Protocol

Example Protocol for the Culture of the MG63 Cell Line on Alvetex™ Scaffold in Well Insert Format

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. MG63 cells were routinely maintained in T-75 flasks.

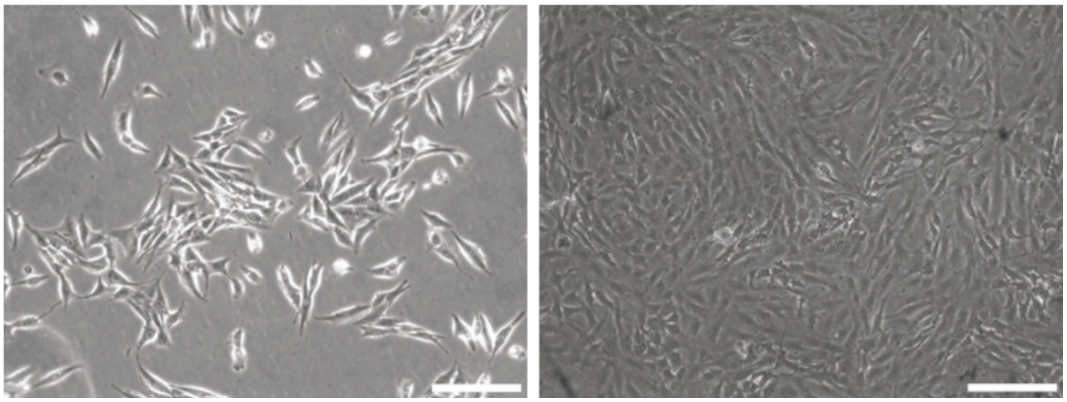

Figure 1. *Phase contrast micrographs of MG63 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 100 µm.*

2. Complete growth media consisted of: DMEM High glucose supplemented with 10 % v/v heat in activated FBS, 1× non-essential amino acids, 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.

2.2. 12-well Insert Format (AVP005)

1. Alvetex Scaffold 12-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 cell suspension was added to the centre of the Alvetex Scaffold disc, which was equivalent to 0.5×10^6 cells per well.

3. 5 mL of media was added to each well taking care not to allow media over the windows of the insert, i.e. independent feeding from below. The plate was incubated overnight at 37 °C with 5 % CO_2 to allow the cells to settle into the scaffold.

4. A further 5 mL of media was added to each well the following morning 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 6-well insert format (AVP004). 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 insert format (AVP005)

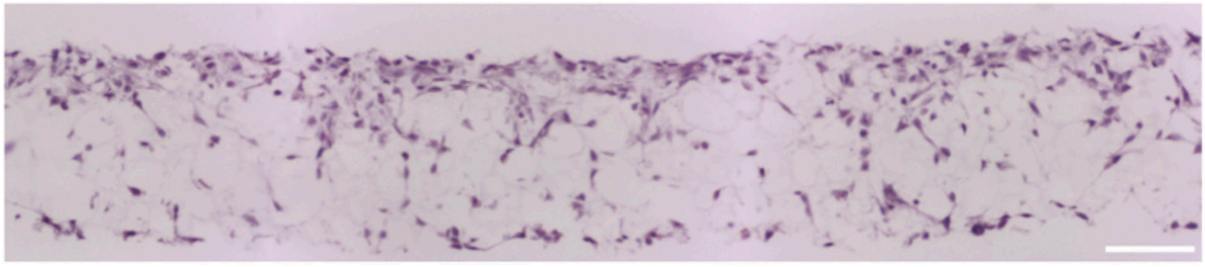

Figure 2. *Brightfield micrographs showing the structure of MG63 cells cultured for 7 days on 15 mm diameter Alvetex Scaffold discs presented in 12-well insert in 6-well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bar 100 µm.*