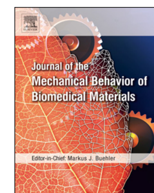
Journal of the Mechanical Behavior of Biomedical Materials 86 (2018) 199–207

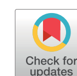
Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

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



An investigation on the correlation between the mechanical property change and the alterations in composition and microstructure of a porcine vascular tissue underwent trypsin-based decellularization treatment



Chih-Hsun Lin[a,b], Yun-Chu Kao[c], Hsu Ma[a,b], Ruey-Yug Tsay[c,d,*]

[a] Division of Plastic Surgery, Department of Surgery, Taipei Veterans General Hospital, No. 201, Section 2, Shipai Rd., Beitou Dist., Taipei City 112, Taiwan, ROC
[b] Department of Surgery, School of Medicine, National Yang-Ming University, No. 155, Section 2, Linong St., Beitou Dist., Taipei City 112, Taiwan, ROC
[c] Institute of Biomedical Engineering, National Yang-Ming University, No. 155, Section 2, Linong St., Beitou Dist., Taipei City 112, Taiwan, ROC
[d] Center for Advanced Pharmaceutics and Drug Delivery Research, National Yang-Ming University, No. 155, Section 2, Linong St., Beitou Dist., Taipei City 112, Taiwan, ROC

ARTICLE INFO

Keywords:
Trypsin
Decellularization
Fiber-progressive-engagement model
Pseudoelastic
Coronary artery

ABSTRACT

Purpose: The nonlinear pseudoelastic behavior of a native/decellularized vascular tissue is closely related to the detailed composition and microstructure of the extracellular matrix and is important in maintaining the patency of a small-caliber vascular graft.

A commonly used enzyme-detergent based decellularization protocol is effective in cell component removal but it also changes the microstructure and composition of the decellularized tissues. Previous studies provide limited information to correlate the mechanical property change with the alterations in composition and microstructure in a decellularization process. In this study, the correlations were studied by implementing a previously established fiber-progressive-engagement model to describe the nonlinear pseudoelastic behavior of a vascular tissue and to evaluate the effects of trypsin concentration and exposure duration on porcine coronary artery decellularization

Results: Results showed that tissue length and width increased and thickness and wet weight decreased with the exposure of trypsin. The effects of trypsin exposure times on the four mechanical parameters, i.e. initial strain, turning strain, initial modulus and stiffness modulus, in the longitudinal and circumferential directions were similar, but stronger in the circumferential direction. Major components of the extracellular matrix were vulnerable to the trypsin-based decellularization process. The decreases in initial and turning strain and the increase in initial modulus in circumferential direction were correlated with the significant decrease of collagen and glycosaminoglycans in the media layer.

Conclusions: Although trypsin-based decellularization achieved cell component removal and preservation of ultimate tensile stress, the microstructure and composition changed with alterations in the pseudoelastic behavior of the porcine coronary artery. Taken together, the current observations suggested less waviness, early engagement, or re-alignment of insoluble collagen fibers in the media layer, which resulted in turning from anisotropic into isotropic uniaxial mechanical property of porcine vascular tissue. Selecting the proper trypsin concentration (< 0.03–0.5%) and duration (< 12 h) of trypsin exposure in combination with other methods will achieve optimal porcine coronary artery decellularization.

1. Introduction

There are clinical demands for developing small-caliber vascular grafts for coronary/peripheral arterial bypass surgery, arteriovenous shunt, transplantation surgery, pediatric surgery, and free flap surgery (Naito et al., 2011). Using autologous vessels is the standard procedure, but there are problems of limited donor sites or poor quality of autologous vessels in patients with co-morbidities. Synthetic vascular grafts, such as Dacron or expanded polytetrafluoroethylene (ePTFE), provide other choices when patients do not have suitable autologous vessels, but a high rate of thrombosis formation has limited the use of these synthetic materials as small-diameter vascular grafts (Xue and Greisler, 2003). A tissue engineering approach using biodegradable polymers has shown some promising results (Pektok et al., 2008), but

