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Inverted orientation improves decellularization of whole porcine hearts

Po-Feng Lee [a], Eric Chau [a], Rafael Cabello [a], Alvin T. Yeh [b], Luiz C. Sampaio [a], Andrea S. Gobin [a], Doris A. Taylor [a,*]

[a] Regenerative Medicine Research, Texas Heart Institute, Houston, TX 77030, USA
[b] Department of Biomedical Engineering, Texas A&M University, College Station, TX 77843, USA

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ABSTRACT

In structurally heterogeneous organs, such as heart, it is challenging to retain extracellular matrix integrity in the thinnest regions (eg, valves) during perfusion decellularization and completely remove cellular debris from thicker areas. The high inflow rates necessary to maintain physiologic pressure can distend or damage thin tissues, but lower pressures prolong the process and increase the likelihood of contamination. We examined two novel retrograde decellularization methods for porcine hearts: inverting the heart or venting the apex to decrease inflow rate. We measured flow dynamics through the aorta (Ao) and pulmonary artery (PA) at different Ao pressures and assessed the heart's appearance, turbidity of the outflow solutions, and coronary perfusion efficiency. We used rectangle image fitting of decellularized heart images to obtain a heart shape index. Using nonlinear optical microscopy, we determined the microstructure of collagen and elastin fibers of the aortic valve cusps. DNA, glycosaminoglycan, and residual detergent levels were compared. The inverted method was superior to the vented method, as shown by a higher coronary perfusion efficiency, more cell debris outflow, higher collagen and elastin content inside the aortic valve, lower DNA content, and better retention of the heart shape after decellularization. To our knowledge, this is the first study to use flow dynamics in a whole heart throughout the decellularization procedure to provide real-time information about the success of the process and the integrity of the vulnerable regions of the matrix. Heart orientation was important in optimizing decellularization efficiency and maintaining extracellular matrix integrity.

Statement of Significance

The use of decellularized tissue as a suitable scaffold for engineered tissue has emerged over the past decade as one of the most promising biofabrication platforms. The decellularization process removes all native cells, leaving the natural biopolymers, extracellular matrix materials and native architecture intact. This manuscript describes heart orientation as important in optimizing decellularization efficiency and maintaining extracellular matrix integrity. To our knowledge, this is the first study to assess flow dynamics in a whole heart throughout the decellularization procedure. Our findings compared to currently published methods demonstrate that continuous complex real-time measurements and analyses are required to produce an optimal scaffold for cardiac regeneration.

1. Introduction

For many patients with advanced heart failure, the only definitive treatment option is cardiac allo-transplantation, which is limited by the number of donor organs and is associated with life-long immunosuppression and potential organ rejection [1]. Creating a biofunctional heart by repopulating a decellularized cardiac scaffold with cells from a recipient could provide a novel treatment option that overcomes both hurdles. However, bioengineering a heart for transplant is fraught with multiple challenges, including developing an efficient decellularization method that protects the most delicate regions of the extracellular matrix (ECM) while fully removing cellular debris from denser areas. Equally important

* Corresponding author at: Regenerative Medicine Research, Texas Heart Institute, TMC MC 2-255, P.O. Box 20345, Houston, TX 77030, USA.
E-mail address: dtaylor@texasheart.org (D.A. Taylor).

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follow on challenges are: identifying appropriate cell sources, optimizing recellularization method(s), and creating adequate closed systems or bioreactors for aseptic, long-term, whole-organ culture.

Physical, chemical, and enzymatic techniques have been used in the cardiac decellularization process to remove cell components and leave the underlying ECM scaffold "intact" [2–5]. This is critical because the ECM provides the biophysical, biochemical, and molecular cues needed for cell survival, migration, proliferation, and differentiation [6–13]. Furthermore, ECM stiffness influences the differentiation and maturation of the cardiomyocytes and other mesodermal cells that give rise to cardiac structures [14–16]. Optimized decellularization methods should provide scaffolds with minimal cellular debris, but with maintaining ECM composition and structure intact to appropriately instruct cell behavior.

Complex whole-organ decellularization is achieved via antegrade or retrograde perfusion through an organ's intrinsic vasculature or other conduits [17]. The goal of this process is to remove the cellular components of the organ while preserving the natural 3D macro- and microstructure and vasculature of the ECM [2–4,18–22]. Maintaining the vasculature is crucial because complex organ integrity requires functional vessels [22]. Coronary perfusion decellularization was first shown to be effective primarily for rat whole-heart decellularization, although decellularization of a porcine heart was preliminarily evaluated [17]. Other groups later focused on the porcine model [39–44]. The porcine heart is an ideal candidate for creating a functional scaffold for human transplantation because it resembles the human heart in size and structure [23].

