Introduction

The following protocol outlines how to coat alvetex membranes with a thin layer of collagen I 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 collagen I coated alvetex® for 7 days in 6-well inserts (AMS.AVP004-32) in 6-well plate format.

Method:

1. *Prepare alvetex® for coating by first treating with 70% ethanol followed by two PBS washes as described in the relevant product information leaflet. Leave alvetex® in the second PBS wash until ready to apply the collagen solution.*
2. *Dilute rat tail collagen I (AMSBIO) to a concentration of 0.8 mg/ml using cell culture grade water. Handle the reagents on ice, using pre-chilled pipette tips to perform the dilution and subsequent application onto alvetex®.*
3. *Aspirate the second PBS wash from alvetex® disc and carefully pipette 500 µl of the diluted collagen solution onto each disc. Replace plate lids and leave to stand for 1 hour at room temperature.*
4. *Remove excess fluid from alvetex® 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® 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 collagen coated alvetex membrane in the volumes relevant for alvetex® product format. Allow the cells to settle for 30-90 minutes in an incubator (5 % CO_2, 37 ℃) before flooding with media.*

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Example: *Growth of HepG2 Hepatocyte Cell Line in Collagen I Coated Alvetex®*

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® 6-well inserts (AMS.AVP004-32) in 6-well plates, were coated in collagen I as described above.
Cells were seeded at a density of 1 x 10^6 cells in 150 µl media suspension per disc and were left to settle for 90 minutes in an incubator (5 % CO_2, 37 ℃). Media was carefully added to each sample (9 ml per well). Cultures were maintained for 7 days, with media changes on days 2, 3, and 6.

Results:

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

Uncoated alvetex® control

3 Day **7 Day Culture**

Collagen I-coated

3 Day **7 Day Culture**

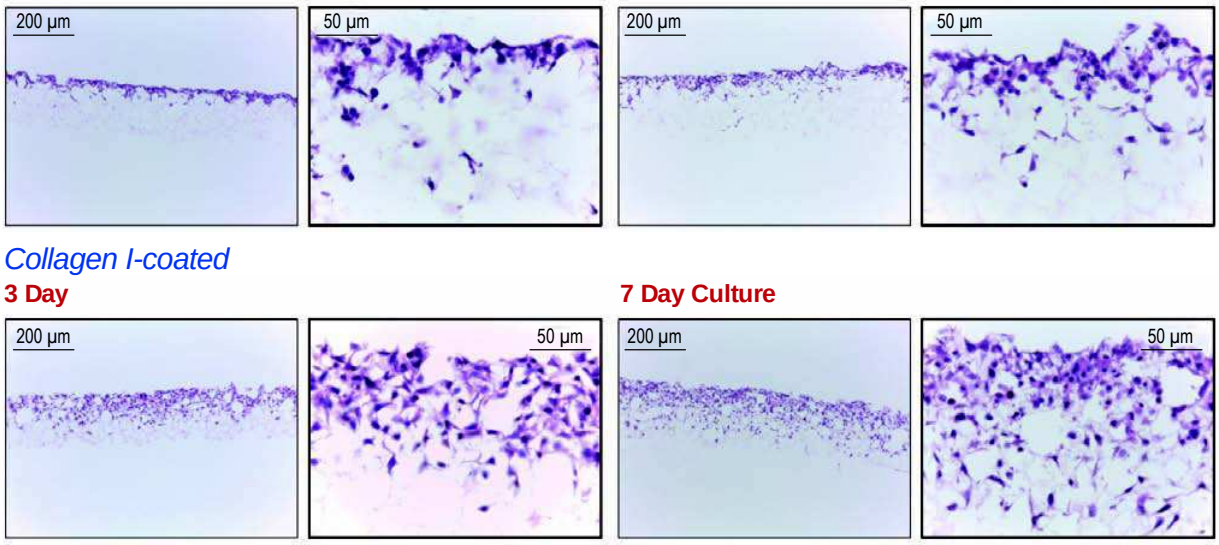

Figure 1. *Brightfield micrographs at low (10x) and high (40x) magnification showing HepG2 cells cultured for up to 7 days on 22 mm diameter alvetex® discs presented in 6-well insert (AMS.AVP004-32) in 6-well plate format. Cells were fixed, sectioned and counterstained with haematoxylin and eosin.*