International Journal of Surgery 25 (2016) 31–37

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

# Comparing the endothelialisation of extracellular matrix bioscaffolds with coated synthetic vascular graft materials

D.N. Coakley [a, *], F.M. Shaikh [a], K. O'Sullivan [b], E.G. Kavanagh [a], P.A. Grace [a], S.R. Walsh [a], T.M. McGloughlin [c]

[a] Department of Vascular Surgery, University Hospital Limerick, Limerick, Ireland
[b] The Statistical Consultancy Unit, School of Mathematical Sciences, University College Cork, Cork, Ireland
[c] Department of Mechanical and Aeronautical Engineering, Materials and Surface Science Institute, University of Limerick, Centre for Applied Biomedical Engineering Research, Limerick, Ireland

## HIGHLIGHTS

- There was no difference in adhesion rates between ECM scaffolds and fibrin-coated ePTFE.
- ECM bioscaffolds offer an improved substrate for promoting rapid endothelialisation compared to fibrin-coated ePTFE.
- ECM materials are promising scaffolds for small vessel tissue engineering.

## ARTICLE INFO

*Article history:*
Received 21 April 2015
Received in revised form
1 November 2015
Accepted 5 November 2015
Available online 12 November 2015

*Keywords:*
Biomaterials
Tissue engineering
Extracellular matrix (ECM)
Endothelialisation
Vascular grafts

## ABSTRACT

*Introduction:* Existing synthetic vascular grafts have unacceptably high failure rates when replacing below knee arteries. In vitro endothelialisation is a technique, which has been shown to enhance the patency rates of below knee vascular grafts. Synthetic materials are however poor cellular substrates and must be combined with coatings to promote cellular growth and attachment. The most common coating clinically is fibrin-coated ePTFE. The aim of our study was to compare the endothelialisation of fibrin-coated ePTFE with novel extracellular matrix (ECM) biomaterials that we hypothesise will provide a superior substrate for cell growth.

*Methods:* Human endothelial cells were cultured on ECM scaffolds and fibrin-coated ePTFE. Uncoated Dacron and ePTFE acted as controls. The cells were examined for viability, phenotype, adhesion and proliferation. Cell morphology was accessed using scanning electron microscopy.

*Results:* Cells remained viable and produced von Willebrand factor on all substrates tested. ECM scaffolds and fibrin-modified ePTFE achieved statistically higher attachment efficiency when compared to both uncoated synthetic graft materials ($p \leq 0.001$). At 90 min 80 ± 3.6% of cells had attached to the ECM scaffold compared to Dacron (30 ± 4.5%, $n = 3$) and ePTFE (33 ± 2.5%, $n = 3$). There was no difference in adhesion rates between ECM scaffolds and fibrin-coated ePTFE ($p = 1.00$). Endothelial cells proliferated fastest on ECM scaffolds when compared to all other materials tested ($p < 0.001$) and reached confluency on day seven.

*Conclusion:* ECM bioscaffolds offer an improved substrate for promoting rapid endothelialisation compared to fibrin-coated ePTFE by combining firm cellular anchorage and superior cell expansion.



## 1. Introduction

Existing synthetic vascular grafts have unacceptably high failure rates when replacing small diameter infrapopliteal vessels [1]. The lack of a confluent endothelial lining is repeatedly cited as the most common cause of conduit failure [2,3]. In vitro graft endothelialisation is an emerging method, which has been shown in several long-term human clinical trials to significantly enhance the patency rates of small caliber synthetic grafts [4–7]. In this technique, autologous endothelial cells (ECs) are harvested from superficial veins or adipose tissue and seeded onto the graft lumen prior to

* Corresponding author.
*E-mail address:* drdanielcoakley@gmail.com (D.N. Coakley).

http://dx.doi.org/10.1016/j.ijsu.2015.11.008
1743-9191/

implantation. Existing synthetic graft materials are however, poor substrates for cell culture and must be combined with coatings to promote cellular proliferation and adhesion [8,9]. To date, a plethora of graft coatings have been investigated with no common consensus as to the most efficacious. The aim of this study is to compare the endothelialisation of the most commonly employed graft coating clinically; fibrin-coated expanded polytetrafluoroethylene (ePTFE) with novel decellularised xenogenic extracellular matrix (ECM) scaffolds. ECM scaffolds support the growth of several cell types [10–12] and have already proven successful in reconstructing a wide range of specialised tissues [13–15]. The ECM scaffolds we are examining are derived from the porcine urinary bladder wall - Urinary Bladder Matrix (UBM) and porcine jejunum - Small Intestine Submucosa (SIS). We hypothesise that an intact ECM scaffold would better approximate the natural vascular wall architecture than existing graft coatings and provide a superior substrate for in vitro endothelial seeding. We are specifically examining cellular viability, phenotype, attachment, growth and morphology.

