Protocol

Example Protocol for the Culture of the Breast Carcinoma MDA-MB-231 Cell Line on Alvetex™ Scaffold in Well Insert and Well Plate Formats

1. Introduction

MDA-MB-231 is a metastatic human breast cancer cell line originally isolated in the early 1970s[1]. MDA-MB-231 cells possess epithelial-like morphology and exhibit invasive properties when cultured *in vitro* and when transplanted into mice. MDA-MB-231 is widely used as a model for estrogen receptor negative breast cancer with applications in the study of tumorigenicity, metastasis, and cell invasion.

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. MDA-MB-231 cells (ATCC, HTB-26) were routinely maintained in T-75 flasks.

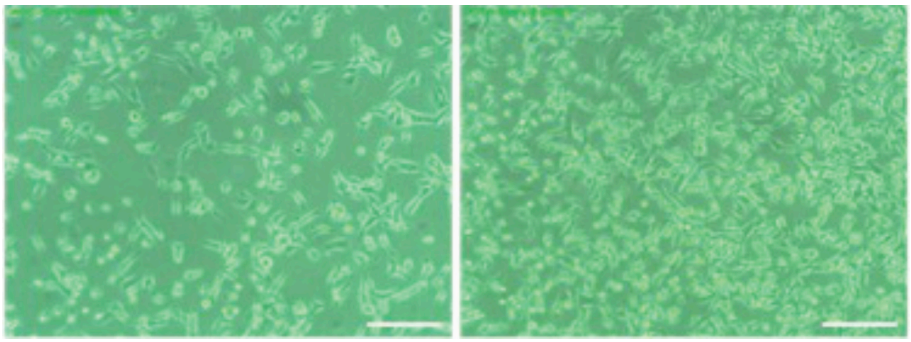

Figure 1. *Phase contrast micrograph of MDA-MB-231 cells grown in conventional 2D culture plates. Image shows cells at low (left) and high (right) confluency. Scale bars: 100 µm.*

2. Complete growth media consisted of: Leibovitz's L-15 medium (ATCC, 30-2008) supplemented with 10 % v/v FBS and 100 U/mL Penicillin/Streptomycin.

Note: The L-15 medium already contains 2 mM L-glutamine and is formulated for use in air of atmospheric composition.

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 3.33×10^6 cells/mL for seeding.

2.2. 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. 75 µL of the cell suspension was added to the centre of each Alvetex Scaffold disc, which was equivalent to 0.25×10^6 cells per well.

3. The plate was incubated for at least 15 minutes at 37 °C without 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 every other day.

Note: This method can be applied to the use of Alvetex Scaffold in 12 well plate format (AVP002). Adjust cell seeding and media volumes according to the guidelines provided in the Alvetex Scaffold Quick Start protocol.

2.3. 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. 75 µL of the cell suspension was added to the centre of each Alvetex Scaffold disc, which was equivalent to 0.25×10^6 cells per well.

3. The plate was incubated for at least 15 minutes at 37 °C without CO_2 to allow the cells to settle into the scaffold.

4. Media was carefully added to the outer wall of each well to a total volume of 4 mL, taking care not to dislodge cells from Alvetex Scaffold.

5. Plates were re-incubated and maintained by complete media exchange every other day.

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. 24 well plate format (AVP006)

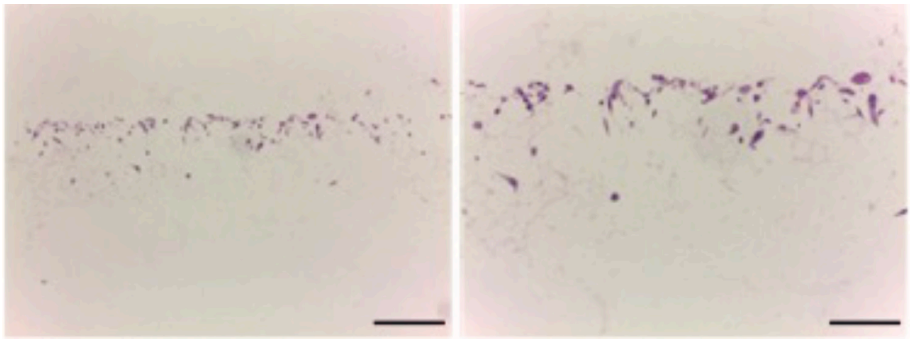

Figure 2. *Brightfield micrograph showing the structure of MDA-MB-231 cells cultured for 3 days on 15 mm diameter Alvetex Scaffold discs presented in 24 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bars: Left: 200 µm, Right: 100 µm.*

