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A multilayered synthetic human elastin/polycaprolactone hybrid vascular graft with tailored mechanical properties

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ABSTRACT

Small-diameter synthetic vascular graft materials fail to match the patency of human tissue conduits used in vascular bypass surgery. The foreign surface retards endothelialization and is highly thrombogenic, while the mismatch in mechanical properties induces intimal hyperplasia. Using recombinant human tropoelastin, we have developed a synthetic vascular conduit for small-diameter applications. We show that tropoelastin enhances endothelial cell attachment (threefold vs. control) and proliferation by 54.7 ± 1.1% (3 days vs. control). Tropoelastin, when presented as a monomer and when cross-linked into synthetic elastin for biomaterials applications, had low thrombogenicity. Activation of the intrinsic pathway of coagulation, measured by plasma clotting time, was reduced for tropoelastin (60.4 ± 8.2% vs. control). Platelet attachment was also reduced compared to collagen. Reductions in platelet interactions were mirrored on cross-linked synthetic elastin scaffolds. Tropoelastin was subsequently incorporated into a synthetic elastin/polycaprolactone conduit with mechanical properties optimized to mimic the human internal mammary artery, including permeability, compliance, elastic modulus and burst pressure. Further, this multilayered conduit presented a synthetic elastin internal lamina to circulating blood and demonstrated suturability and mechanical durability in a small scale rabbit carotid interposition model.

1. Introduction

Despite the increasing incidence of cardiovascular disease, there are few effective biomaterials currently available for clinical vascular applications such as small-diameter (<6 mm) bypass grafts [1]. Surgical management of occlusive atherosclerotic disease involves placement of a conduit to bypass blood flow around the diseased arterial segment. Autologous vessels harvested from the patient for bypass surgery include the saphenous vein (SV) from the leg and the internal mammary artery (IMA) from the chest wall. The IMA has greater elasticity, maintains the ability to vasoregulate and is less prone to atherosclerosis [2]. Consequently, the IMA demonstrates superior patency over the SV (up to 95% 10-year patency for IMA, 71% for SV) [3] and is the gold standard for autologous grafting in coronary bypass surgery. Its use is limited by unavailability of suitable vessels due to previous harvest or anatomical variability [4].

The two clinically available synthetic conduit materials are polyethylene terephthalate (Dacron) and expanded polytetrafluoroethylene (ePTFE). The foreign surfaces of these materials fail to establish a luminal coating of endothelial cells, remain thrombogenic and generate an unfavorable immune response that leads to chronic inflammation [5]. Additionally, these polymers are inelastic, resulting in compliance mismatch at the anastomoses between the stiff synthetic conduits and native vessels. This results in anastomotic intimal hyperplasia and leads to graft occlusion [6]. While Dacron and ePTFE perform satisfactorily as large-diameter high-flow conduits, their use in small-diameter (<6 mm) applications has produced poor patency rates and an ideal vascular graft for these situations is lacking [7].

Elastin is one of the major structural components of the vessel wall, comprising up to 50% of the dry weight of major arteries [8]. As a key component of the vascular extracellular matrix, elastin imparts elastic recoil, resilience and durability. In addition to its favorable mechanical properties, elastin acts as an important regulator of vascular cells, inhibiting smooth muscle cell migration and proliferation [9] and enhancing attachment and proliferation of endothelial cells [10]. Elastin is also known to demonstrate low

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thrombogenicity, and vascular devices coated with elastin-based polypeptides have reduced platelet adhesion and activation, translating to improved patency in vivo [11,12]. In summary, elastin demonstrates characteristics that are deficient in current graft materials and are implicated in their failure, including elasticity, favorable cellular interactions and blood compatibility.

The combination of strength and elasticity has been a particular challenge common to all synthetic graft development, with modification of traditional materials such as segmented polyurethanes allowing an improvement in one at the expense of the other [13]. Recognizing the distinct biological and mechanical advantages of elastin as a biomaterial, previous work had sought to electrospin elastin derivatives into synthetic conduits [14,15]. These efforts relied on animal-derived, mature elastin due to the difficulty in obtaining the purified tropoelastin monomer. The low ultimate tensile strength of elastin has most commonly seen it used in combination with synthetic polymers and strengthening matrix proteins such as collagen in the synthesis of arterial substitutes [16–18]. Grafts containing multiple layers have also been produced, in an attempt to separate out the biological advantages of elastin from the mechanical necessity of co-polymerization [19,20].

Our approach has been to electrospin recombinant human tropoelastin (rhTE), the soluble precursor of elastin [21], in combination with polycaprolactone (PCL) to produce a conduit that mimics the mechanical properties of the IMA. PCL degrades slowly and is well known for its excellent mechanical properties and favorable cell interactions [22]. We show here the development of multilayer synthetic elastin/PCL vascular conduits, characterized by an all SE luminal layer and mechanical properties matched to those of the human IMA. We have produced a fully synthetic graft comprised primarily of recombinant human tropoelastin (rhTE), which responds elastically at low pressures and has a burst pressure equivalent to the human IMA. In vitro, both soluble rhTE and cross-linked rhTE, known as synthetic elastin (SE), demonstrated enhanced endothelial cell interactions and low thrombogenicity. These grafts sutured well, were impermeable and maintained their mechanical properties after a 1 month in vivo pilot study.

