

## Introduction

The following protocol outlines how to coat Alvetex® Scaffold membranes with fibronectin in order to facilitate and enhance cell attachment and migration within the scaffold. Example data shown herein was obtained using this protocol to grow HepG2 hepatocytes on fibronectin coated Alvetex® Scaffold for 7 days in 6-well inserts (AMS.AVP004-32) in a 6-well plate format.

## Method

1. Prepare Alvetex® Scaffold for coating by first treating with 70 % ethanol followed by two PBS washes as described in the relevant product information leaflet. Leave Alvetex® Scaffold in the second PBS wash until ready to apply the fibronectin solution.
2. Reconstitute fibronectin (BD Biosciences, 356008) to a concentration of 0.5 mg/ml using PBS.
3. Aspirate the second PBS wash from Alvetex® Scaffold disc and carefully pipette 300 µl of the diluted fibronectin solution onto each disc. Replace plate lids and leave to stand for 1 hour at room temperature.
4. Remove excess fluid from Alvetex® Scaffold in well insert format by gently tapping the plate or Petri dish on the worktop. Check that no residual fluid is hanging from the base of the well inserts. Aspirate to remove any residual coating agent from the bottom of the wells. If using Alvetex® Scaffold in 12-well plate format, tilt the plate and gently aspirate any excess fluid from the edge of the wells.
5. Prepare cells for seeding in the appropriate culture media and seed directly on the wet fibronectin coated Alvetex® Scaffold membrane. Seed cells in volumes relevant for the specific Alvetex® Scaffold format being used (see product information booklet for volume details). Allow the cells to settle for 30-90 minutes in an incubator (5 % CO<sub>2</sub>, 37 °C) before flooding with media.

**Example** Growth of HepG2 Hepatocyte Cell Line in Fibronectin Coated Alvetex® Scaffold

### Cell Culture details:

HepG2 cells (ATCC, HB-8065) were routinely maintained in T-75 flasks. HepG2 complete media consisted of: MEM media (Gibco, 21090) supplemented with 10 % v/v FBS, 2 mM L-glutamine and 100 U/ml Penicillin/Streptomycin. Alvetex® Scaffold 6-well inserts (AMS.AVP004-32) in 6-well plates, were coated in fibronectin as described above.

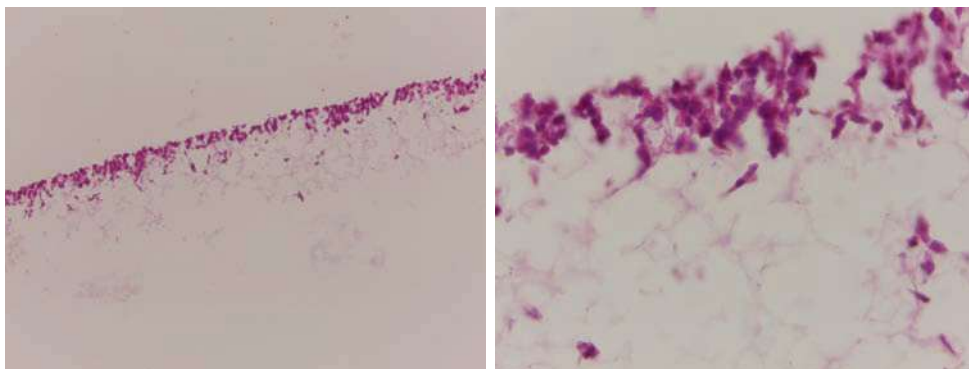
Cells were seeded at a density of  $1 \times 10^6$  cells in 150 µl media suspension per disc and were left to settle for 60 minutes in an incubator (5 % CO<sub>2</sub>, 37 °C).

Media was carefully added to each sample (10 ml per well). Cultures were maintained for 7 days with media changes on days 2 and 5.

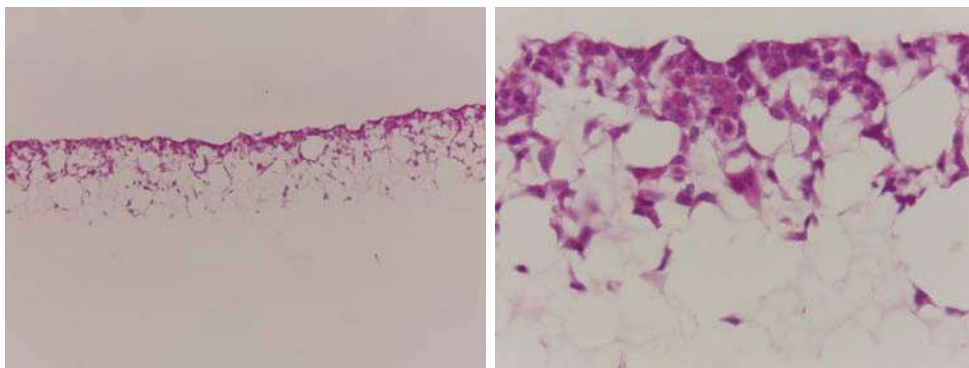
**Results**

Pre-coating of Alvetex® Scaffold discs with fibronectin resulted in enhanced infiltration of HepG2 cells into the scaffold compared with control cultures in untreated Alvetex® Scaffold. Cells were seen to have reached deeper within the scaffold after 7 days of growth in treated discs, while cells grown in untreated Alvetex® Scaffold occupied only the upper part of the scaffold. These findings indicate that pre-treatment of Alvetex® Scaffold with extracellular matrix products is able to enhance the attachment and growth of appropriate cell types into the 3D structure.

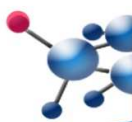
**Uncoated Alvetex® Scaffold control  
7 Day**



**Fibronectin coated Alvetex® Scaffold  
7 Day**



**Figure 1.** Brightfield micrographs at low (10x) and high (40x) magnification showing HepG2 cells cultured for up to 7 days on 22 mm diameter Alvetex® Scaffold discs presented in 6-well insert (AMS.AVP004-32) in 6-well plate format. Cells were fixed, sectioned and counterstained with Haematoxylin and Eosin.



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