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# Acellular vascular grafts generated from collagen and elastin analogs

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ABSTRACT

Tissue-engineered vascular grafts require long fabrication times, in part due to the requirement of cells from a variety of cell sources to produce a robust, load-bearing extracellular matrix. Herein, we propose a design strategy for the fabrication of tubular conduits comprising collagen fiber networks and elastin-like protein polymers to mimic native tissue structure and function. Dense fibrillar collagen networks exhibited an ultimate tensile strength (UTS) of 0.71 ± 0.06 MPa, strain to failure of 37.1 ± 2.2% and Young's modulus of 2.09 ± 0.42 MPa, comparing favorably to a UTS and a Young's modulus for native blood vessels of 1.4–11.1 MPa and 1.5 ± 0.3 MPa, respectively. Resilience, a measure of recovered energy during unloading of matrices, demonstrated that 58.9 ± 4.4% of the energy was recovered during loading–unloading cycles. Rapid fabrication of multilayer tubular conduits with maintenance of native collagen ultrastructure was achieved with internal diameters ranging between 1 and 4 mm. Compliance and burst pressures exceeded 2.7 ± 0.3%/100 mmHg and 830 ± 131 mmHg, respectively, with a significant reduction in observed platelet adherence as compared to expanded polytetrafluoroethylene (ePTFE; 6.8 ± 0.05 × $10^5$ vs. 62 ± 0.05 × $10^5$ platelets $mm^{-2}$, $p < 0.01$). Using a rat aortic interposition model, early in vivo responses were evaluated at 2 weeks via Doppler ultrasound and CT angiography with immunohistochemistry confirming a limited early inflammatory response ($n = 8$). Engineered collagen–elastin composites represent a promising strategy for fabricating synthetic tissues with defined extracellular matrix content, composition and architecture.



## 1. Introduction

The design of a tissue-engineered vascular graft to replace diseased arteries requires consideration of mechanical, biological and clinical factors that influence behavior in vitro and in vivo [1–3]. While much progress has been made in determining the key factors that contribute to the eventual success of a graft, widespread acceptance of a tissue-engineered conduit as an acceptable alternative to either an autologous artery or a vein has yet to be achieved. Mechanical requirements for an arterial substitute include sufficient burst pressure to prevent catastrophic failure and long-term fatigue resistance; compliance, which approximates that of native vessels to prevent mechanical mismatch; and suitable suture retention to permit implantation in a manner that tolerates hydrodynamic and mechanical forces at the anastomoses. Biological and clinical considerations have focused on the generation of a non-fouling luminal surface to prevent thrombosis and the minimization of inflammatory events due to either surgery or graft properties that may contribute to early or late graft failure [1,4].

Several groups have demonstrated the efficacy of various strategies that vary from modification of existing **e**xpanded polytetrafluoroethylene (ePTFE)/Dacron™ grafts, acellular or cellularized constructs, to de novo engineering of tissue substitutes that mimic native vessels [5]. Tissue-engineered blood vessels derived from cell-sheet tissue engineering and degradable synthetic polymer scaffolding have demonstrated early clinical success, and continued progress with several additional systems suggests that these technologies will continue to evolve [3,6,7]. We believe that clinical success will ultimately require utilizing a "bottom-up" approach where recapitulation of the fundamental features of the vascular wall, incorporation of key elements that obviate thrombosis and acute graft failure and potentially the addition of a cellular component to provide a means for self-repair and other functional properties required for long-term graft patency [8–10]. The strategy reported herein does not rely on the process of seeded cells to produce extracellular matrix (ECM) or bioreactor conditioning of cell-containing constructs, which typically requires weeks to months of process time and mandates either the use of autologous cells or the removal of allogeneic cells [11–13]. We believe that avoiding

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these steps will shorten fabrication time, enhance tissue integrity and improve biological responses after in vivo implantation.

Our lab and others have recently synthesized and characterized a series of elastin-like protein polymers that consist of sequentially repeated amino acid blocks [14–17]. With the ability to easily modify peptide chain length, consensus repeat sequence and introduce additional oligopeptide units, protein polymers can be produced with enhanced biological, thermodynamic and mechanical properties. We have designed a series of elastin-like polypeptides that can be fabricated as films or other geometrical constructs, have robust mechanical properties, a high degree of resilience, minimal thrombogenicity and long-term stability in vivo [18–26].

Collagen, a vital component of the ECM, is required both as a load-bearing element and as a mediator of local biological responses. Electrospinning, casting gels and wet spinning have been the mainstay for large-scale production of collagen matrices for tissue engineering. Although much progress has been made in the field of electrospinning, the use of organic solvents leads to collagen denaturation [27]. Likewise, the lack of mechanical integrity of collagen hydrogels precludes their use as a structural component. The objective of this study was to develop a strategy for processing dense collagen fiber networks, embedded with an elastin-like protein matrix, to function as a mechanical and tissue-mimetic analog for fabrication of an arterial substitute. Composite structures were fabricated with defined composition and microarchitecture with preservation of native collagen structure.

This paper describes the rapid fabrication of protein-based matrices of high strength and stiffness approximating native tissue. Mechanical characterization of non-cross-linked matrices illustrates the potential to modulate and tailor mechanical strength for a variety of vascular and other soft tissue engineering applications. We hypothesize that these composites may prove useful for blood contacting applications given their hemocompatibility and in vivo stability.

