetex Scaffold.
6. Plates were re-incubated and maintained by complete media exchange after every 2-3 days.

12 well Insert Format, AVP005

1. Alvetex Scaffold 12 well inserts in 12 well plate format were prepared for seeding by dipping in 70 % ethanol followed by media washes (twice with 4 ml per well).
2. Wells were filled from the outside of the insert with enough medium to allow it to rise inside the insert and cover the substrate, but not to go over the sides of the inserts, i.e. 2.4 mL ± 0.2 mL, as described for feeding above and below separately in the Quick Start Protocol.
3. 50 µL of the cell suspension was added in droplets over the surface of the Alvetex Scaffold disc, which was equivalent to 0.5×10^6 cells per well.
4. The plate was incubated overnight at 37 °C with 5 % CO_2 to allow the cells to settle into the scaffold.
5. Media was added to each well to a total volume of 4 mL the following morning taking care not to dislodge cells from Alvetex Scaffold.
6. Plates were re-incubated and maintained by complete media exchange after every 2-3 days.

Example Data: 24 well plate format, AVP006

Figure 2. Brightfield micrographs showing the structure of SW620 cells cultured for 7 days on 15 mm diameter Alvetex Scaffold discs presented in the 24 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bar: 300 µm.

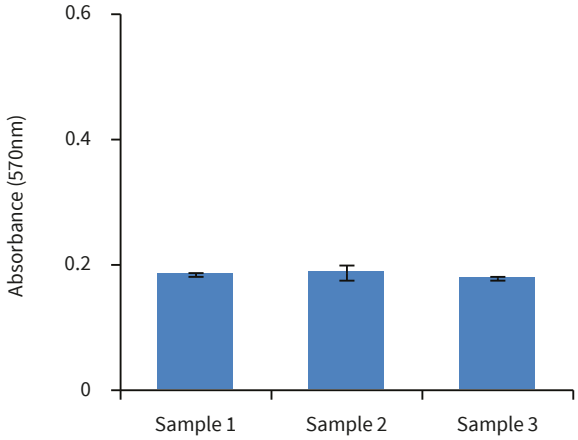

Figure 3. Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of SW620 cells are shown (n = 3, mean ± SD). Cells were cultured for 3 days on 15 mm Alvetex Scaffold discs presented in the 24 well plate format.

Example Data: 6 well insert format, AVP004

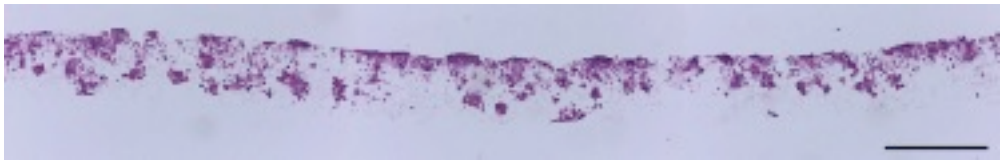

Figure 4. Brightfield micrographs showing the structure of SW620 cells cultured for 7 days on 22 mm diameter Alvetex Scaffold discs presented in 6 well inserts in the 6 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bar: 300 µm.

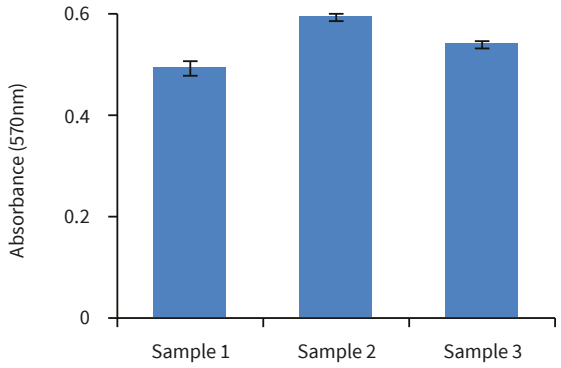

Figure 5. Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of SW620 cells are shown (n = 3, mean ± SD). Cells were cultured for 3 days on 22 mm Alvetex Scaffold discs presented in 6 well inserts in the 6 well plate format.

Example Data: 12 well insert format, AVP005

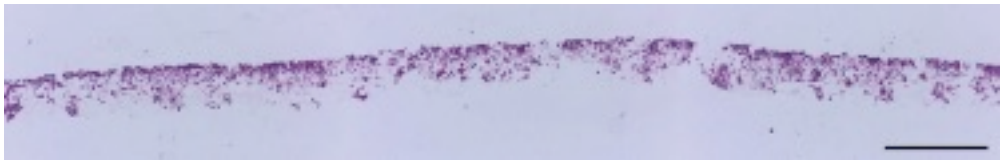

Figure 6. Brightfield micrographs showing the structure of SW620 cells cultured for 7 days on 15 mm diameter Alvetex Scaffold discs presented in 12 well insert in 12 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bar: 300 µm.

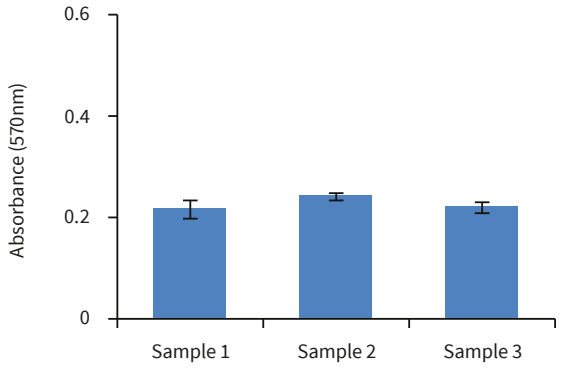

Figure 7. Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of SW620 cells are shown (n = 3, mean ± SD). Cells were cultured for 3 days on 15 mm Alvetex Scaffold discs presented in 12 well insert in 12 well plate format.

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