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REVIEW

Cell-matrix biology in vascular tissue engineering

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Abstract

We are developing biocompatible small-calibre vascular substitutes based on polymeric scaffolds that incorporate cell-matrix signals to enhance vascular cell attachment and function. Our graft scaffold comprises an outer electrostatically spun porous polyurethane layer seeded with smooth muscle cells, and a luminal polycaprolactone layer for endothelial cell attachment. Vascular cell adhesion properties of three vascular elastic fibre molecules, tropoelastin, fibrillin-1 and fibulin-5, have been defined, and adhesion fragments optimized. These fragments are being used to coat the scaffolds to enhance luminal endothelial cell attachment, and to regulate smooth muscle cell attachment and function. Tropoelastin-based cell seeding materials are also being developed. In this way, vascular cell-matrix biology is enhancing graft design.

Key words arteries; cell adhesion; endothelial cells; extracellular matrix; smooth muscle cells; tissue engineering.

Introduction

Vascular disease is the largest killer in Western society, and bypass grafting is a common treatment. However, there is a great need for tissue-engineered small-diameter grafts as many patients do not have adequate autologous vessels. The development of biocompatible small-calibre vascular grafts for coronary and peripheral arterial replacement is thus a major goal in vascular tissue engineering (L'Heureux et al. 1998; Niklason et al. 1999; Mitchell & Niklason, 2003; Daly et al. 2004; Borschel et al. 2005; Swartz et al. 2005; Kielty et al. 2006). However, problems with tissue-engineered grafts include thrombogenicity due to poor endothelial cell (EC) attachment, inappropriate burst strengths, and compliance mismatch between arteries and grafts which contributes to anastomotic myointimal hyperplasia.

Most vascular tissue engineering approaches utilize *ex vivo* approaches to generate living prostheses (L'Heureux et al. 1998; Niklason et al. 1999; Daly et al. 2004; Borschel et al. 2005; Swartz et al. 2005; Kielty et al. 2006). Constructs are often based on a synthetic polymer 'tunica media equivalent' with elastic and non-porous properties essential for immediate graft patency, and controlled biodegradation characteristics to allow long-term remodelling. Grafts are commonly designed to be populated with smooth muscle cells (SMCs) that, during graft preconditioning, are encouraged to deposit extracellular matrix (ECM) with native architecture and the essential biomechanical properties of elastic recoil (elastic fibres) and tensile strength (collagen fibres) to stabilize the synthetic scaffold. Subsequently, SMCs may adopt a quiescent contractile phenotype. A stable EC monolayer is essential to line the luminal surface of grafts to provide physiological vasoactive and anti-thrombotic properties. Such engineered substitutes should mimic natural vessels and be able to undergo remodelling within the patient.

In normal blood vessels, vascular cells and elastic fibres have critical structure–function relationships. Vessels, especially elastic arteries and the aorta, contain abundant elastic fibres, which endow vessel walls with the essential property of elastic recoil (Kielty et al. 2002; Miao et al. 2005; Kielty, 2006). SMCs and ECs deposit elastic fibre layers during development, and interact with

