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Mechanical Properties and Compositions of Tissue Engineered and Native Arteries

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Abstract

With the goal of mimicking the mechanical properties of a given native tissue, tissue engineers seek to culture replacement tissues with compositions similar to those of native tissues. In this report, differences between the mechanical properties of engineered arteries and native arteries were correlated with differences in tissue composition. Engineered arteries failed to match the strengths or compliances of native tissues. Lower strengths of engineered arteries resulted partially from inferior organization of collagen, but not from differences in collagen density. Furthermore, ultimate strengths of engineered vessels were significantly reduced by the presence of residual polyglycolic acid polymer fragments, which caused stress concentrations in the vessel wall. Lower compliances of engineered vessels resulted from minimal smooth muscle cell contractility and a lack of organized extracellular elastin. Organization of elastin and collagen in engineered arteries may have been partially hindered by high concentrations of sulfated glycosaminoglycans. Tissue engineers should continue to regulate cell phenotype and promote synthesis of proteins that are known to dominate the mechanical properties of the associated native tissue. However, we should also be aware of the potential negative impacts of polymer fragments and glycosaminoglycans on the mechanical properties of engineered tissues.

Keywords

Tissue engineering; Blood vessels; Mechanics; Extracellular matrix; Collagen; Elastin; Glycosaminoglycans; Smooth muscle contractility; Polyglycolic acid

INTRODUCTION

Engineered arteries show great promise as a source of vascular grafting material for patients who need coronary artery or peripheral bypass surgeries, but who lack healthy autologous replacement artery or vein. In the field of tissue engineering, it is commonly thought that the best way to successfully mimic the mechanical behavior of a native tissue is to engineer a tissue with similar composition and structure. In this report, we measure the mechanical properties of engineered vessels, compare the mechanical properties of engineered vessels to those of native vessels, and seek to understand similarities and differences between the mechanical properties of engineered and native vessels based on their compositions.

The stress–strain curves of native arteries have been described by the contributions of collagen, elastin, and smooth muscle, which are prevalent in the arterial wall.^{1,2} Strong collagen networks in blood vessels provide good surgical handling properties and prevent rupture *in vivo*. Elastin, which is arranged as extracellular concentric cylinders in the native artery, provides recoil after each cycle of vessel inflation and thereby prevents arterial dilation *in vivo*.^{2,15} Elastin and contractile smooth muscle cells (SMCs) regulate arterial compliance.² Native arteries also contain glycosaminoglycans (GAGs) and water, yet their contribution to arterial mechanics is frequently considered negligible.

Polyglycolic acid (PGA) scaffolds are used for many tissue engineering applications because their large void volumes support cellular infiltration, cellular attachment, and extracellular matrix deposition.^{8,16} Given that PGA degrades over time and loses its mechanical integrity during the first 3 weeks in tissue culture or other aqueous conditions,^{8,19} the impact of partially degraded remnant polymer fragments on the mechanical properties of engineered tissues generally is considered negligible by the tissue engineering community.¹⁹ In this paper we show that engineered tissues would be stronger without residual polymer.

METHODS

Culture of Porcine Tissue Engineered Vessels

Engineered arteries having dimensions of 3 mm in diameter and 7 cm in length were grown as previously described.¹⁶ Briefly, porcine carotid SMCs (5×10^6 cells/ml) were seeded onto a PGA scaffold (Albany International, Mansfield, MA) that was sewn into a cylindrical construct with 6–0 PGA suture (US Surgical, Norwalk, CT) around a silicone tube for support. Sewing with PGA suture created an axially aligned suture line on each vessel. Vessels were cultured in a bioreactor connected to a peristaltic pump (vessels were strained 1.5% at 2.8 Hz) for 7–8 weeks in low glucose Dulbecco's Modified Eagle's Medium (JRH Biosciences, Lenexa, Kansas) with 10% fetal bovine serum (FBS, GibcoBRL), 10% porcine serum (PS, GibcoBRL, Grand Island, NY), basic fibroblast growth factor (10 ng/ml), platelet derived growth factor (10 ng/ml), L-ascorbic acid, copper sulfate, HEPES, L-proline, L-alanine, L-glycine, and Penicillin G (Sigma, St. Louis, MO). Because endothelial cells do not contribute to passive vascular mechanics, no endothelial cells were seeded onto tissue engineered arteries in this study.