## 2. Materials and methods

### 2.1. Isolation and purification of monomeric Type I collagen

Monomeric Type I rat tail tendon collagen was obtained by acid extraction from Sprague–Dawley rats (Pel-Freez Biologicals, Rogers, AR) following a procedure adapted from Silver and Trelstad [28]. Briefly, rat tail tendons were extracted with the aid of autoclaved pliers and dissolved in 10 mM HCl for 4 h at 25 °C to dissolve the proteinaceous components. Insoluble tissue and other contaminants were removed by centrifugation at 30,000*g* at 4 °C for 30 min with subsequent vacuum filtration through 20 μm, 0.45 μm and 0.2 μm filters. The sterile filtered collagen in HCl was precipitated from solution by adding NaCl to a final concentration of 0.7 M. The precipitated collagen was pelleted by centrifugation, redissolved in 10 mM HCl and dialyzed first against 20 mM phosphate buffer at room temperature, then at 4 °C against 10 mM HCl at 4 °C and finally against deionized water at 4 °C. The collagen was then frozen and lyophilized until use.

### 2.2. Synthesis of a recombinant elastin-like protein polymer (ELP)

Development and production of the ELP, LysB10, has been described elsewhere [24]. Briefly, a triblock amphiphillic copolymer was designed to contain hydrophobic endblocks and a hydrophilic midblock. The 75 kDa endblock comprises 33 repeats of the pentapeptide sequence [IPAVG]5 and the 58 kDa midblock comprises 28 repeats of the sequence $[(VPGAG)_2VPGEG(VPGAG)_2]$. Flanking both the hydrophobic, plastic endblocks and the hydrophilic, elastic midblock, were cross-linkable amino acid sequences [KAAK], which allow for amine-based cross-linking. Subsequent to expression in *E. coli*, protein was extracted and purified using hot/cold centrifugation cycles and nucleic acid removal. Protein solutions were then dialyzed against water and lyophilized.

### 2.3. Production of dense collagen networks

Monomeric rat tail tendon collagen and Lys-B10 were dissolved in 10 mM HCl at a concentration of 2.5 mg $ml^{-1}$ . Solutions were neutralized using a gelation buffer (4.14 mg $ml^{-1}$ monobasic sodium phosphate, 12.1 mg $ml^{-1}$ dibasic sodium phosphate, 6.86 mg $ml^{-1}$ TES (N-tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid sodium salt, 7.89 mg $ml^{-1}$ sodium chloride, pH 8.0) at 4 °C and were poured immediately into rectangular molds ($10 \times 7 \times 0.4$ cm) for 24 h. Gels were subsequently placed in a fiber incubation buffer (7.89 mg $ml^{-1}$ sodium chloride, 4.26 mg $ml^{-1}$ dibasic sodium phosphate,10 mM Tris, pH 7.4) at 37 °C for 48 h to promote collagen fibrillogenesis [29]. Gels were then dried at room temperature under a steady air stream.

### 2.4. Imaging of composite architecture

Optical microscopy, fluorescence microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to analyze the collagen micro- and ultrastructure prior to and after embedding in elastin. For SEM studies, dry collagen mats were hydrated in water for 24 h and dehydrated in serial exchanges of ethanol–water mixtures from 30% to 100%. The samples were then critical-point-dried (Auto Samdri 815 Series A, Tousimis, Rockville, MD), sputter-coated with 6 nm of Pt/Pd (208HR Cressington, Watford, UK) and imaged at an accelerating voltage of 10 keV using a field emission scanning electron microscope (Zeiss Supra 55 FE-SEM, Center for Nanoscale Systems, Harvard University). For TEM studies, samples in PBS were washed in 0.1 M cacodylate buffer and fixed in glutaraldehyde. After washing in water, samples were partially dehydrated in ethanol and stained with uranyl acetate. Samples were then fully dehydrated in ethanol, embedded in resin and polymerized. Ultrathin (60–80 nm) sections were cut using a RMC MT-7000 ultramicrotome (Boeckeler, Tucson, AZ). Post-staining with uranyl acetate and lead citrate was followed by imaging using a JOEL JEM-1400 TEM (JOEL, Tokyo, Japan) at 90 kV.

### 2.5. Fabrication collagen–elastin nanofibrous grafts

Lys-B10, dissolved in molecular grade water at 4 °C at a concentration of 100 mg $ml^{-1}$, was used to embed acellular collagen matrices in a sandwich molding setup (Fig. 1). Briefly, collagen matrices were dried on 0.45 μm filter membranes (Millipore, MA), and cooled to 4 °C. Plastic shims (height 72.6 μm, Precision Brand, IL) were placed around the collagen sheet. Cold elastin was spread on top of the collagen matrix and a capping glass layer was added for 20 min. The setup was warmed to 25 °C to allow the liquid elastin to gel. The collagen–elastin composites were then removed from the glass support, the long sheets were rolled on 1.3 mm or 4 mm ID stainless steel mandrels, rotated at 60 rpm at 4 °C for 10 min to allow the elastin to undergo a phase transition to a liquid state and then rotated at 60 rpm at 25 °C for 5 min to gel the elastin into one continuous layer.

### 2.6. Mechanical testing of composites

#### 2.6.1. Planar constructs

Collagen sheets were cut using a dog-bone punch (13 mm gage length), mounted onto a Dynamic Mechanical Thermal Analyzer V (DMTA V, Rheometric Scientific, Piscataway, NJ) and immersed in