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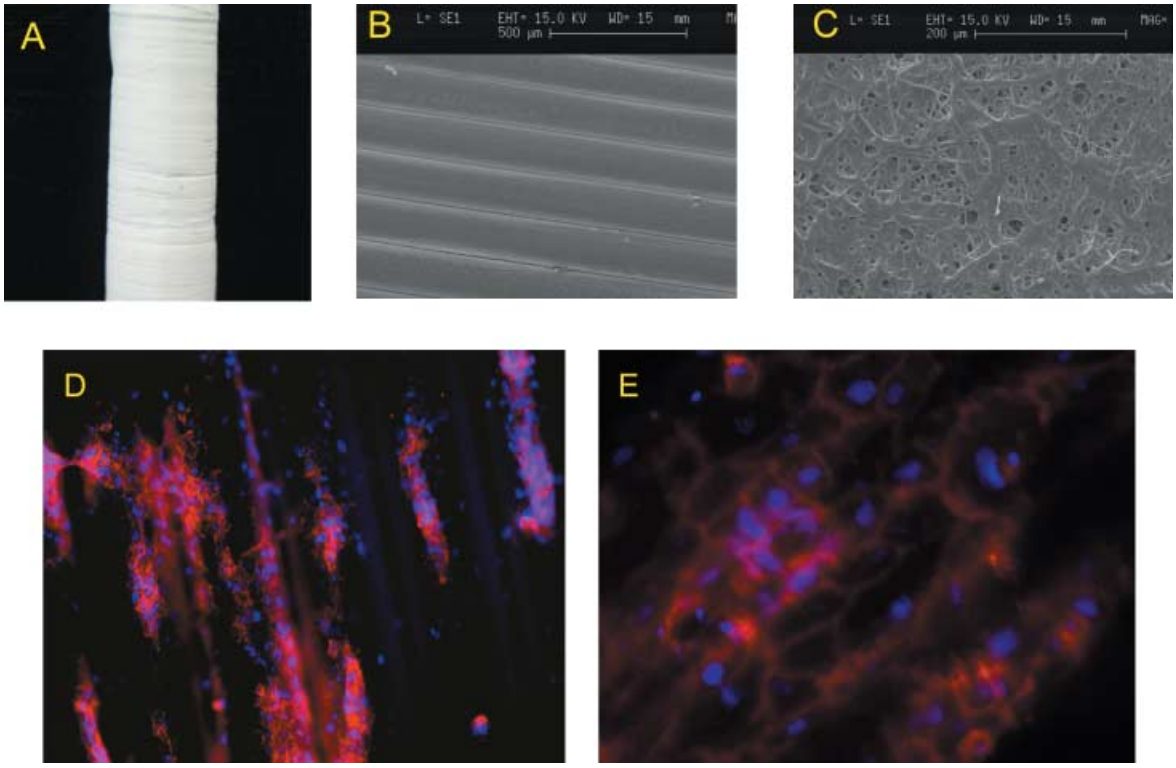

Fig. 1 (A) Image of a PCL–PU composite scaffold. (B) Scanning electron micrograph of the lumen surface. (C) Scanning electron micrograph of the porous anti-lumen surface. (D) Image of endothelial cells (HUVECs) on the lumen surface of the PCL–PU composite stained for PECAM-1 (red) and nuclei (DAPI, blue) after 24 h. (E) Coverage of HUVECs on the lumen surface of the PCL–PU composite after 7 days stained for PECAM-1 (red) and nuclei (DAPI, blue).

elastic fibres throughout the vessel wall (Davis, 1995). In the adult media, elastic laminae intercalate with SMCs, whereas the internal and external elastic laminae separate intima and media, and media and adventitia, respectively. During development, ECs may contribute to the deposition of the internal elastic lamina (Davis, 1993). Following vascular damage to the intima/media 'barrier', SMCs migrate into the neointima where they revert to synthetic, migratory and proliferative phenotype and contribute to intimal hyperplasia.

Vascular graft model

Our vascular graft model is based on a composite polymer scaffold comprising a luminal polycaprolactone (PCL) layer and an outer porous, electrostatically spun, polyurethane (PU) 'medial' layer ('composite PCL–PU scaffold', Fig. 1A–C) (Williamson et al. 2006). The PCL layer supports EC attachment and full coverage of the lumen surface (Fig. 1D,E). Sources of human allogeneic vascular cells for seeding vascular scaffolds include saphenous vein, umbilical artery and vein, and coronary artery and aorta. Bone marrow-derived mesenchymal stem cells (MSCs) represent an alternative (potentially autologous) source of smooth muscle-like cells (see below) (Ball et al. 2004, 2006). Our grafts are being modified by incorporation of selected vascular matrix signals that have the potential to regulate SMC and EC attachment, survival and phenotypic state.

Endothelial cell attachment to composite PCL–PU scaffold

Human umbilical vein endothelial cells (HUVECs) bind strongly to the PCL luminal surface of our composite PCL–PU scaffold in static conditions, and form a stable monolayer expressing EC markers such as PECAM-1 (Williamson et al. 2006) (Fig. 1D,E). The adherent ECs retain vasoactive and immunoreactive characteristics, expressing von Willebrand factor (vWf) and secreting it upon stimulation with histamine, secreting nitric oxide particularly in response to vascular growth factors, and inducing ICAM-1 expression following lipopolysaccharide stimulation. Another advantageous feature of the