Native Porcine Arteries

Common carotid arteries were excised in sterile fashion from anesthetized 25–30 kg Duroc swine (Lee Brothers, Four Oaks, NC). All animals were treated in accordance with standard NIH protocols.

Histological Analysis

Vessel segments were fixed in 10% neutral buffered formalin for histochemistry. After paraffin embedding, vessels sections (5 μ m thick) were stained with Masson's trichrome stain for collagen (collagen stains blue), Movat's stain for glycosaminoglycans (GAGs stain blue), or Verhoeff–Van Gieson's stain for elastin (elastin stains black). Wall thicknesses of fixed vessels were measured using NIH Image.

Mechanical Analysis

Vascular mechanical properties were assessed during inflation of a vessel until failure, as previously described.⁵ According to linear elastic theory for an isotropic, thick-walled cylinder, stress (σ) and strain (ϵ) were calculated as:

$$\sigma = \frac{8P(r_{\text{external}} \times r_{\text{internal}})^2}{(r_{\text{external}}^2 - r_{\text{internal}}^2)(r_{\text{external}} + r_{\text{internal}})^2} \quad (1)$$

$$\varepsilon = \frac{(r_{\text{external}} + r_{\text{internal}})}{(r_{0,\text{external}} + r_{0,\text{internal}})} - 1 \quad (2)$$

where P is the pressure inside the vessel, r is the measured radius and r_0 is the radius at zero pressure.¹ Mean physiological compliance (measured between 70 and 120 mmHg) was measured as a percent per 100 mmHg:

$$\text{Compliance} = \frac{10^4 (D_{\text{sys}} - D_{\text{dias}})}{D_{\text{dias}} (P_{\text{sys}} - P_{\text{dias}})} \quad (3)$$

where D_{sys} and P_{sys} are the systolic vessel diameter and pressure, respectively, and D_{dias} and P_{dias} are the diastolic diameter and pressure. The maximal incremental modulus, E_{max} , was calculated as the slope of the stress-strain curve at the four highest values of strain before failure. We considered E_{max} to represent E_{collagen} for engineered vessels.

Tissue Composition

Collagen in engineered and native vessels was calculated as 10 times the amount of hydroxyproline, as previously described.^{18,28}

DNA in engineered and native vessels was measured fluorometrically with Hoechst 33258 dye (Polysciences, Warrington, PA), as previously described,¹¹ and we calculated a value of 8.5 pg DNA/porcine carotid SMC.

SMC contractility was measured by mounting ring segments of engineered vessels around wire hooks.^{23,24} Each ring segment was adjusted to a baseline tension of 0.25 g and pre-tested with potassium chloride (24 mM) as a non-receptor mediated agonist. Contraction was measured in response to 10^{-4} M prostaglandin $F_{2\alpha}$ (Sigma, St. Louis, MO) with a digital data acquisition analysis and archive system (Gould Instrument Systems, Inc., Valley View, OH).

Sulfated GAG density was measured colorimetrically with the Blyscan Sulfated Glycosaminoglycan Assay, using chondroitin sulfate as a standard (Biocolor Ltd., Newtownabbey, Northern Ireland).⁴ Sulfated GAG measurement included chondroitin sulfates, keratan sulfates, dermatan sulfates, and heparan sulfates.

Water in engineered and native arteries was calculated as

$$\% \text{ Water} = \left(1 - \frac{\text{Dry Weight}}{\text{Wet Weight}} \right) \times 100 \quad (4)$$

Volume fractions of polymer (area of PGA as a fraction of vessel area) were measured from histological images of engineered vessels at suture line and non-suture line regions using NIH Image. See Appendix for stress concentration calculations.

Statistics

Statistical significances were determined by Student's two-sample t -tests, assuming unequal variances. The reported p values are one-sided. All values are presented as the mean $\pm$ the standard error of the mean.

RESULTS

Mechanical Properties of Tissue Engineered and Native Arteries

The mechanical properties of engineered vessels differed from those of native vessels, both at failure and in the *in vivo* pressure range. Specifically, burst pressures, maximum stresses, and mean compliances of engineered vessels were significantly lower than those of native porcine carotid arteries (Table 1). Additionally, maximum moduli of engineered vessels appeared lower than those of native vessels, though the difference was not statistically significant (Table 1). Interestingly, burst pressures and maximal stresses of engineered vessels were less than those of native vessels, despite similar collagen densities between engineered and native tissues (Table 2).

