Introduction:

The following protocol outlines a method for the retrieval of cells cultured in Alvetex®Scaffold. Example data were obtained using this protocol to extract HepG2 hepatocyte cells cultured in Alvetex®Scaffold for 7 days in 6-well inserts (AMS.AVP004-32) in the Well Insert Holder in a Deep Petri Dish (AMS.AVP015-2) format. Two methods of cell extraction may be employed; a standard method and a syringe-based method which produces higher average cell yields. Both methods demonstrate that partial retrieval of cells from Alvetex® Scaffold is possible.

Methods:

1.0. Standard Enzymatic Method

1.1. Unclip inserts and carefully remove the Alvetex®Scaffold discs using flat-ended forceps.

1.2. Gently wash each disc in PBS, and transfer to a new 6-well plate.

1.3. 3 ml of 0.25 % Trypsin-EDTA (Sigma, T4049).

1.4. Incubate plate at 37 °C, 5 % CO_2 on a shaking platform set to 100 rpm, for 15 minutes.

1.5. Transfer the resulting cell suspension to a 15 ml centrifuge tube.

1.6. Add 3 ml medium to the well containing the Alvetex®Scaffold membrane and gently triturate to remove residual detached cells.

1.7. Combine the two solutions in the 15 ml centrifuge tube to neutralise the trypsin.

1.8. Centrifuge at 1000 rpm for 5 minutes, to pellet the cells.

1.9. Resuspend the cell pellet in an appropriate volume of medium for downstream processes.

2.0. Enzyme and Syringe Method

2.1. Unclip inserts and carefully remove the Alvetex®Scaffold discs using flat-ended forceps.

2.2. Gently wash each disc in PBS, and transfer to a new 6-well plate.

2.3. 3 ml of 0.25 % Trypsin-EDTA (Sigma, T4049).

2.4. Incubate plate at 37 °C, 5 % CO_2 on a reciprocating shaker set to 100 strokes/min, for 15 minutes.

2.5. Transfer the resulting cell suspension to a 15 ml centrifuge tube.

2.6. Transfer the Alvetex®Scaffold membrane to the bottom of a 30 ml plastic syringe barrel. Add 3 ml medium and depress the plunger slowly to force residual detached/loose cells from the scaffold.
Note: Care should be taken not to force the solution through the membrane too quickly as this is liable to induce considerable shear stress upon the cells.

2.7. Combine the two solutions in the 15 ml centrifuge tube to neutralise the Typsin.

2.8. Centrifuge at 1000 rpm for 5 minutes, to collect the cells.

2.9. Resuspend the cell pellet in an appropriate volume of medium required for the downstream processes.

Example: HepG2 cells grown for 7 days in Alvetex®Scaffold 6-well insert in a Petri dish format.

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. Cells were seeded onto Alvetex®Scaffold discs in 6-well inserts (AMS.AVP004-32) in Well Insert Holders in Deep Petri dishes (AMS.AVP015-2), at a density of 1x 10^6 cells in 100 µl media per insert. After settling for 1 hour in an incubator (5 % CO_2, 37 °C), complete media was carefully added (70 ml per Petri dish). Cultures were maintained for 7 days, with media changes on days 2 and 4. After 7 days, cultures were processed according to the protocol described above.

The total number, and number of viable cells retrieved were counted using Trypan Blue exclusion method. Alvetex®Scaffold discs were analysed post-retrieval by MTT assay (Figure 1.) and histological staining to estimate cell numbers remaining in the 3D scaffold (Figure 2.).

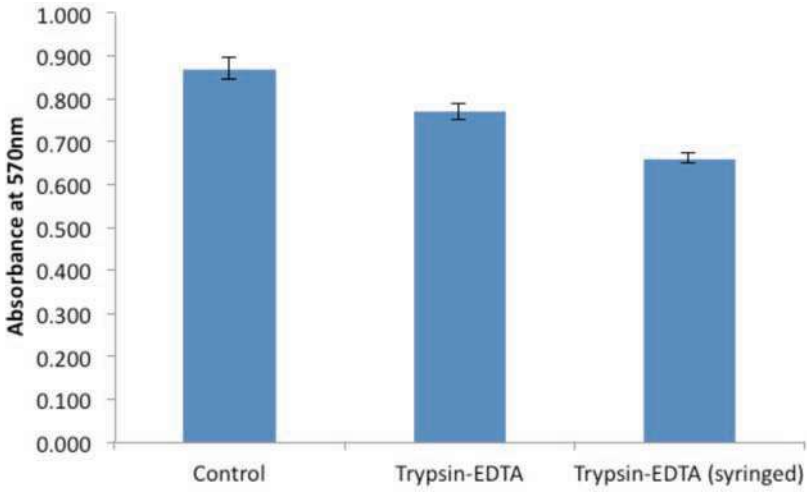

Figure 1. *Biochemical analysis of cell viability using a standard MTT assay on Alvetex®Scaffold discs post-cell extraction using trypsin-EDTA. Data from 3 sample replicates of HepG2 cultures are shown (n=3, mean ± SE).*

On the basis of residual in-scaffold MTT assay data, 12 % of cells were retrieved (compared to untreated control discs) using the standard protocol described above. When the syringe-based method was employed, cell recovery increased to 24 %. Cell viability post-retrieval was 99 % for both methods, as determined by cell counting in conjunction with Trypan Blue exclusion (data not shown).