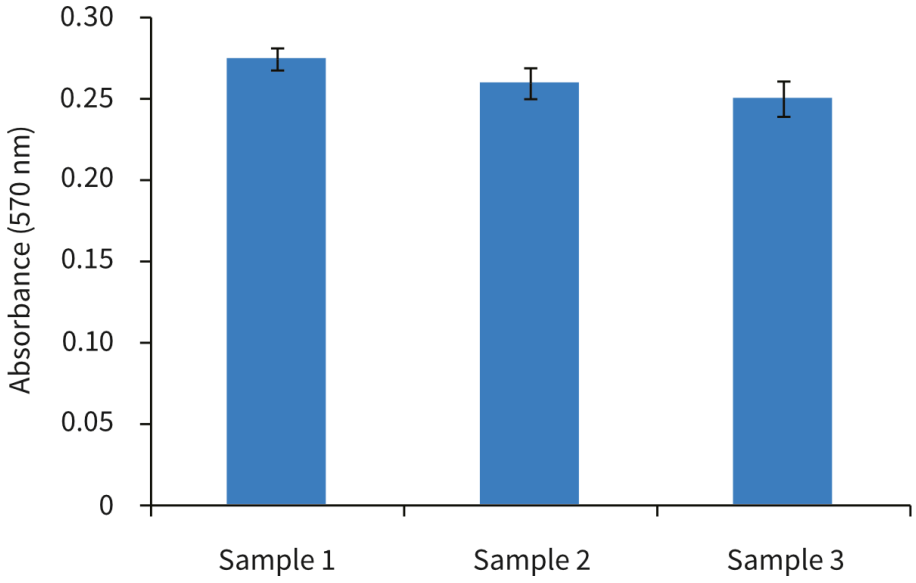

Figure 3. *Biochemical analysis of cell viability using a standard MTT assay. Data from 2 sample replicates of MDA-MB-231 cells are shown, each sampled in triplicate (n=3, mean ± SD). Cells were cultured for 3 days on 15 mm Alvetex Scaffold discs presented in 24 well plate format.*

3.2. 12 well insert format (AVP005)

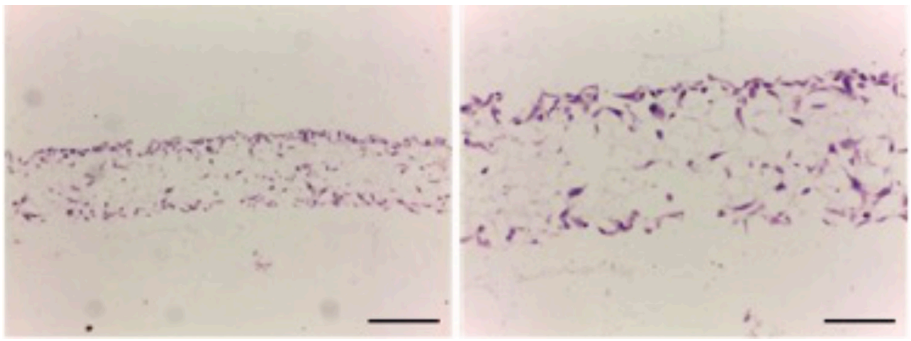

Figure 4. *Brightfield micrograph showing the structure of MDA-MB-231 cells cultured for 3 days on 15 mm diameter Alvetex Scaffold discs presented in 12-well insert in 12 well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bars: Left: 200 µm, Right: 100 µm.*

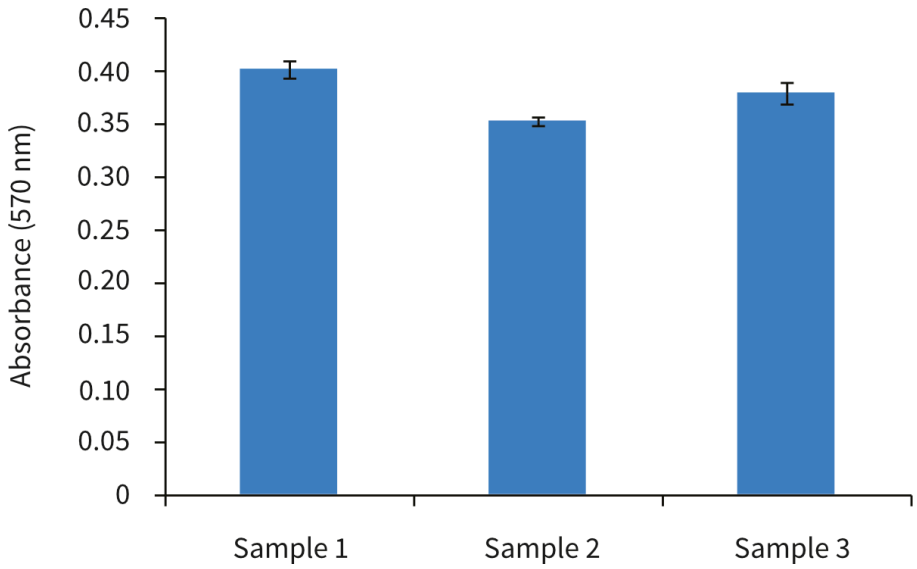

Figure 5. *Biochemical analysis of cell viability using a standard MTT assay. Data from 2 sample replicates of MDA-MB-231 cells are shown, each sampled in triplicate (n = 3, mean ± SD). Cells were cultured for 3 days on 15 mm Alvetex Scaffold discs presented in 12 well inserts in 12 well plate format.*

4. References

1. Cailleau RM *et al*, 1974. Breast tumor cell lines from pleural effusions. *J Natl Cancer Inst* 53(3): 661-74.