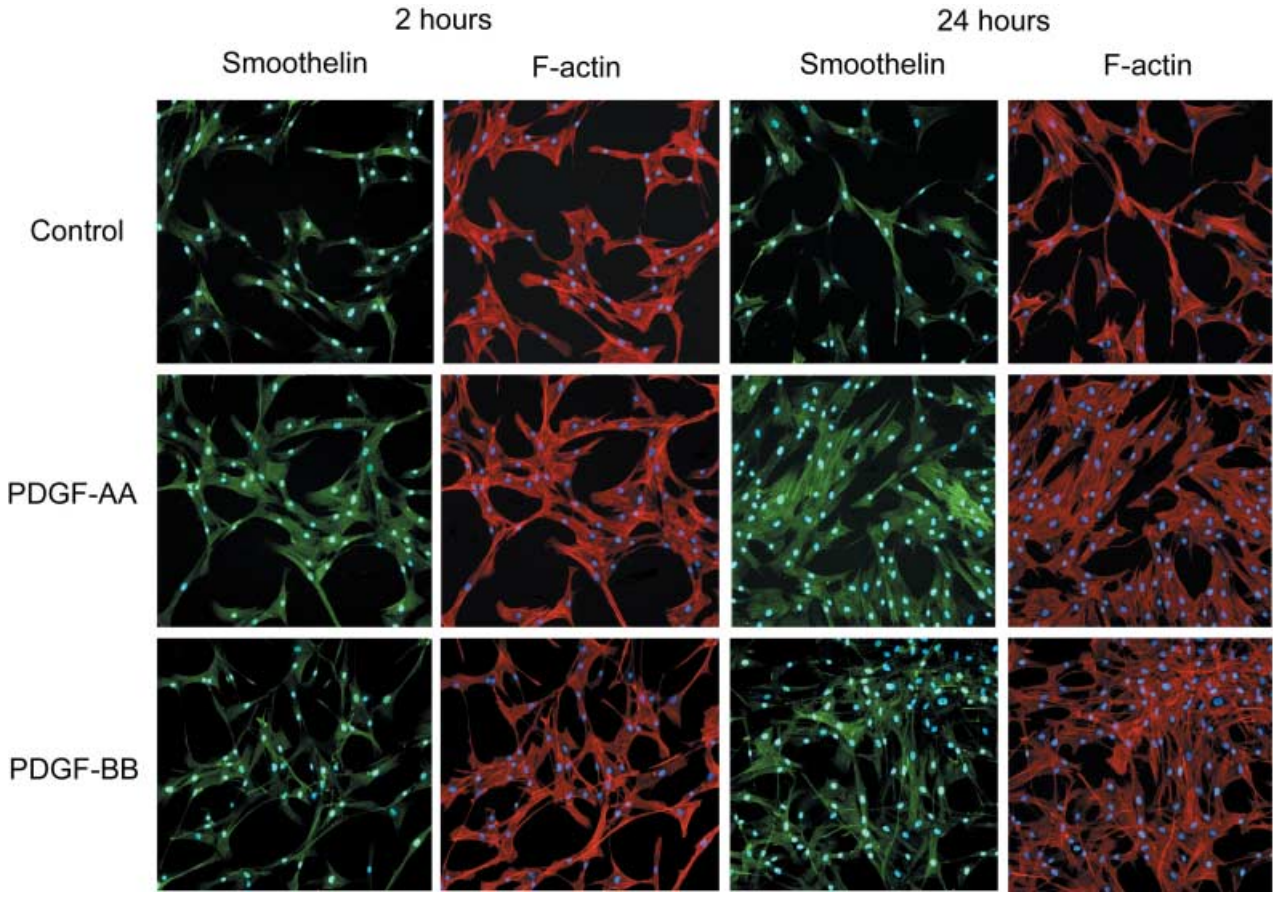

Fig. 2 PDGF-AA enhanced the expression of smoothelin filaments. MSCs were cultured for 24 h in serum-free medium, then either untreated (control) or exposed to 75 ng mL^{-1} PDGF-AA or PDGF-BB for 2 h or 24 h at 37 °C. Cells were immunostained for smoothelin (green), nuclei were stained with DAPI (blue), and F-actin counterstained with phalloidin (red). Cells were visualized using a ×10 objective. A representative of four independent experiments is shown.

PCL–PU composite scaffold is its ability to release small molecules such as growth factors in a controlled manner during early graft preconditioning (Williamson et al. 2006). We are now investigating the stability of ECs on PCL in flow, and how modification of the PCL surface with vascular matrix molecules (see below) may enhance EC attachment and function during graft preconditioning.

