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Altered structural and mechanical properties in decellularized rabbit carotid arteries

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Abstract

Recently, major achievements in creating decellularized whole tissue scaffolds have drawn considerable attention to decellularization as a promising approach for tissue engineering. Decellularized tissues are expected to have mechanical strength and structure similar to the native tissues from which they are derived. However, numerous studies have shown that mechanical properties change after decellularization. Often, tissue structure is observed by histology and electron microscopy, but the structural alterations that may have occurred are not always evident. Here, a variety of techniques were used to investigate changes in tissue structure and relate them to altered mechanical behavior in decellularized rabbit carotid arteries. Histology and scanning electron microscopy revealed that major extracellular matrix components were preserved and fibers appeared intact, although collagen appeared looser and less crimped after decellularization. Transmission electron microscopy confirmed the presence of proteoglycans (PG), but there was decreased PG density and increased spacing between collagen fibrils. Mechanical testing and opening angle measurements showed that decellularized arteries had significantly increased stiffness, decreased extensibility and decreased residual stress compared with native arteries. Small-angle light scattering revealed that fibers had increased mobility and that structural integrity was compromised in decellularized arteries. Taken together, these studies revealed structural alterations that could be related to changes in mechanical properties. Further studies are warranted to determine the specific effects of different decellularization methods on the structure and performance of decellularized arteries used as vascular grafts.

Keywords: Decellularized artery; Extracellular matrix; Small-angle light scattering; Fiber orientation; Mechanical properties

1. Introduction

Decellularized tissues have gained significant attention in the field of tissue engineering, especially for their promise in whole organ transplant and grafting [1,2]. Although most applications are still far from clinical use, numerous tissue types have been successfully decellularized, including heart [3], heart valve [4–7], bladder [1,8], blood vessel [9–11], skeletal muscle [12,13], tendon [14] and ligament [15,16]. A major motivation for using decellularized tissues is that they are expected to mimic closely the complex 3D structure and mechanical properties of the native tissues from which they are derived [17,18]. It is well established that the mechanical properties of a tissue are intimately linked to its structure [19], and this relationship is especially important for load-bearing tissues such as the artery [20].

Decellularized blood vessels have been studied extensively [9–11,21–34], mostly for their potential as scaffolds

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for small diameter vascular grafts (SDVG). There is a great need for tissue-engineered SDVG, as many patients do not have autologous vessels available and synthetic grafts are prone to failure in small diameter applications [35]; in many cases, this has been linked to inappropriate structure and/or mechanical performance [36,37]. If decellularized vessels do indeed maintain native tissue architecture and mechanical properties, these challenges could be overcome.

A majority of the literature regarding decellularized vessels tends to focus on cell seeding and implantation; while such studies are important and encouraging, there is still a lack of fundamental study of decellularized vessel structure–function relationships. Interestingly, many studies have reported that decellularized vessels have significantly altered mechanical characteristics compared with native vessels [10,11,21–23,29]. Histology and/or electron microscopy images are often included, but changes (or similarities) in extracellular matrix (ECM) structure are not necessarily evident. While these imaging techniques are commonly used and do provide some useful information on structure, they have limitations: specifically, they do not reveal whether fiber–fiber interactions, fiber orientation or fiber mobility changes as a result of decellularization, which are important to the structural integrity of tissue.

Preservation of ECM does not necessarily correspond to preservation of tissue architecture. Although the intention of most decellularization procedures is to minimize disruption to the ECM, the removal of cells inevitably results in changes to native ECM structure [17]. Therefore, the goal of the present study was to use a variety of characterization techniques to investigate tissue structure in decellularized rabbit carotid arteries and to relate structural changes to altered mechanical properties. Histology, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to confirm removal of cells and to assess qualitatively ECM composition and ultrastructure. Mechanical properties were determined using stress–strain analysis and stress relaxation tests; additionally, opening angle studies were used as a measure of residual stress in the vessel wall. Finally, small-angle light scattering (SALS), a quantitative technique that measures the average local fiber orientation throughout the tissue thickness [38], was used to determine gross fiber architecture and changes in ECM fiber kinematics (e.g., fiber mobility and organization). SALS was included to elucidate changes in structural integrity that would not be revealed from histology and EM alone. Together, these data provided insight into how altered structural properties could be related to changes in mechanical properties as a result of decellularization.

2. Materials and methods

2.1. Tissue harvest

All procedures were performed in accordance with the Institutional Animal Care and Use Committee at Boston University and the NIH Guide for the Care and Use of Laboratory Animals. Healthy male New Zealand white rabbits (2.5–3 kg, Pine Acres Rabbitry, Brattleboro, VT) were euthanized, and carotid arteries were harvested using sterile tools. Vessels were immediately placed in cold Hanks' Balanced Salt Solution (HBSS: 137 mM NaCl, 5.4 mM KCl, 0.42 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.17 mM $NaHCO_3$, 10 mM HEPES, 5.55 mM glucose; 1% penicillin–streptomycin; pH 7.4) and stored on ice until use. For several samples, the length of the artery segment to be harvested was measured *in vivo* using digital calipers. The length was again measured after harvest, with the vessel in the unloaded state. The ratio of *in vivo* length to post-harvest length was determined (~1.7) and used for adjusting pressure-fixed samples to approximate *in vivo* length for the SALS studies described below.

2.2. Decellularization of carotid arteries

The decellularization procedure was modified from a previously published protocol [10]. First, carotid arteries were cleaned of blood and excess surrounding tissue. Vessels were then immersed in distilled water for 24 h at 4 °C to rupture cell membranes. Next, the vessels were treated with 0.025% trypsin (Gibco) diluted in Dulbecco's phosphate buffered saline (PBS; Gibco) for 24 h at 37 °C. After removal of trypsin, the vessels were treated with a solution of 1% TritonX-100 (Sigma) and 0.1% ammonium hydroxide (EM Science, Gibbstown, NJ) in distilled water for 72 h at 4 °C to remove nuclear components and lyse cell membranes and cytoplasmic proteins. Vessels were then transferred to a solution of Dulbecco's Modified Eagle Medium (DMEM; Gibco) with 5% bovine calf serum (BCS) to deactivate any residual trypsin for 24 h at 37 °C. Vessels were washed in distilled water for 24 h at 4 °C and, lastly, were washed overnight in PBS at 4 °C. All steps of the procedure were performed with mechanical agitation using a rotating platform.

2.3. Histology

For histological analysis, samples were fixed in 4% glutaraldehyde in PBS for 4 h, dehydrated with graduated concentrations of ethanol and embedded in paraffin. Cross-sections of the artery were cut to 5 μm thick. To elucidate changes in tissue components, Movat's Pentachrome stain was used to identify cells, collagen, elastin and PG with red, yellow, black and blue color, respectively. Stained tissue sections were imaged using bright field microscopy (Nikon EC600).

2.4. Transmission electron microscopy

Samples for TEM studies were fixed in 2% glutaraldehyde diluted in PBS overnight at 4 °C. For staining of PG, the specimens were fixed overnight in 1% Cupromeronic Blue (CB) under critical electrolyte concentration (CEC) conditions [39,40]. The 1% CB was in solution with

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