Decellularization of whole porcine hearts has been achieved by using pressure control [24–27] or perfusion flow rate control [28,29]. However, the effects of each of these methods on the integrity of the more delicate aspects of the scaffold are unclear. Generally, hearts decellularized in a conventional Langendorff upright perfusion have a high inflow rate during the decellularization process, which allows the left ventricle (LV) to fill, potentially causing deterioration of the aortic valve and decreasing its ability to seal properly. Aortic valve patency during decellularization is important to maintain coronary perfusion and to prevent retrograde filling of the LV and atria which in turn stresses the mechanical integrity of thin-walled atria. Monitoring the fluid dynamics of coronary perfusion throughout the cardiac decellularization process could enable investigators to evaluate aortic valve status and, ultimately, to optimize decellularization efficiency. However, this monitoring requires continuous complex real-time measurements and analyses.

Our laboratory uses a simple decellularization protocol in which only osmotic shock (high- and low-molarity ionic solution perfusion) is used to rupture cells; then, an ionic detergent (1% SDS) is used to solubilize the cell lipid membrane; finally, the cell debris is removed by simple perfusion. In the current study, we examined two unique heart preparations to relieve pressure on the aortic valve while maintaining intact coronary flow: 1) a vented preparation – in which a small hole in the apex of heart is made to maintain a pressure differential across the aortic valve and 2) an inverted preparation – in which the heart is inverted at −45° to reduce LV filling with perfusate during the decellularization process. We compared these approaches with the traditional "upright" Langendorff perfusion decellularization method. Throughout decellularization, we monitored the flow across the coronary bed and calculated perfusion efficiency and visualized cell debris removal. After decellularization, we evaluated the retention of heart shape, assessed aortic valve deterioration, and quantified DNA, glycosaminoglycan (GAG), and detergent levels in the constructs via biochemical analyses of the ECM.

2. Materials and methods

2.1. Materials and reagents

For heart decellularization, connectors were purchased from NovoSci (Conroe, TX). The 1% SDS solution was prepared from 20% SDS (Affymetrix, Santa Clara, CA). Hypertonic and hypotonic solutions were made from sodium chloride (Affymetrix). The phosphate-buffered saline (PBS) was prepared with sodium chloride (Affymetrix), sodium phosphate (Sigma-Aldrich, St. Louis, MO), potassium chloride (Sigma-Aldrich), and potassium phosphate (Sigma-Aldrich).

2.2. Preparation of porcine hearts for decellularization via the upright, vented, and inverted methods

All tissue was obtained through an approved IACUC tissue sharing resource between the University of Texas Health Science Center at Houston (UTHSC) and the Texas Heart Institute. Porcine hearts were isolated from freshly heparinized and euthanized pigs (25–50 kg) at UTHSC. The hearts (180–260 g) were removed aseptically via thoracotomy and then perfused with a heparinized 0.9% saline solution to clear residual blood within the heart. Cadaveric non-decellularized hearts after heparinization were used as controls. Table 1 shows the preparation details for the upright, vented, and inverted hearts (n = 6 each).

Briefly, upright hearts were prepared for decellularization by using the Langendorff method. Connectors were placed into the descending aorta (DA) for perfusion, the brachiocephalic artery (BA) for support, and the pulmonary artery (PA) for outflow monitoring.

In vented hearts, connectors were placed into the BA for perfusion and support and into the PA for outflow monitoring, the DA was ligated. A 7-mm diameter hole was created in the LV apex and a sterile polypropylene cannula was placed within the vent to keep it open.

In the inverted hearts connectors were placed into the DA for perfusion and into the PA for outflow monitoring. The inverted hearts were affixed to a polystyrene block and angled at −45°; a polyester-nylon blend mesh (1.5 cm wide by 3.7 mm thick) was tied lightly to support the heart around the base to prevent LV filling. The −45° angle enabled the hearts to maintain aortic trunk pressure with the lowest inflow rate and the highest coronary perfusion efficiency as compared with angles of 90°, 45° and 0° (Appendix Fig. 1).

All hearts were placed within a bioreactor (Harvard Apparatus Regenerative Technologies, Holliston, MA), which is a cylindrical shaped polycarbonate container, with three ports on the lid for heart support, fluid infusion and fluid draining (from PA); and one port on the bottom for fluid draining (from non-PA areas).

2.3. Decellularization protocol

All hearts (except cadaveric controls) underwent the same decellularization procedure: a 4-h hypertonic (500 mM NaCl) perfusion, 2-h hypotonic (20 mM NaCl) perfusion, 60-h 1% SDS perfusion, and a final 40-liter 1X PBS wash. The total amount of 1% SDS perfused was 1 L per gram of heart weight, and the final 60 L was recirculated until the end of the 60-h SDS perfusion period. A constant perfusion pressure of 60 mmHg was maintained at the aortic root throughout the decellularization process by using a PID (Potential-Integral-Derivative) controller (Harvard Apparatus, Cambridge, MA), which controlled the perfusion flow rate through a peristaltic pump via instantaneous pressure monitoring with a pressure transducer.