

Introduction:

Alvetex[®] Scaffold is currently available in four different cell culture formats: 24-well plate (AMS.AVP006), 12-well plate (AMS.AVP002), 6-well insert (AMS.AVP004), and 12-well insert (AMS.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 cell penetration into the scaffold. They also provide for conveniently tailored media set ups (see Quick Start Protocol, www.reinnervate.com/alvetex/workflow). 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 (AMS.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.

Methods:

Preparation for 3D cell culture on Alvetex[®] Scaffold

1. MCF-7 cells were routinely maintained in T-75 flasks.

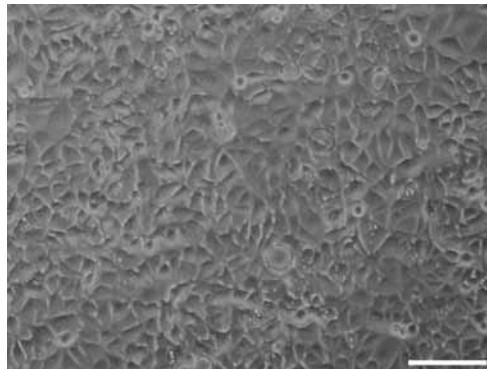


Figure 1. Phase contrast micrograph of MCF-7 cells grown in conventional 2D culture plates. Scale bar: 200 μ m.

2. Complete media consisted of: Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10 % v/v 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.



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

6-well Insert format **AMS.AVP004:**

- a. Cells were re-suspended at a concentration of 10×10^6 cells/ml for seeding.
- b. Alvetex® Scaffold 6-well inserts were prepared for seeding with a 70 % ethanol wash (7ml) and subsequent media washes (twice, with 7ml media each).
- c. 100 μ l of the cell suspension was added to the centre of the Alvetex® Scaffold, which was equivalent to 1×10^6 cells per well.
- d. The plate was incubated for 60 minutes at 37 °C with 5 % CO₂ to allow the cells to settle into the scaffold.
- e. 10 ml of media was added to each well taking care not to dislodge cells from the membrane.
- f. 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, **AMS.AVP005**. Adjust cell seeding and media volumes according to the guidelines provided in the Quick Start Protocol.

24-well Plate Format, **AMS.AVP006:**

- a. Cells were re-suspended at a concentration of 4×10^6 cells/ml for seeding.
- b. Alvetex® Scaffold 24-well plate were prepared for seeding with a 70 % ethanol wash (2 ml per well) and subsequent media washes (twice, with 2 ml media each).
- c. 50 μ l of the cell suspension was added to the centre of the Alvetex® Scaffold, which was equivalent to 0.2×10^6 cells per well.
- d. The plate was incubated for 60 minutes at 37 °C with 5 % CO₂ to allow the cells to settle into the scaffold.
- e. 2 ml of media was added to each well taking care not to dislodge cells from the membrane.
- f. Plates were re-incubated and maintained by complete media exchange every 2-3 days for the first 10 days, then every day.

Note: This method can be applied to the use of Alvetex® Scaffold in 12-well plate format, **AMS.AVP002**. Adjust cell seeding and media volumes according to the guidelines provided in the Quick Start Protocol.



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Example Data: 6-well Insert Format, AMS.AVP004:

MCF-7 cells were maintained on Alvetex® Scaffold 6-well inserts for up to 21 days. Cultures underwent complete media change every 2-3 days. At specific time points, cultures were harvested and analysed by histological staining (Figure 2) and MTT viability assay (Figure 3) to monitor cell survival and proliferation within the scaffold.

The cells showed good linear growth and expansion in 3D which plateaued between 14 and 21 days. We would not recommend growing cultures of MCF-7 cells beyond 14-18 days as when they reach confluency in 3D they can become overcrowded and the quality of the culture is reduced resulting in cell death.