Compositional Properties of Tissue Engineered and Native Arteries

Collagen was prevalent in engineered and native vessels (Fig. 1). Although engineered vessels were significantly thinner than native vessels, their collagen densities were similar (Table 2). Nonetheless, maximal moduli of engineered vessels were lower than those of native vessels, implying some difference in the organization of the collagenous vascular wall.

Elastin was less prevalent in engineered vessels than in native vessels (Fig. 2). Further, elastin in engineered vessels was predominantly intracellular and did not form concentric sheets. In contrast, elastin in native vessels was organized into clearly identifiable concentric sheets.

SMC density was lower in engineered vessels than in native vessels, and the contractilities of SMCs in engineered vessels were less than 2% of those in native vessels in response to 10^{-4} M prostaglandin $F_{2\alpha}$ (Table 2; native vessel contractilities previously reported by Schoeffter and Muller-Schweinitzer²¹). Hence, the lack of organized elastin, combined with the low level of intrinsic contractility of the engineered SMCs, led to decreased compliance of engineered vessels at physiological pressures.

The density of sulfated GAGs was significantly higher in engineered vessels than in native vessels (Fig. 3, Table 2). Higher GAG densities in engineered vessels may explain their significantly higher water content (Table 2).

Lastly, PGA fragments were present throughout the engineered vessel wall (Region 1, Fig. 4), but were more densely packed and more prevalent at the suture line (Region 2, Fig. 4; Table 3) as a result of using dense PGA suture to sew a porous PGA scaffold into a tubular structure. In contrast, of course, native vessels do not contain polymer. The densely packed region of PGA fragments at the suture line may have acted as a large circular void in engineered tissue (see Appendix), thereby forcing tissue adjacent to polymer to see local maximum stresses higher than those reported in Table 1 (Table 3). Polymer remnants in normal areas of engineered tissues (Region 1) also caused local stress concentrations (Table 3). Our calculations (Appendix) show that stresses borne by collagenous tissue that neighbors polymer remnants are approximately three times those that would be calculated based on simple assumptions of tissue homogeneity and isotropy. Stated differently, this means that engineered collagen fails at pressures that are one-third those that would induce failure if polymer were not present in the wall. This in turn means that polymer residuals are an important factor in reducing the ultimate strengths of engineered tissues. In addition, it is noteworthy that engineered vessels primarily failed by tearing along the suture line, at the region of highest polymer concentration and therefore highest stress.

DISCUSSION

The ultimate mechanical properties of arterial tissues are generally associated with collagen density.^{1,16} In this study, however, densities of collagen in engineered vessels matched those of native arteries. Thus, collagen density cannot be used to explain differences in the ultimate mechanical properties of engineered and native vessels observed in this study. Theoretical models suggest that alignment of collagen also impacts a tissue's mechanical properties.^{10, 12,20} In another study, we have shown that engineered vessels have less circumferentially aligned collagen than native vessels, (S. L. M. Dahl, Submitted) which may contribute to lower ultimate mechanical strengths in engineered vessels. We have also shown that collagen fibrils and fibers in engineered vessels are thinner than those in native vessels (S. L. M. Dahl, Submitted). Further, collagen fibers are less dense (collagen fibrils pack less closely together to form fibers) in engineered tissues than in native tissues. Given that thicker fibrils and fibers have been correlated with increased ultimate tissue strengths,¹⁷ thinner fibrils and fibers may explain, in part, the inferior ultimate mechanical properties of engineered tissues. Growth of collagen fibrils may be inhibited by overabundant GAGs.¹⁴ In our system, synthesis of chondroitin sulfate proteoglycans is likely promoted by PDGF and bFGF,^{22,27} which are used as mitogens in the culture of porcine engineered vessels. Reducing the use of growth factors that stimulate GAG synthesis may allow formation of thicker collagen fibrils, and better packing of fibrils into thick and dense collagen fibers, which in turn, may increase ultimate strengths of engineered tissues.

Ultimate mechanical properties of engineered vessels may be inferior to those of native vessels in part because engineered tissues were thinner. Nutrient diffusion is often blamed for limitations on thicknesses of engineered tissues cultured *in vitro*. Developing tissues with a network of capillary-type structures to allow enhanced nutrient diffusion may promote formation of thicker (and thus, likely stronger) engineered tissues.