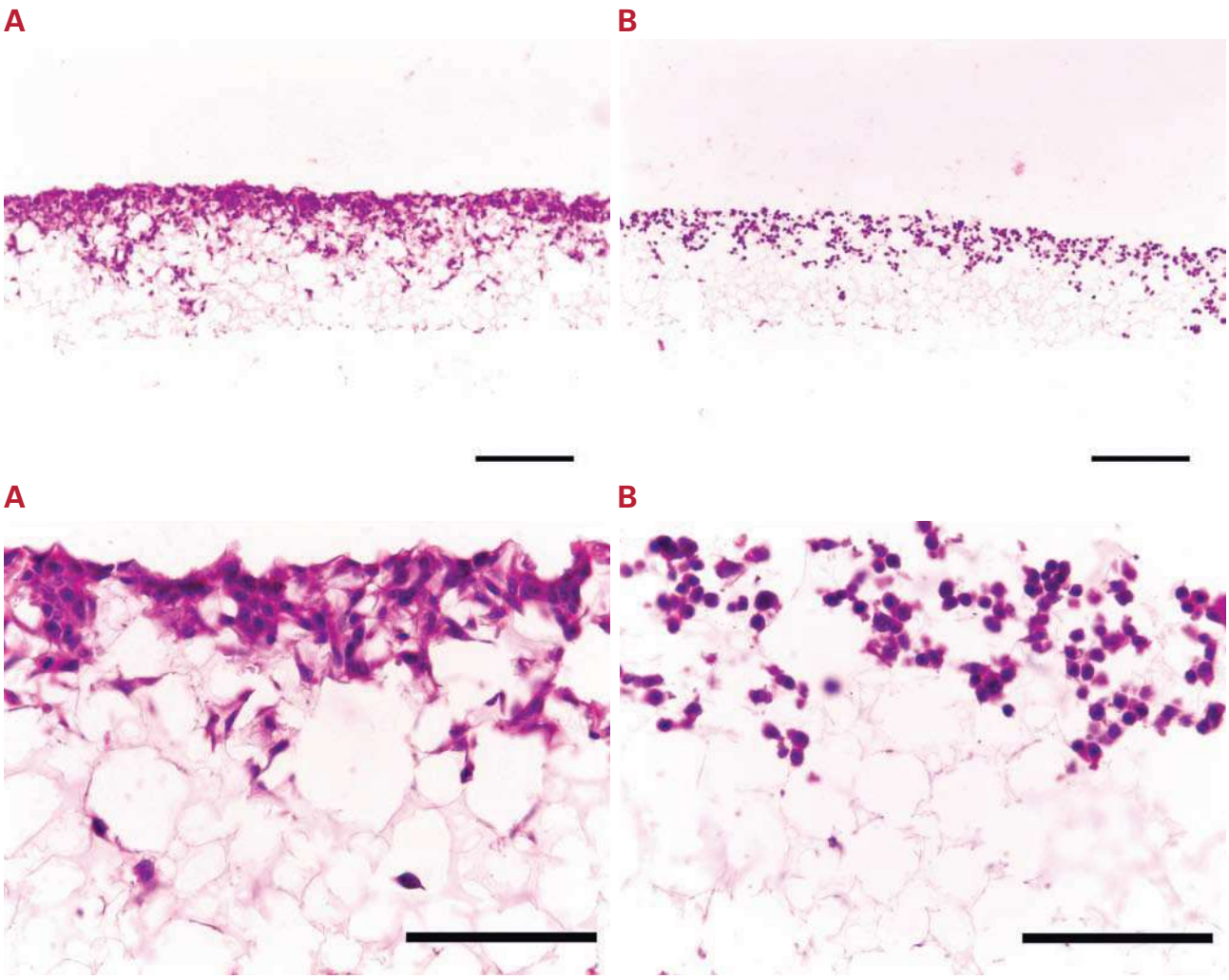

Figure 2. *Bright field micrographs showing the morphology of HepG2 cells cultured for 7 days on Alvetex®Scaffold in 6-well insert within a Petri dish format (x10 and x40 objective).* ***A:*** *untreated HepG2 cells and* ***B:*** *HepG2 cells after Trypsin-EDTA treatment for 15 minutes. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with Haematoxylin and Eosin. The effect of Trypsin action can clearly be seen on residual cells which lose contact with each other and adopt a rounded morphology.*

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