Bone marrow-derived mesenchymal stem cells

We have shown that bone marrow-derived human MSCs exhibit some SMC cytoskeletal characteristics (Ball et al. 2004; S. G. Ball, C. A. Shuttleworth, C. M. Kielty, unpublished data). They may thus be a suitable source of cells for seeding graft scaffold walls. When MSCs were directly, but not indirectly, co-cultured with ECs, their smooth muscle (SM) α-actin cytoskeleton was markedly disrupted (Ball et al. 2004). Thus, our scaffolds are designed to ensure that MSCs are physically separated from the luminal EC monolayer, whilst allowing diffusion of soluble factors. We have further analysed how the MSC cytoskeleton is regulated, focusing on SM α-actin, and found that PDGF-AA signalling through PDGF receptor-α (PDGFRα) is essential for the appearance of organized SM α-actin filaments (S. G. Ball, C. A. Shuttleworth, C. M. Kielty, unpublished data). PDGFRα stimulation leads to activation of RhoA and ROCK, leading in turn to phosphorylation of cofilin, which stabilizes the actin filaments. By contrast, PDGFRβ signalling inhibits SM α-actin filament, mainly through activation of RhoE, which blocks ROCK activity. TGFβ1 also regulates SM α-actin filaments, but mainly by stimulating the expression of SM α-actin and the PDGFRα system. PDGF-AA also enhances filaments of smoothelin-B, a specific SMC cytoskeletal marker (Fig. 2). Knowledge of the importance of PDGF and TGFβ growth factors is being incorporated into graft design in the form of controlled growth factor delivery.

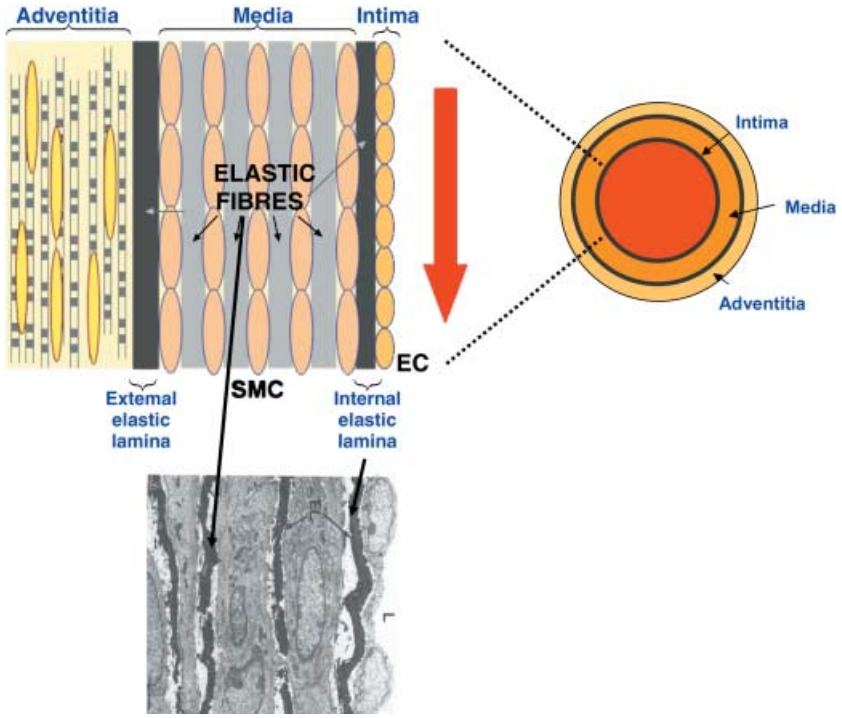

Fig. 3 Cartoon and transmission electron micrograph of elastic fibres within an artery wall.

Exploiting vascular elastic fibre molecules in graft design

We have focused on exploiting elastic fibre molecules as cell-matrix elements in our graft model, because elastic fibres are major structural and cell adhesion elements of the vasculature (Kielty et al. 2005, 2006) (Fig. 3). These molecules, normally laid down by SMCs and ECs during blood vessel development, endow vessels with elastic recoil and they may profoundly influence vascular cell adhesion and function. The major components of elastic fibres are crosslinked elastin core surrounded by a mantle of fibrillin microfibrils. During elastic fibre deposition, tropoelastin (soluble secreted form of elastin) is deposited on a fibrillin microfibril template (Mecham & Davis, 1994; Kielty et al. 2002; Czirok et al. 2005; Kozel et al. 2003, 2005). Fibulin-5 co-localizes with elastic fibres and is essential for their normal assembly (Nakamura et al. 2002; Yanagisawa et al. 2002). We are utilizing tropoelastin, fibrillin-1 and fibulin-5 in our graft design.