Residual polymer fragments have a significant negative impact on the ultimate mechanical properties of engineered tissues. After exhaustively studying factors that have the potential to positively impact the ultimate strengths of engineered tissues (including collagen density, collagen crosslink density,⁶ alignment of collagen fibrils, (S. L. M. Dahl, Submitted) and synthesis and packing of collagen fibrils (S. L. M. Dahl, Submitted)), and finding that engineered vessels still failed to match the ultimate strengths of native tissues, we considered whether any components of engineered vessels negatively impacted ultimate tissue strengths. The negative impact of remnant polymer fragments was striking. Our observation that engineered vessels routinely rupture at the suture line supports the theory that polymer fragments may act as voids in engineered tissues and thereby greatly increase stresses and strains in adjacent tissue. Our estimation that stresses in tissues adjacent to polymer fragments are 3-fold higher than measured stresses suggests that the quality of proteinaceous extracellular matrix synthesized in engineered tissues may be comparable in strength to native tissue (in Table 1, burst pressures and maximum stresses of native tissues are approximately 3–5 times greater than those of engineered tissues).

The low compliances of engineered vessels compared to those of native vessels likely result from poor development of elastin fibers and negligible contractilities of SMCs. Low levels of staining for elastin in engineered vessels may be explained by the use of factors that promote collagen synthesis (e.g. ascorbic acid and bFGF), but simultaneously inhibit tropoelastin gene expression.⁷ Further, intracellular staining suggests that tropoelastin in engineered vessels was not effectively transported to microfibrillar networks in the extracellular matrix.^{9,13} High densities of GAGs, which aid in extracellular elastin assembly,^{9,13} may have contributed in part to ineffective extracellular elastin organization. Controlling *in vitro* development of

functional concentric sheets of extracellular elastin, which has yet to be fully elucidated in the field of elastin biology, would greatly enhance the compliance of engineered tissues.

Low compliances of engineered vessels also resulted in part from minimally contractile SMCs. These SMCs were guided towards a proliferative and synthetic phenotype by the use of PDGF and bFGF, both of which cause SMCs to lose their contractile phenotype.^{25,26} Tissue engineers may be able to guide SMCs towards a more contractile phenotype, and thereby increase tissue compliances, by eliminating growth factors from culture medium after the desired synthesis of tissue is complete.

The lessons learned herein are transferable across the field of tissue engineering. It is likely that small improvements in the ultimate mechanical properties of engineered tissues would be achieved by better collagen alignment, thicker collagen fibrils and fibers, or more dense packing collagen of collagen fibrils into collagen fibers. However, larger improvements in ultimate mechanical properties and tissue compliances may be made by focusing on other efforts. Specifically, biomedical and tissue engineers may benefit by focusing future efforts towards (1) eliminating polymer fragments from engineered tissues, (2) generating thicker tissues, and (3) developing approaches to stimulate formation of mature elastin in the desired configuration (concentric sheets for tissue engineered vessels). Focusing on these goals will promote the development of engineered tissues that better mimic their native counterparts.

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APPENDIX

Calculation of Stress Concentrations Caused by Polymer Fragments

At the suture line, regions of densely packed PGA fragments (e.g., circled area in Fig. 4) were approximated as a single circular stress concentration in the vessel wall. The behavior of this stress concentration during mechanical testing was assumed to be analogous to a void within a plate being pulled in tension.³

To calculate the maximum local stress seen by engineered tissue bordering a polymer “void,” vessel wall thickness, h , and radius of the polymer void, r_{void} , were used to calculate the thickness of the vessel wall not containing polymer, d :

$$d = h - 2r_{\text{void}} \quad (A1)$$

The ratio r_{void}/d was used to calculate a stress concentration factor, K_T , which ranged from 2.0 to 3.0:

$$K_T = \frac{\sigma_{\text{max}}}{\sigma_{\text{avg}}} \quad (A2)$$

where σ_{max} was the maximum stress seen by a tissue at a void edge, and σ_{avg} was the average stress across d (Fig. 5).

