Acta Biomaterialia 8 (2012) 225–233

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Mechanical property characterization of electrospun recombinant human tropoelastin for vascular graft biomaterials

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ARTICLE INFO

Article history:
Received 21 April 2011
Received in revised form 8 July 2011
Accepted 1 August 2011
Available online 6 August 2011

Keywords:
Tropoelastin
Electrospinning
Mechanical properties
Vascular grafts
Tissue engineering

ABSTRACT

The development of vascular grafts has focused on finding a biomaterial that is non-thrombogenic, minimizes intimal hyperplasia, matches the mechanical properties of native vessels and allows for regeneration of arterial tissue. In this study, the structural and mechanical properties and the vascular cell compatibility of electrospun recombinant human tropoelastin (rTE) were evaluated as a potential vascular graft support matrix. Disuccinimidyl suberate (DSS) was used to cross-link electrospun rTE fibers to produce a polymeric recombinant tropoelastin (prTE) matrix that is stable in aqueous environments. Tubular 1 cm diameter prTE samples were constructed for uniaxial tensile testing and 4 mm small-diameter prTE tubular scaffolds were produced for burst pressure and cell compatibility evaluations from 15 wt.% rTE solutions. Uniaxial tensile tests demonstrated an average ultimate tensile strength (UTS) of 0.36 ± 0.05 MPa and elastic moduli of 0.15 ± 0.04 and 0.91 ± 0.16 MPa, which were comparable to extracted native elastin. Burst pressures of 485 ± 25 mm Hg were obtained from 4 mm internal diameter scaffolds with 453 ± 74 μm average wall thickness. prTE supported endothelial cell growth with typical endothelial cell cobblestone morphology after 48 h in culture. Cross-linked electrospun rTE has promising properties for utilization as a vascular graft biomaterial with customizable dimensions, a compliant matrix and vascular cell compatibility.

1. Introduction

Heart disease remains the leading cause of death in the Western world with nearly 81 million people affected in the United States in 2006 [1]. Bypass surgery using autografts of saphenous veins or mammary arteries remains the gold standard treatment for severe cases, but can be limited by previous vessel harvest or preexisting disease. Synthetic graft materials, such as expanded poly(tetrafluoroethylene) (ePTFE) and poly(ethylene terephthalate) (PET or Dacron®), work well for large-diameter vessels, but are not viable options for grafts smaller than 6 mm in diameter [2].

The failure modes of small-diameter vascular grafts have primarily been thrombosis, aneurysm and intimal hyperplasia [3]. To address these failure modes many researchers have studied both synthetic and natural biomaterial scaffolds. Synthetic biomaterials have controllable physical and mechanical properties that are highly reproducible and are easily manufactured in large-scale quantities, but many lack the elasticity of native arteries and biocompatibility for long-term vascular cell functionality. Considerable efforts have been made to functionalize synthetic surfaces [4,5]. Natural biomaterial scaffolds, including well-studied grafts of decellularized blood vessels [6–8], have had limited success. Although the decellularized bovine [6] and human [7] blood vessels had promising 5 year patency rates, aneurysm formation due to in vivo degradation has limited their widespread use [7,8]. Decellularized arteries, from allogenic and xenogenic sources, are attractive scaffolds for tissue-engineered vascular grafts due to their mechanical and biological properties [9]; however, these natural scaffolds are limited by the lack of precise manufacturing control of the physical and mechanical properties, as well as problems with inflammation and calcification [18]. To reduce concerns of inflammation of the allogenic and xenogenic sourced vascular biomaterials, the biomaterials are frequently cross-linked; yet this has led to problems of limited cell repopulation and increased stiffness of the biomaterials [19]. While stiffness and compliance mismatch alone may not lead to vascular graft failure [27–29], graft compliance has correlated to the formation of intimal hyperplasia and should be considered in biomaterial scaffold design along with the potential of cellular remodeling of the graft and cell signaling capability [20–26]. The search for a viable off-the-shelf small diameter vascular graft that can match an autograft's

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doi:10.1016/j.actbio.2011.08.001

performance in terms of mechanical properties, cell compatibility, and vascular healing has been the focus of many research efforts, but has remained an elusive target.

The incorporation of vascular cells with biomaterial scaffolds to produce tissue engineered grafts have been successful in animal and, recently, human trials [10–15]. Production of the scaffold by the vascular cells has been accomplished using both in vivo [11,16–17] and in vitro [12,13] methods. The in vivo methods require the graft to be grown in the recipient's peritoneal cavity. Recent advancements of this technique include improved scaffold design to produce multilayered scaffolds and the use of cyclic stretch to improve the assembly time and organization of the extracellular matrix [16,17]. However, these in vivo methods require a second surgical site for autologous use. The in vitro methods of L'Heureux et al., in which the tissue-engineered vascular grafts were produced from autologous cells without a scaffold, have advanced to clinical trails using an arteriovenous shunt model [12,13]. These autologous tissue-engineered grafts have shown promising results with primary patency rates of 78% at 1 month and 60% at 6 months, and limited failures due to thrombosis, dilation and aneurysm [12]. The in vitro cell-produced scaffolds are elegant in design, but require lengthy production times, potentially limiting their clinical use.

