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Limits to the durability of arterial elastic tissue

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Abstract

To engineer a better blood vessel, we must identify which structural, mechanical, and biological features of the native vessel must be replicated to ensure long-term survival of the implant. In this study, we tested autoclave-purified elastic tissue from along the pig thoracic aorta under long-term static and cyclic loading to identify factors that affected its durability. Samples were tested in water or in sucrose, which enhances viscoelasticity. Samples failed between 50% and 80% extension, which is lower than the failure extension in shorter, quasi-static tensile tests. Cyclic loading had a small effect on the durability of samples tested in water. Samples from the distal thoracic aorta and samples pre-treated in 70% ethanol showed enhanced durability. Failure between 50% and 80% extension appears associated with structural features of the individual fibre, and indirect evidence suggests it may be due to failure of the microfibrils, not the elastin. Cross-linked elastin may be necessary but insufficient to prevent failure. Durability appears also affected by regional differences in tissue structure, possibly the three-dimensional fibre organization. These results suggest ensuring normal fibre synthesis and organization may be crucial to the design of a successful vascular implant.

Keywords: Elastin; Microfibril; Arterial tissue engineering; Fatigue; Mechanical test

1. Introduction

Arterial elastic tissue is a crucial regulatory and structural component of the vascular wall required for development and mechanical functioning. The elastic tissue provides long-range extensibility and recoil that must be reproduced in vascular implants to present the vascular smooth muscle with the proper mechanical environment and to avoid vessel dilation and structural failure due to creep. The incorporation of elastic tissue into implants has proven difficult. In tissue-engineered constructs, elastic tissue can be introduced either as part of the initial supporting scaffold or synthesized later as new material. Recent attempts to introduce elastin as part of the scaffold have met with varying success [1–6], but calcification and enzymatic degradation remain a problem [2,3,7,8]. Initiating elastogenesis has been generally unsuccessful [5,9–15], due in part to the inability to adequately reproduce the spatially complex cues required for growth and remodelling [15,16]. Cyclic mechanical conditioning increases the production of elastin [17,18], but the optimal conditions are not yet known. The inability to incorporate mature functional elastic fibres, therefore, presents a serious limitation to the tissue engineering of blood vessels [15].

In order to adequately reproduce the local mechanical environment, we must understand the relationship between the structure and function of the elastic tissue in the native vessel. The elastic fibre is a composite of a cross-linked elastin network assembled on a fibrillin-rich microfibrillar scaffold. Fibrillins are required for fibre assembly, wall maturation and development [19,20], and there is some direct mechanical evidence [21,22] and indirect histopathological evidence [20,23] that they also provide load-bearing structural support in the adult fibre. The individual elastic fibres interconnect to form a three-dimensional meshwork throughout the arterial wall. Although, the general structure and function of the elastic meshwork has been understood since the papers of Wolinsky and Glagov [24–26], little work has been done on how the organization of this structure is modulated to provide the mechanical strains appropriate for the local hemodynamic loads. Even

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within the thoracic aorta, the mechanical properties of the elastic tissue changes with position [27], but the structural basis for this is unknown. It is unlikely that a tissue-engineered vessel will mimic all features of the elastic tissue; so identifying which of these structural features must be replicated in a tissue-engineered blood vessel is essential for producing a successful replacement with a long service lifetime [28]. We have, therefore, been studying the elastic tissue in the thoracic aorta, a vessel particularly rich in elastic tissue, to identify the fundamental principles of its design.

The current experiments were designed to examine factors that affect the durability of aortic elastic tissue. Durability can be quantified by the fatigue lifetime of a sample under long-term static or cyclic loading, and in most materials lifetime depends on the loading level. We hoped to identify the limit to the strains that can be safely placed on elastic tissue within the vascular wall, to determine whether any specific structural element constitutes a potentially vulnerable point in an implant, and to identify factors that increase or decrease durability and that therefore should be optimized or avoided in an implant. We found that durability was affected by several factors, suggesting that both the elastic fibre and the meshwork of elastic fibres must be appropriately assembled to produce a successful, tissue-specific implant.

2. Materials and methods

2.1. Rationale for using sucrose and 70% ethanol

Sucrose-induced viscoelasticity increased elastic tissue strength in quasi-static tests [29], and so we developed a sucrose protocol to establish whether viscoelasticity also increased durability through a similar mechanism. Elastin forms a series of kinetically mobile molecular chains that are cross-linked into a network. Adjacent chains interact periodically and this ''internal friction'' slows the rate at which a chain moves, making the network viscoelastic [30]. Water inhibits these interactions, reducing viscoelasticity [31], and so osmotic agents such as sucrose increase viscoelasticity [32,33]. Sucrose had no measurable effects on elastic tissue other than those secondary to the slowing of chain motion [33], so it should have no effect on non-viscoelastic processes. The impact of sucrose on durability cannot be predicted a priori. Chain interactions stiffen the network, generating higher stresses, which should shorten fatigue lifetime, but they also dissipate strain energy and distribute internal loads more evenly, which should strengthen the network and lengthen lifetime. Comparing the durability in water and sucrose may reveal information on the molecular processes involved in failure.