* Corresponding author at: Institute of Biomedical Engineering, National Yang-Ming University, No. 155, Section 2, Linong St., Beitou Dist., Taipei City 112, Taiwan, ROC.
E-mail address: tsay@ym.edu.tw (R.-Y. Tsay).

https://doi.org/10.1016/j.jmbbm.2018.06.029
Received 18 April 2018; Received in revised form 14 June 2018; Accepted 19 June 2018
Available online 27 June 2018
1751-6161/ © 2018 Elsevier Ltd. All rights reserved.

there are numerous technical hurdles, e.g., modulating the mechanical, chemical, and biological properties and clinical challenges, e.g., the occurrence of neo-intimal hyperplasia and aneurysmal dilatation, that remain (Khademhosseini et al., 2006).

Recently, decellularization has become an important technology in the field of tissue engineering for biological scaffold preparation (Gilbert et al., 2006). The ideal decellularization process should remove most of the antigenic components from a tissue or an organ and leave the complex mixture of structural and functional proteins in the extracellular matrix (ECM). A decellularized extracellular matrix will provide an appropriate microenvironment for cell adhesion, migration, proliferation, and differentiation (Gong et al., 2008). A variety of tissues, including skin (Prasertsung et al., 2008), cornea (Márquez et al., 2009), nerves (Nectow et al., 2011), adipose tissue (Yu et al., 2013), heart valves (Grauss et al., 2005), tendons (Schulze-Tanzil et al., 2012), ligaments (Woods and Gratzer, 2005), placenta (Flynn et al., 2006), urinary bladder (Bolland et al., 2007), small intestine submucosa (Badylak et al., 1995), liver (Zhou et al., 2011), and facial composite tissue (Duisit et al., 2017), have been evaluated for tissue engineering and tissue repair applications. In addition, naturally occurring small-caliber tubular structures, either from vascular or other tissues from allogenic or xenogenic sources, have been investigated for use in decellularized small-caliber vascular grafts (Dahan et al., 2012).

As a vascular graft, the decellularized extracellular matrix of vascular tissues should have sufficient strength to withstand the hemodynamic cyclic strain, matched compliance with the adjacent native vascular tissue, and a lining of the lumen with non-thrombogenic properties (Sheridan et al., 2012). To mimic the native arterial wall's unique mechanical properties, which are derived from the interaction between two major ECM proteins, collagen and elastin (Holzapfel et al., 2002), the decellularized scaffolds should maintain their ECM components – not only in sufficient quantities, but also in their complex structure – as much as possible.

Notably, the mechanical properties and the amount of structural proteins remaining in the scaffold after decellularization depend greatly on the process of decellularization. Currently, the most effective decellularization protocols entail a combination of physical, chemical, detergent, and enzymatic treatments (Gilbert et al., 2006; Badylak et al., 2009). Trypsin is commonly used in enzyme-detergent decellularization processes. Its function depends on cleavage of the peptide bonds of protein in the extracellular matrix on the carboxyl side of arginine and lysine, if the next residue is not proline. This action of trypsin will separate the cellular components from the structural proteins (Grauss et al., 2005; Voet D and Pratt, 2002). There have been studies using trypsin in the decellularization protocols for vascular and other tissues, such as heart valve, myocardium, or muscle (Grauss et al., 2005; Dahan et al., 2012; Luo et al., 2014). Treatments with trypsin alone or combined with various detergents like Triton X-100 were reported to be sufficient for cell removal (Dahan et al., 2012; Mancuso et al., 2014).

It is known that prolonged exposure of 15–24 h to trypsin at concentrations of 0.05–0.5% may also disrupt ECM structure, remove biomolecules, and change mechanical properties (Grauss et al., 2005; Dahan et al., 2012; Merna et al., 2013). Some studies investigated the structural change of the matrix in decellularized vascular or cardiac tissues using scattered-angle light spectroscope (SALS) or multi-photon microscope. The results indeed showed a change in fiber alignment, distribution, and motility (Merna et al., 2013; Williams et al., 2009).