The use of electrospinning to make biomaterials has the capability of combining natural proteins with controllable physical and mechanical properties. Electrospinning produces sub-micron-sized fibers from suspensions of monomers or polymers from both natural proteins and synthetic polymers [30–34]. Fibers produced from monomer suspensions can then be cross-linked to produce stable polymeric structures with customizable dimensions in terms of fiber diameter and overall graft dimensions. Electrospun fibrous scaffolds made from biodegradable polymers, such as poly(ε-caprolactone) (PCL), polylactic acid (PLA), polyglycolic acid (PGA) and poly(lactide-co-glycolide) (PLGA) [35–38] have been proposed for use in bone, cardiac, blood vessel and wound-dressing applications [39–45]. Several groups have successfully electrospun elastin for use in tissue-engineered grafts [46–52] and support material for vein grafts [53]. Most, however, have used animal-sourced elastin that is [20,54–56] extracted from already assembled and cross-linked protein forms. While these forms of elastin may provide the biochemical signaling of elastin, they remain an animal-sourced material with the associated potential for immunorejection leading to structural degradation and ultimate aneurismal graft failure.

Our aim is to electrospin small-diameter vascular grafts containing recombinant human tropoelastin (rTE), the monomer unit of elastin, that when cross-linked mimic native elastin fibers. Elastin is the principal structural component of elastic arteries responsible for energy storage and recovery, and contributes to their unique mechanical properties [60]. End-stage aneurysm disease and supravalvular aortic stenosis have been associated with the pathologic loss of elastin or deficiency in elastin expression [61–68]. Elastin, as a blood-contacting surface on stents and grafts, reduces thrombus adherence and has demonstrated good long-term patency [69,70]. The importance of establishing an elastic fiber structure in a vascular scaffold that is similar to the arterial wall has been clearly recognized, as the depletion or loss of elastin has been correlated to both aneurysmal progression and severe smooth muscle cell hyperplasia in both animals and humans [61,64,65,67,70,82–84]. Thus, elastin is a promising, and perhaps necessary, component in vascular graft development [71,72]. Recent work has examined a class of elastin-like recombinant polymers with self-assembly properties and cross-link sites designed into the peptide sequence [57]. The elastin-like polymer has been used to produce organized multilayer collagen-reinforced vascular grafts and abdominal wall repair tissue constructs with customizable mechanical properties, which make this technology promising for many soft tissue applications [58,59]. Our use of electrospinning will enable the customization of the dimensions and mechanical properties for the vascular graft biomaterial, and the use of tropoelastin may impart critical cell signaling to the biomaterial.

2. Materials and methods

2.1. Materials

Human tropoelastin was optimized and expressed from a synthetic gene codon in gram quantities in a 10 l *Escherichia coli* fermentation system. Gel electrophoresis determined that the purification procedure resulted in a greater than 99% pure product (Fig. 1) as well as low endotoxin levels with an average of 0.2 EU · mg^{-1} (1 EU = 100 pg of endotoxin) as determined by the Kinetic-QCL Assay (Cambrex). All chemical reagents were acquired from Sigma–Aldrich unless otherwise noted.

The purified human tropoelastin protein includes all of the functional exons with the exception of exons 1, 22 and 26A (Fig. 2). Exon 1 contains the signal sequence, while hydrophobic exon 22 and hydrophilic exon 26A are rarely expressed in mature elastin. The resultant tropoelastin exon structure used is the same as a natural isoform produced by normal human fetal heart cells.

Extracted porcine elastin was isolated as previously described [69]. Briefly, porcine carotid arteries were obtained from domestic swine weighing 250 lb and 6–9 months of age (Animal Technologies, Tyler, TX) to size match the diameters. The arteries were shipped overnight in phosphate-buffered saline (PBS) on ice. The gross fat was dissected away and, using aseptic techniques, the arteries were placed in 80% ethanol for a minimum of 72 h at 4 °C and subsequently treated with 0.25 M NaOH for 70 min with sonication at 60 °C, followed by two 30 min, 4 °C washes in 0.05 M HEPES (pH 7.4). The extracted elastin tubular conduits were then autoclaved at 121 °C for 15 min and stored at 4 °C in 0.05 M HEPES buffer.

2.2. Electrospinning of rTE

Tropoelastin solutions of 15 wt.% rTE in 1,1,1,3,3,3-hexafluro-2-propanol (HFP) were prepared and loaded into 2 or 5 ml glass

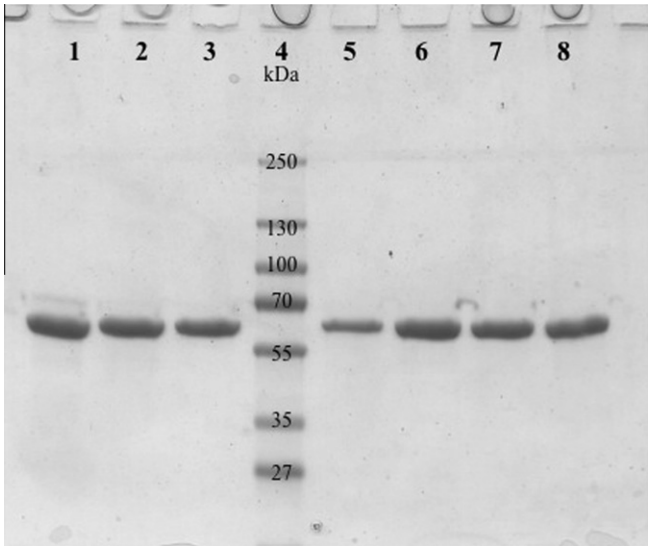

Fig. 1. Stained electrophoresis gel showing purified human tropoelastin from seven different batches (lanes 1–3 and 5–8), illustrating the purity of the product and reproducibility of the purification process. Lane 4 is a molecular weight standard.