Vascular cell attachment to elastic fibre molecules

Fibrillin-1

Fibrillin-1 is the major structural element of elastin-associated microfibrils (Kielty et al. 2005). It is a large multidomain glycoprotein (350 kDa), with multiple calcium-binding epidermal growth factor (cbEGF)-like domains interspersed with eight-cysteine motifs (also known as TB motifs). It contains a single arg–gly–asp (RGD) cell attachment motif within TB4, which we and others have shown to support cell attachment (Pfaff et al. 1996; Sakamoto et al. 1996; Bax et al. 2003; Lee et al. 2004). We showed that human dermal fibroblast cells adhere to fibrillin-1 through integrin receptors $\alpha5\beta1$ and $\alpha v\beta3$ (Fig. 4A,B) (Bax et al. 2003). We have found that fibrillin-1 RGD-containing fragments starting at TB4 and containing only downstream domains had poor cell attachment activity compared with RGD fragments containing more than one upstream cbEGF-like domain. SMCs attach very strongly to the latter, and spread well with well-organized focal adhesions and stress fibres. In this way, we have optimized fibrillin-1 fragments for use in graft scaffolds. Cell adhesion to fibrillin-1 can also modify gene expression levels. We showed, at mRNA and protein levels, that fibrillin-1 is auto-up-regulated when cells adhere to fibrillin-1 (Bax et al. 2003). Enhanced matrix metallo-proteinase (MMP) expression has also been shown in cells on fibrillin-1 RGD peptides (Booms et al. 2005). Fibrillin microfibrils are subendothelial matrix elements (Davis, 1993). Our pilot data indicate that HUVECs attach and spread well on fibrillin-1.