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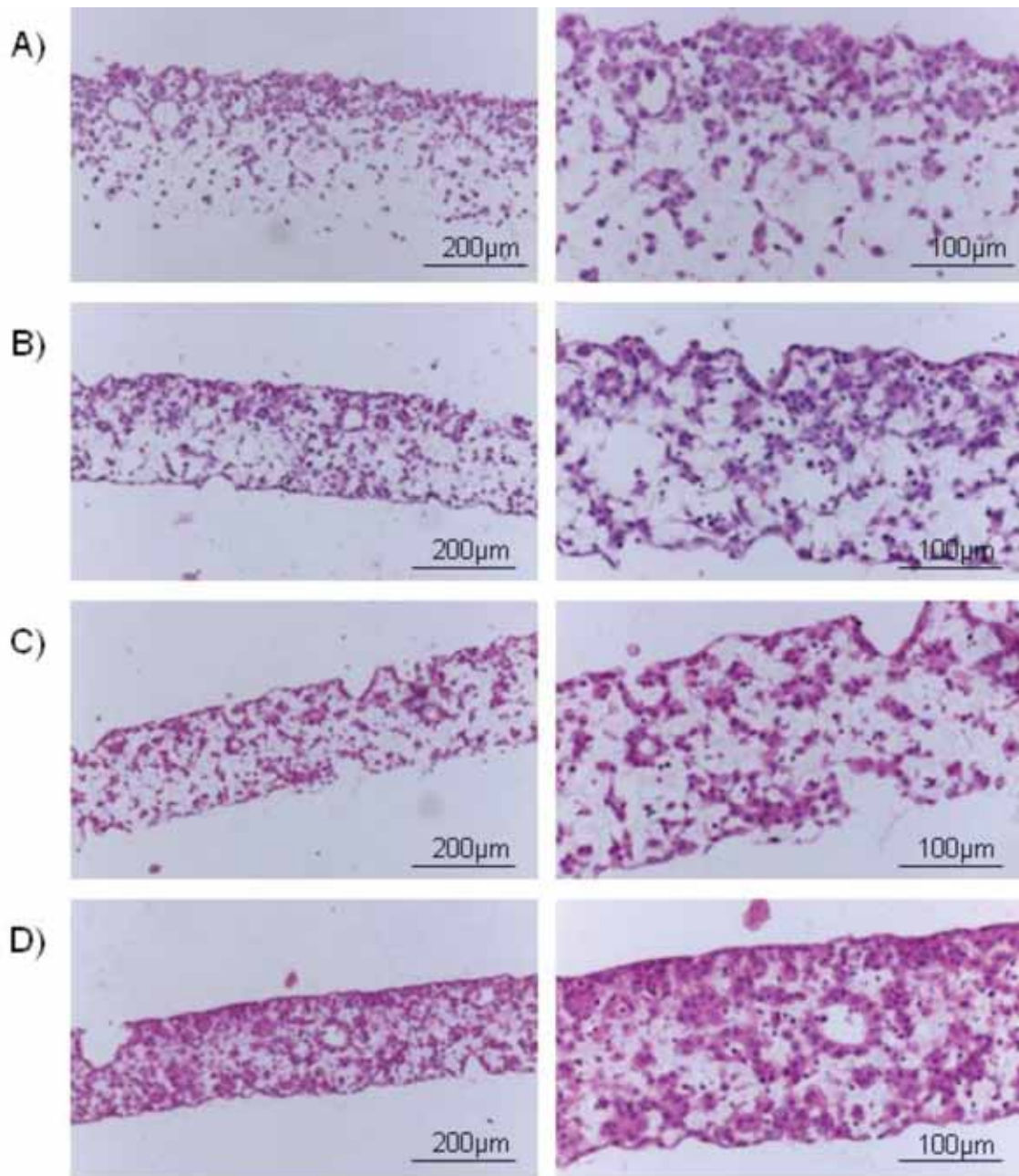


Figure 2. Brightfield micrographs showing the structure of MCF-7 cells cultured for A) 3 days, B) 7 days, C) 10 days or D) 14 days on 22 mm diameter Alvetex® Scaffold presented in 6-well inserts. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.