2. Materials and methods

2.1. Reagents

Recombinant human tropoelastin corresponding to amino acid residues 27–724 of GenBank entry AAC98394 (gi 182020) was expressed and purified as previously described [23]. Human umbilical vein endothelial cells (HUVECs) were harvested enzymatically from umbilical cords as previously described [24]. HUVECs from passages 2–4 were used. All other reagents were purchased from Sigma–Aldrich unless otherwise stated.

2.2. Electrospinning

Solutions dissolved in 1,1,1,3,3,3-hexafluro-2-propanol were electrospun by loading into a syringe equipped with a blunt 18G needle. Blends of rhTE (5–9%) and polycaprolactone (PCL, 1–5%, *Mn* = 70,000–90,000) were generated, maintaining a total solution concentration of 10% (w/v). Constant flow rates (1 ml h^{-1}) were achieved using a syringe pump and a needle connected to the positive output of a high voltage power supply. Fibers were electrospun onto a flat metal target to produce rectangular sheets, or onto a custom rotating mandrel system for conduit manufacture. Bi-layered conduits were generated by sequential delivery of distinct SE/PCL solutions onto the mandrel [25]. Scaffolds were cross-linked using glutaraldehyde vapors (6–18 h), quenched with 0.02 M glycine, washed with PBS and stored in a sterile environment [26,27]. Conduits ultimately comprised a luminal layer of 100% SE (0.25 ml) and an outer layer of hybrid SE/PCL (80:20 w/w, 0.6 ml).

2.3. Endothelial cell adhesion and proliferation

2.3.1. Response to rhTE-coated tissue culture plastic

For attachment studies, HUVECS (300 cells mm^{-2}) were allowed to attach for 15, 30 and 60 min. For proliferation assays, HUVECs (150 cells mm^{-2}) were plated for 3 and 5 days. Attachment and proliferation of HUVECs to rhTE-coated wells were analyzed in comparison to tissue culture plastic alone and to wells coated with fibronectin and bovine serum albumin (BSA) (10 μg $well^{-1}$). At each time point, cells were fixed with 3.7% formaldehyde, washed and stained with 0.1% (w/v) crystal violet solution for 1 h at room temperature [28]. The dye was washed with distilled H_2O, solubilized with 10% (v/v) acetic acid, and the absorbance measured at 570 nm ($n = 3$ per group per time point for all cell experiments). For attachment studies, the data from defined cell percentage standards were plotted and the straight-line slope of the graph was used to convert experimental absorbances into percent attachment.

2.3.2. Response to SE/PCL scaffolds

Endothelialization was qualitatively assessed on PCL, SE and SE/PCL (50:50 w/w) scaffolds (0.25 ml solution). Fibers were cross-linked with glutaraldehyde vapors for 3 h, before quenching with 0.2 M glycine. HUVECs (300 cells mm^{-2}) were seeded onto the scaffolds and incubated for 1 and 3 days. Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB), post-fixed with 1% (v/v) osmium tetraoxide in 0.1 M PB and dehydrated in ascending grades of ethanol before drying with hexamethyldisilasane . The samples were sputter-coated with 20 nm gold and imaged with a Philips XL 30 CP scanning electron microscope (SEM) ($n = 3$ per group; representative images shown).

For quantification, thin layers of fibers either SE or SE/PCL hybrid (50:50 w/w) were electrospun onto glass coverslips (0.1 ml solution) [29,30]. Fibers were cross-linked with glutaraldehyde vapors for 3 h, before quenching with 0.2 M glycine. HUVECs (300 cells mm^{-2}) were seeded onto the fibers and incubated for 1 and 3 days. Cell number was quantified by counting the number of 4′,6-diamidino-2-phenylindole (DAPI) stained nuclei in 20 fields of view/sample ($n = 3$ per group, per time point) using a Nikon Eclipse E800 fluorescent microscope.

2.4. Blood compatibility assays

2.4.1. Plasma interaction

Blood was drawn from healthy adult volunteers, with informed consent and in accordance with the Declaration of Helsinki. Blood was collected in a syringe pre-loaded with 1/10th volume 0.1 M sodium citrate after discarding the first 1 ml to avoid thromboplastin contamination [31]. Platelet-poor plasma (PPP) was obtained and plasma clotting time profiles measured as previously described [32]. Tissue culture plastic (TCP) wells were coated with 0.1 mg ml^{-1} rhTE or BSA ($n = 6$) before being rinsed with PBS. PPP and 25 mM $CaCl_2$ in PBS was added to each well. Negative controls were not protein-coated and received no $CaCl_2$. Absorbance was recorded every 5 min for a total of 65 min at 405 nm, with plates kept at 37 °C between readings.

2.4.2. ^{111}Indium-labeled platelet attachment

Blood was taken from a healthy adult volunteer into a syringe pre-loaded with 1/5th volume acid-citrate-dextrose (ACD) after discarding the first 1 ml collected. Platelet adhesion was assessed as previously described [33] using platelets labeled with ^{111}indium oxine [34] ($n = 3$ per group per time point). Adhesion was mea-