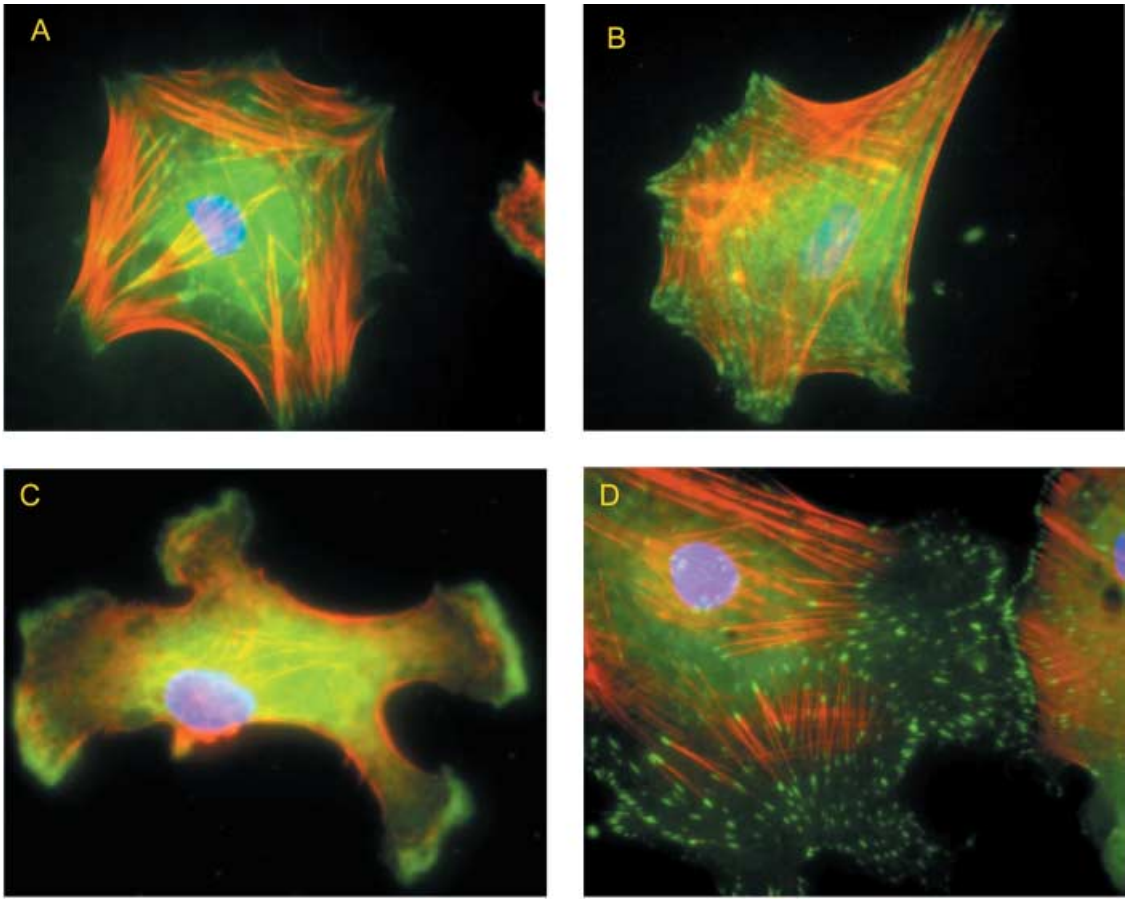

Fig. 4 (A) Human dermal fibroblasts on fibrillin-1 immunostained with DAPI nuclei (blue), actin (red), and MAB 16 $\alpha5\beta1$ antibody (green). (B) Human dermal fibroblasts immunostained with DAPI nuclei (blue), actin (red) and LM 609 $\alpha v\beta3$ antibody (green). (C) Human aortic SMCs on fibulin-5 immunostained with DAPI nuclei (blue), actin (red) and α-paxillin antibody (green). (D) Human aortic SMCs on fibronectin immunostained with DAPI nuclei (blue), actin (red) and α-paxillin antibody (green).

Fibulin-5

Fibulin-5 interacts with cells in an RGD-dependent manner (Yanagisawa et al. 2002; A. Lomas, D. V. Bax, C. A. Shuttleworth, C. M. Kielty, unpublished data). CHO cells over-expressing certain integrins bound to fibulin-5 through $\alpha v\beta3$, $\alpha v\beta5$ and $\alpha9\beta1$ (Yanagisawa et al. 2002). Knock-out mice studies showed that fibulin-5 is essential not only for normal elastic fibre deposition *in vivo* (Nakamura et al. 2002; Yanagisawa et al. 2002; Chu & Tsuda, 2004), but also to regulate SMC proliferation and migration (Spencer et al. 2005). We have shown that human SMCs and ECs bind recombinant human fibulin-5 in an integrin-dependent manner (A. Lomas, D. V. Bax, C. A. Shuttleworth, C. M. Kielty, unpublished data). On fibulin-5, both cell types exhibit a characteristic morphology with no stress fibres or focal adhesions, in contrast to well-spread cells on fibronectin (another RGD-containing adhesive glycoprotein) (Fig. 4C,D). Molecular signalling underlying these fibulin-5-mediated effects, and possible applications in vascular tissue engineering are under investigation.

Tropoelastin

Once deposited on the fibrillin microfibril template, tropoelastin is crosslinked by lysyl oxidase to form the insoluble core of mature elastic fibres. SMCs are juxtaposed to elastic fibres in the medial layer of vascular walls, and ECs to the internal elastic lamina (particularly during blood vessel formation). Several groups have shown that purified elastin and recombinant tropoelastin expressed in bacterial systems can support cell adhesion (Grosso et al. 1991; Jung et al. 1999; Broekelmann et al. 2005), and that elastin can profoundly influence SMC morphology, proliferation and phenotype (Karnik et al. 2004). The elastic binding protein (EBP, an alternatively spliced form of β-galactosidase; 67 kDa)