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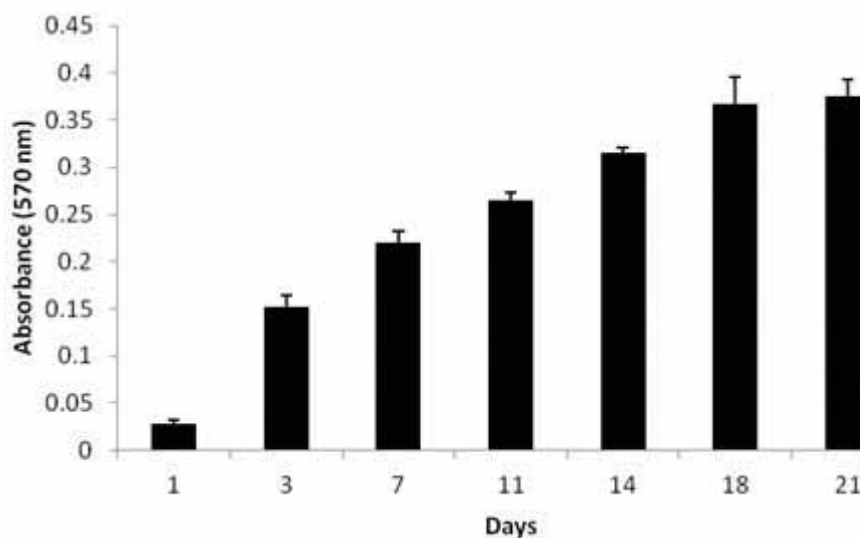


Figure 3. Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of MCF-7 cells are shown (mean \pm SD). Each well was sampled in triplicate with mean value shown. Cells were cultured for up to 21 days on 22 mm Alvetex[®] Scaffold presented in 6-well inserts.

Example Data: 24-well Plate Format, AMS.AVP006:

MCF-7 cells were maintained on Alvetex[®] Scaffold 24-well plates for up to 18 days. Cultures underwent complete media change every 2-3 days until day 10, then every day. At specific time points, cultures were harvested and analysed by histological staining (Figure 2) and MTT viability assay (Figure 3) to monitor cell survival and proliferation within the scaffold.

The cultures showed good linear growth and expansion in 3D which plateaued between 10 and 18 days. If cells are grown longer than 10 days, we would recommend changing the medium every day. When the cells reach confluency in 3D they become overcrowded and the quality of the culture is reduced, resulting in cell death, therefore we would not recommend culturing these cells for longer than 14 days in this culture format.