A similar logic underlay the use of 70% ethanol, which swells elastic tissue. We previously found elastic tissue exposed to the swelling agent guanidine became stiffer at high extensions, and we suggested that swelling altered load transmission amongst elastic fibres [21]. We therefore pre-treated tissue with a swelling agent to test whether the distribution of loads throughout the fibre mesh plays a significant role in fatigue failure. In this study, we used 70% ethanol as the swelling agent, and 100% ethanol, which does not swell elastic tissue, as a control.

2.2. Aortic ring preparation

Thoracic aortas from about 100 kg pigs were collected from a local abattoir, transported on ice and were either frozen in excess distilled water or purified immediately. The tissue was purified by repeated autoclaving in distilled water [34] and stored under sterile conditions until used. Purified aortas were carefully cut into rings averaging 100 mg (dry weight). Branches or irregular sections were avoided. The position, z, of the proximal edge of each ring along the aorta was measured with callipers. The position of the proximal edge of the ring immediately distal to the first intercostal branch was designated as 0 mm. Rings proximal to the first branch were given a negative position value.

All rings were subjected to quasi-static testing, described below, and were then divided into three pre-treatment groups. Rings that received no pre-treatment (NT) were placed in distilled water containing sodium azide (0.02% w/v). Rings to be pre-treated were put in individual glass vials containing 15–20 ml of either 70% aqueous ethanol (70 Et) or 100% (nominal) ethanol (100 Et). Vials were gently shaken for 28–31 days at 37 °C with a weekly change of solution. Rings were removed from solution, rinsed exhaustively in distilled water and azide and stored in distilled water with azide.

2.3. Quasi-static tensile tests

Rings were subjected to quasi-static tests to obtain the load required to produce the desired static extension in the subsequent durability test. The tensile mechanical properties of the tissue were measured as previously described [34]. Rings were mounted around two parallel steel bars and immersed in distilled water at 37.0 ± 0.5 °C. The samples were deformed in tension in an Instron 5500R tensile testing machine at a crosshead rate of 20 mm/min. Samples were taken through two pre-conditioning cycles to between 40% and 75% extension. Force, F, and extension data were collected on the third cycle. Force was converted to Lagrangian stress, σ_{Lag}, based on the undeformed tissue dimensions, $\sigma_{\mathrm{Lag}} = F/(2wh)$, where w is the ring width, measured with callipers, and h is wall thickness. Thickness was determined initially with callipers and finally from the submerged weight of the ring, as previously described [27].

The midwall length at which the bulk of the tissue first started to resist deformation, L_0, was determined by fitting a linear regression to the stress-length data between stresses of 50 and 100 kPa and regressing to zero load. The applied circumferential stretch ratio, $\lambda = L/L_0$, was calculated from the midwall circumferential length, L [27]. To avoid damaging the tissue, samples were rarely taken to high extensions in these tests. The load required to produce a given extension was either obtained directly from these quasi-static tests or was extrapolated from the measured mechanical behaviour using a general stress–strain relationship for our pig tissue that takes into account tissue position.

In a second group of tests we determined the mechanical and failure properties of untreated samples and samples pre-treated with 70% ethanol. Tissue was taken to failure at 20 mm/min without pre-conditioning. Some untreated samples were tested at a cross-head speed of 0.1 mm/min. The circumferential tangent modulus was calculated as $E = \Delta\sigma/\Delta\varepsilon$, using Cauchy stress, $\sigma = \sigma_{\mathrm{Lag}}\lambda$, Green strain, $\varepsilon = 0.5(\lambda_\theta^2 - 1)$, and $\Delta\varepsilon = 0.01$.

2.4. Cyclic loading tests

2.4.1. Apparatus

Each ring was mounted in the cyclic loading apparatus immersed in either distilled water or 2 M sucrose containing 0.02% sodium azide at 37 ± 0.5 °C (Fig. 1). A thin layer of mineral oil or paraffin was floated on the surface of the liquid to prevent evaporation. The ring was mounted vertically around two horizontal bars so that it hung from the upper bar and was stretched by the weight of the lower bar and attached aluminium frame. The frame was stabilized by two shims but its weight was supported by the ring. The weight of the frame was adjusted by adding masses to provide the desired static tensile load on the ring. The ring gradually stretched or crept under the static load, allowing the frame to move slowly downwards. A linear variable displacement transducer (LVDT) attached to the frame monitored this movement. The LVDT output was corrected for long-term drift in its power supply, allowing measurement of creep