For a void space covering 15% of the vessel area, $r_{\text{void}}/d = 0.118$, and thus, $K_T = 2.5$.³ With

$$\sigma_o = \frac{Pr_i}{h} = \frac{\text{Force}}{\text{SurfaceArea}} \quad (A3)$$

where r_i was the vessel inner radius, and

$$\sigma_{\text{avg}} = \frac{\text{Force}}{(h - 2r_{\text{void}}) \times \text{length}} = \frac{\text{SurfaceArea} \times \sigma_o}{(h - 2r_{\text{void}}) \times \text{length}} \quad (A4)$$

where length was the axial length of the tested vessel segment, σ_{max} could be calculated from Eq. (A2). The ratio $\sigma_{\text{max}}/\sigma_o$ described the factor of increased stress seen by engineered tissue near the polymer void (Table 3). Thus, in the absence of polymer fragments at the suture line and elsewhere, engineered tissues may withstand pressures and stresses greater than 3 times their reported burst pressures and maximum stresses.

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FIGURE 1. Representative histological sections stained with Masson's trichrome showed similar densities of collagen (blue) throughout (a) engineered and (b) native arterial tissue (scale bars = 100 μm).

FIGURE 2.

Representative Verhoeff-Van Gieson's stains showed (a) small amounts of elastin (black) that was not organized into concentric sheets in engineered vessels (enlarged in b) and (c) clearly identifiable concentric sheets of mature elastin in native arteries (enlarged in d). Scale bars = 100 μm .

FIGURE 3. Representative Movat's staining of GAGs (blue–green) in (a) engineered and (b) native vessels (scale bar = 100 μm).

FIGURE 4.

PGA polymer remnants (arrow) remaining at the end of engineered vessel culture are shown in a representative Masson's trichrome stain (scale bar = 100 μm) throughout the engineered tissue (Region 1) and at the suture line (Region 2).

FIGURE 5.

Engineered vessels sense higher stresses near polymer fragments, which act as voids in the tissue, than at tissue sites far away from polymer fragments. σ_0 was the stress seen across the vessel wall when stress distribution was not disrupted by polymer fragments. σ_{avg} was the average stress seen across d , the thickness of the vessel wall without the polymer particle. σ_{max} was the maximum stress seen by the tissue at the edge of the polymer or void space. P was the pressure exerted on the vessel lumen.

TABLE 1

Mechanical properties of engineered and native vessels.

	Engineered vessels (<i>n</i> = 8)	Native vessels (<i>n</i> = 3)	<i>p</i> value
Burst pressures (kPa)	107 ± 14	443 ± 55	<0.05
Maximum stresses (MPa)	1.40 ± 0.25	6.58 ± 0.97	<0.05
Maximum moduli (MPa)	11.8 ± 2.7	45.1 ± 16.8	0.09
Mean compliances (% per 100 mmHg)	3.5 ± 0.2	18.7 ± 4.1	<0.05

TABLE 2

Compositional properties of engineered and native vessels.

	Engineered vessels	Native vessels	p value
Wall thicknesses (μm)	220 $\pm$ 22 (n = 8)	614 $\pm$ 52 (n = 3)	< 0.05
Collagen (% of dry weight)	43.9 $\pm$ 1.6 (n = 8)	39.5 $\pm$ 2.5 (n = 3)	NS
SMCs (10^6 cells/ml tissue)	136 $\pm$ 5 (n = 8)	214 $\pm$ 17 (n = 6)	< 0.05
Contractility of SMCs (g)	0.11 $\pm$ 0.04 (n = 4)	~6.6 $\pm$ 0.6 (n = 8)	NA
Sulfated GAGs (μg /mg tissue)	2.70 $\pm$ 0.18 (n = 11)	1.28 $\pm$ 0.04 (n = 5)	< 0.05
Water (% wet weight)	86.0 $\pm$ 0.3 (n = 35)	81.0 $\pm$ 0.8 (n = 24)	< 0.05

NS, not significant; NA, not available.

TABLE 3

PGA in engineered vessels.

	Non-suture line regions	Suture line region	<i>p</i> value
Volume fraction PGA (%)	9.0 ± 1.9 ($n = 3$)	15.0 ± 1.6 ($n = 3$)	<0.05
Theoretical maximum stresses /Reported maximum stresses	3.05 ± 0.02 ($n = 8$)	3.32 ± 0.03 ($n = 8$)	<0.05