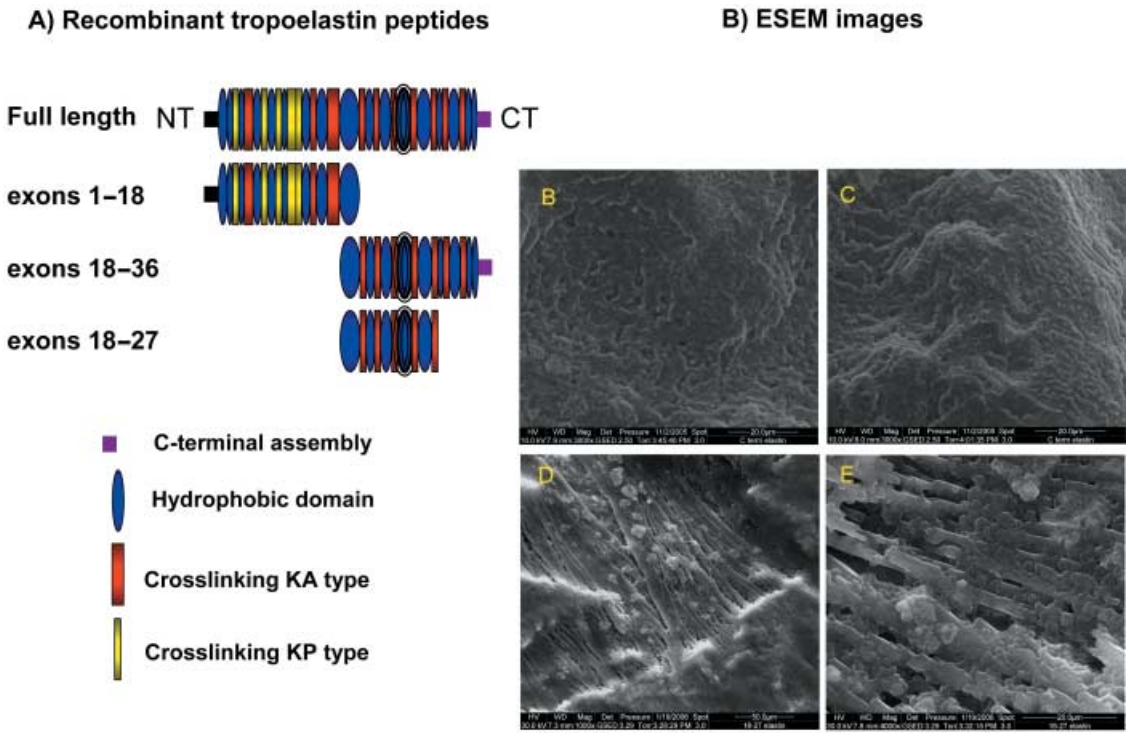

Fig. 5 (A) Recombinant tropoelastin fragments expressed in our laboratory using a mammalian expression system. (B,C) Environmental scanning electron microscopy (ESEM) images of tropoelastin fragment 18–36. ESEM images of tropoelastin peptide 18–27. These fragments form sheets and ordered linear arrays.

binds elastin through the VGVAPG motif (repeat hexapeptide in exon 24), and signalling through this receptor influences SMC proliferation and differentiation (Mochizuki et al. 2002; Karnik et al. 2004). Integrin $\alpha v\beta 3$ is reported to recognize an RKRK sequence close to the C-terminus of elastin, in a saturable, divalent cation-dependent, single-site binding manner (Rodgers & Weiss, 2005). Certain elastin proteolytic fragments are highly chemotactic (Bisaccia et al. 1994; Uemura & Okamoto, 1997). We have expressed recombinant human tropoelastin in a mammalian system, as N- and C-terminal regions. These large overlapping fragments, in their soluble secreted form with charged lysines at physiological pH, support adhesion of human SMCs (S. Stephan, C. A. Shuttleworth, C. M. Kielty, unpublished data).

Coating scaffolds with elastic fibre molecules

Having characterized vascular cell adhesion properties of fibrillin-1, fibulin-5 and tropoelastin, we have developed strategies to adsorb these recombinant elastic fibre molecules onto PU and PCL–PU scaffolds. Efficient coating of fibrillin-1, tropoelastin and fibulin-5 fragments onto the scaffolds, and cell attachment to the coated scaffolds has been demonstrated. Thus, we can begin to exploit these molecules to regulate cell adhesion and behaviour in our grafts.

Tropoelastin-based materials for vascular tissue engineering

Tropoelastin comprises alternating hydrophobic and lysine-rich crosslinking domains. *In vitro*, it undergoes the well-characterized process of coacervation, in which molecules become increasingly ordered at increased temperature (Vrhovski et al. 1997; Bellingham et al. 2003; Mithieux & Weiss, 2005; Tamburro et al. 2005). This process, which is influenced by salt concentration and pH, involves inter- and intra-molecular interactions between hydrophobic and cross-linking domains (Miao et al. 2005). Using our recombinant tropoelastin N- and C-terminal regions, we have generated stable crosslinked sheets and fibres (Fig. 5A–E). We are now developing elastin-based composite materials incorporating other elastic fibre molecules (e.g. fibrillin-1 RGD fragments) that may be used to seed vascular cells within porous PU scaffolds.

Concluding remarks

Cell-matrix biology applications in vascular tissue engineering are enhancing our ability to regulate EC and SMC proliferation and phenotype, and advancing small-diameter graft design.

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