It is important to notice that the alternations of the wall structure and mechanical properties of inappropriately decellularized vascular grafts will cause the weakness of the vascular wall and possibly result in early intimal hyperplasia, graft occlusion, failure, or aneurysmal dilatation while implanted in vivo (Borschel et al., 2005). Hence, stringent surveillance in the alteration of structure, composition, and mechanical properties in decellularization processes is necessary for optimization. In our previous study, we proposed a fiber-progressive-engagement model for evaluating non-linear pseudoelastic behavior of porcine arterial tissue (Lin et al., 2016). In this study, our aim is to systemically evaluate the impact of trypsin concentration and treatment duration on the composition, structure, and mechanical properties of porcine vascular tissue. The correlation between model parameters and composition is further studied. The results should provide useful information not only on the properties of vascular extracellular matrix but also on the development of an ideal protocol for porcine vascular tissue decellularization.

2. Material and methods

2.1. Porcine artery harvest and preparation

The porcine hearts were purchased from a local slaughterhouse. The specimens were kept on ice for transportation and temporary storage before proceeding. The main trunk of the right coronary artery was harvested with surgical instruments. Excessive adventitial and connective tissues were removed using sterile delicate instruments. The samples were washed with phosphate buffer solution (PBS) to remove blood clots. The dimensions including the outer diameter, wall thickness, length, and width were measured from the proximal, middle, and distal part of each artery segment by the Mitutoyo ruler (Mitutoyo Taiwan Co., ± 0.01 mm). The lengths and widths were measured from plane tissue. The wet weight/dry weight ratio of the native tissue for every harvested arteries was recorded. This was done by taking a small piece (> 20mg in wet weight) of the newly harvested blood vessel and measured both of its wet weight and dry weight. For dry weight measurement, the specimen was frozen in liquid nitrogen and lyophilized for more than 10 h. The vessels were placed in sterile PBS with penicillin (Sigma-Aldrich, St Louis, MO) and streptomycin (Sigma-Aldrich, St Louis, MO) and stored at 4 °C. Decellularization of blood vessels was started within 24 h from sacrifice.

2.2. Decellularization

For decellularization, the coronary arteries were washed with hypotonic Tris buffer solution (10 mM Tris/0.1 M NaCl) for 30 min and then changed to hypertonic solution (50 mM Tris/0.5 M NaCl) for another 30 min. This step was repeated twice after the initial wash for a total of three washes. The sample was agitated in the PBS solution 30 min for a total of three times. After that step, the segment was put in acetone (50%)/ethanol (50%) solution at 25 °C for 1 h and followed by a wash in PBS solution 30 min for a total of three times. The samples were then kept in trypsin solutions (in 2% EDTA/PBS solution) of two different concentrations for four different durations: 0.03% for 6, 12, 18 and 24 h, and 0.05% for 3, 12, 24 and 48 h at 37 °C. Samples were then washed in PBS solution for 30 min for a total of three times and stored at 4 °C until tissue sections were taken for histology, biochemical, or biomechanical tests. The mechanical tests were conducted within 24 h.

2.3. Histology

Ring segments measuring ~ 5 mm were taken from native coronary arteries and decellularized arterial tissue for histological analysis. Samples were embedded in paraffin wax in an automatic tissue processor (Tissue-Tek TEC 5, Sakura Finetek Japan). All samples were sectioned axially or circumferentially using a rotary microtome (Sakura Finetek Japan). Sections of 5 µm were cut and collected on glass slides and subsequently washed through a graded series of ethanol from 100% to 70% (v/v). Samples were stained with hematoxylin and eosin (H&E) and 2-(4-amidinophenyl)-1H -indole-6-carboxamidine (DAPI) stain.

2.4. Mechanical testing

Uniaxial tensile tests were performed in both the axial and

Download English Version:

https://daneshyari.com/en/article/7206904

Download Persian Version:

https://daneshyari.com/article/7206904

Daneshyari.com