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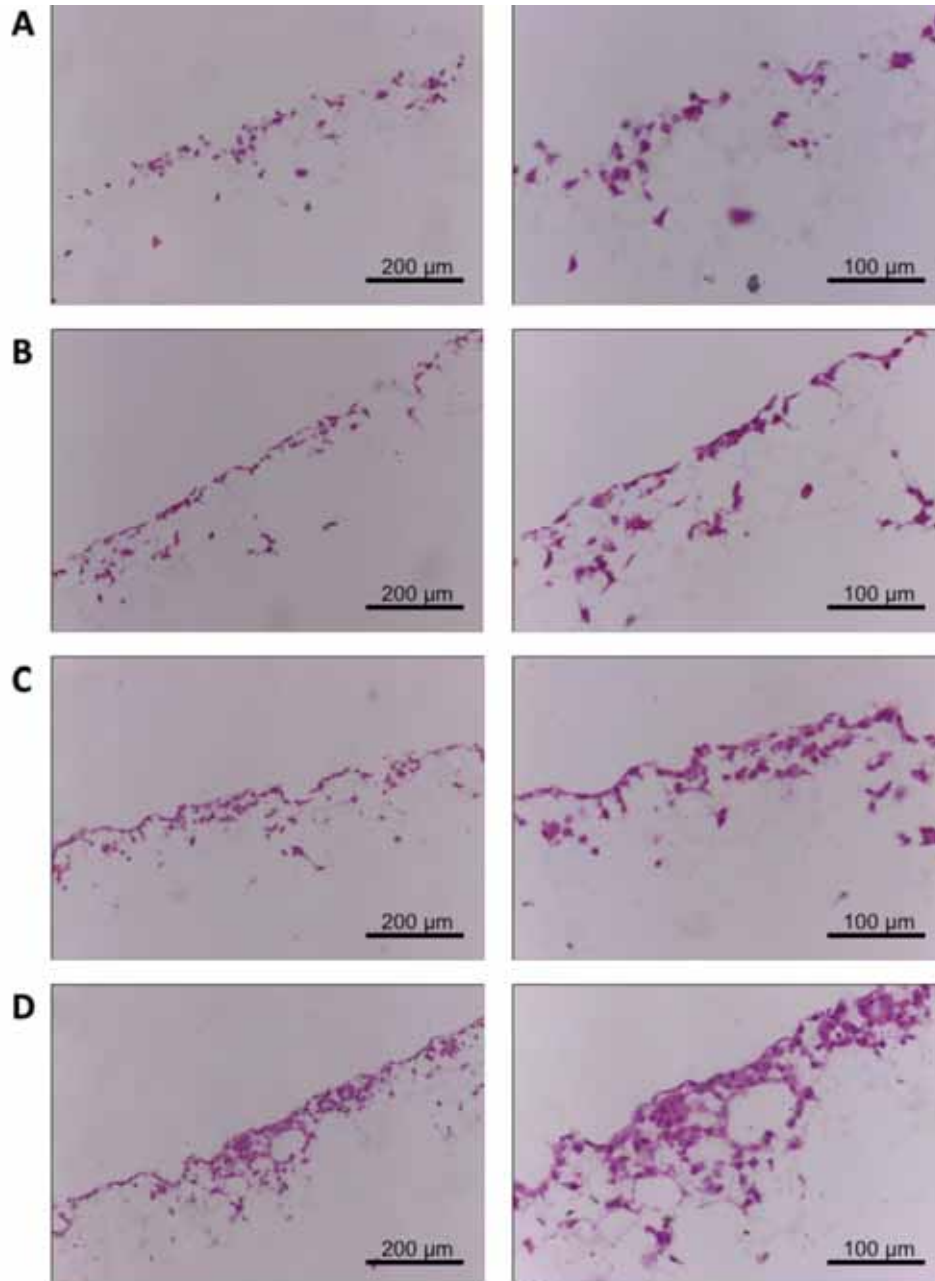


Figure 4. Brightfield micrographs showing the structure of MCF-7 cells cultured for A) 3 days, B) 7 days, C) 10 days or D) 14 days on 15 mm diameter Alvetex[®] Scaffold presented in 24-well plates. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.


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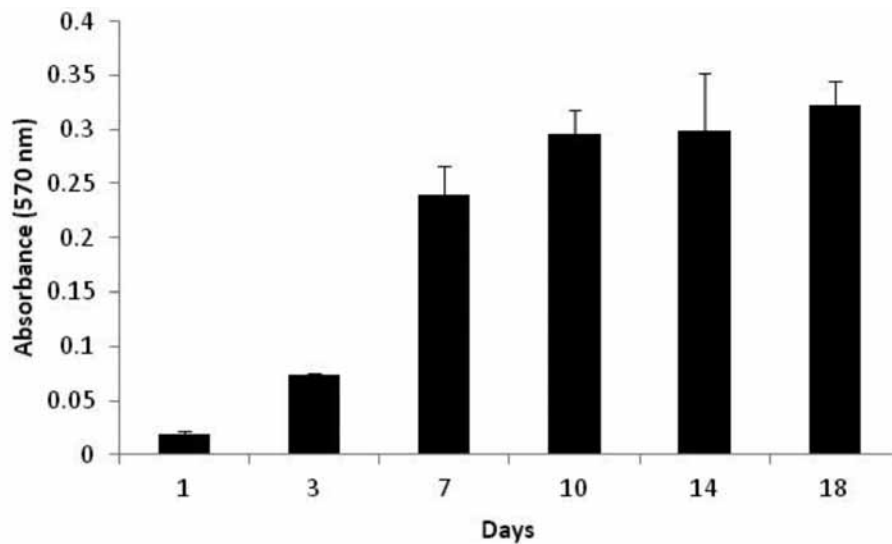
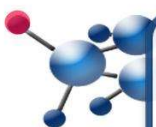


Figure 5. Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of MCF-7 cells are shown (mean \pm SD). Each well was sampled in triplicate with mean value shown. Cells were cultured for up to 18 days on 15 mm Alvetex[®] Scaffold presented in 24-well plate format.

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