## 2. Methods

### 2.1. Preparation of scaffolds

#### 2.1.1. Urinary bladder matrix (UBM) bioscaffolds

A urinary bladder was obtained from market-weight pigs following euthanasia. Urothelial cells were removed by soaking the bladder in normal saline solution. The bladder was then incised via its apex and halved. The external layers of the bladder wall (tunica serosa, tunica muscularis externa, tunica submucosa, and the muscularis mucosa) were removed by mechanical delaminated. The remaining bilayered material including the basement membrane of the tunica mucosa layer (luminal surface) and the subadjacent tunica propria layer (abluminal surface) constitute UBM. Decellularisation was achieved by soakage in 0.1% (v/v) peracetic acid, 4% (v/v) ethanol and 95.9% (v/v) sterile water. The sheet was then soaked in distilled water with phosphate buffered saline to return the pH to neutrality. Finally UBM was terminally disinfected by 10-kGy-gamma irradiation.

#### 2.1.2. Preparation of small intestine submucosa (SIS) bioscaffolds

SIS was harvested from the porcine jejunum. Sections of rinsed jejunum were longitudinally split to form an elongated sheet. The superficial mucosal and external muscular layers with surrounding serosa were extracted by physical delamination. The remaining layers; submucosa, muscularis mucosa and basilar layers of the mucosa, the most superficial of which is the stratum compactum (the luminal layer), constitute SIS. The side from which the muscular layers were removed is the abluminal surface. This tissue was rinsed with phosphate buffered saline (pH = 7.0) and distilled water to lyse any remaining cells and remove residual cellular debris. This was then sterilised with 0.1% peracetic acid and 20% ethanol and finally 1.5 MRad gamma irradiation.

#### 2.1.3. Fibrin gel preparation

The formation of fibrin gel has already been documented [16]. The production method is summarized here. Fibrinogen in tissue buffered saline (TBS) solution at a concentration of 10 mg/ml was prepared. Thrombin solution was then made to get the final concentration of 40 IU/ml of thrombin. Prior to use, 75 ul of 50 mM $CaCl_2$ in tissue buffered saline was added to 75 μl of 40 IU/ml thrombin and 350 μl of TBS. Then 500 μl of fibrinogen solution was added and mixed with gentle shaking in 24 well plates. The gel was left for 1 h to polymerise in an incubator at 37 °C with 5% $CO_2$ and 95% $O_2$ environment. Fibrin glue was applied evenly to the graft surface using a sterile syringe.

### 2.2. Sample preparation

2.5 cm diameter circular segments of each material was placed under sterile conditions between two stainless steel rings with inner diameters of 2.2 cm such that 3.8 $cm^2$ of the luminal graft surface was exposed. The luminal surfaces of UBM and SIS were used for seeding of cells.

### 2.3. Cell culturing technique

Human umbilical vein endothelial (HUVEC) cell lines were purchased from Cascade Biologics/Invitrogen®. HUVEC culture medium 200 was used and supplemented with low serum growth supplement (Cascade Biologics/Invitrogen). The media was replaced every 48 h until cells reached confluency. The cells were then split in a ratio 1:3 with 3 mls buffered saline solution containing 0.25% trypsin and 0.09% ethylenediaminetetraacetic acid (EDTA).

### 2.4. Seeding protocols

2.5 cm diameter discs of each material were placed in six well culture dishes. The Sterile stainless-steel culture rings were placed over the scaffolds to prevent cell leakage. The cells were seeded at a density of $7 \times 10^4$ cells per $cm^2$ in 3 mls of culture media. The constructs were then left in an incubator for 2 h to allow for cell adhesion. Thereafter the chambers were flooded with media and the constructs were returned to the incubator.

### 2.5. Cellular viability

The cytotoxicity of the constructs to HUVECs was assessed by determining cellular viability using a live/dead viability assay (Invitrogen™). The methodology of this assay has been prescribed previously [17]. Images were captured using a Nikon Eclipse TE200 inverted microscope.

### 2.6. Immunofluorescence analysis of von Willebrand factor (VWF)

Endothelial cells seeded on each substrate were fixed with 3.7% para formaldehyde in phosphate buffered saline (PBS). These were then rinsed with PBS and mixed for 20 min with 0.27% $NH_4Cl$/0.38% glycine in PBS and permeabilised with Triton X- 100 (0.5%) in PBS. Von Willebrand Factor was confirmed via fluorescein-labelled Antibodies-conjugated mouse anti-human VWF antibodies ($2\ \mu gmL^{-1}$).

### 2.7. Cellular attachment

Cellular adhesion was determined by examining the percentage of attached cells over time, seventh passage cells were used in each experiment. Uniform passages HUVECs (P7) of $7 \times 10^4$ cells per $cm^2$ were seeded separately onto each material surface in a drop wise manner and incubated for a maximum of 120 min. At 30 min intervals, the surface of the constructs were rinsed with 5 ml phosphate buffer solution (PBS) containing 0.4% v/v trypan blue to wash off any unattached cells. The detached cells were counted with a haemocytometer and expressed as a fraction of the original seeding density.

### 2.8. Cellular proliferation

Cellular proliferation was assessed on days 1,3,5 and 7 using the