

RESEARCH ARTICLE

A Standardized Dissection Protocol to Generate Aortic Valvular Scaffolds from Porcine Hearts

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Objective: To describe a particular harvesting procedure for isolating intact porcine aortic heart valve roots as potential sources for biologic scaffolds. **Methods:** Fresh porcine hearts were brought to the Tissue Engineering and Regenerative Medicine Laboratory at the University of Medicine and Pharmacy in Targu Mures. The aortic roots were extracted from the porcine hearts by anatomical dissection. For this purpose, we used a basic surgical instrument kit. This initial phase was the first step in obtaining acellular extracellular matrix as a biologic scaffold material. **Results:** Aortic roots were isolated with preservation of the ascending aorta as well as the intact aortic sinus and coronaries together with the adjacent myocardial tissue and anterior leaflet of the mitral valve. This approach allowed for safe mounting of roots into mounting rings for perfusion decellularization. **Conclusions:** The described procedure is a feasible protocol for obtaining intact biological valvular scaffolds from porcine hearts. Reduced requirements regarding tools and personnel underline the easiness of aortic root harvesting using this particular procedure.

Keywords: scaffold, valve, extraction, decellularization

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Introduction

Aortic valve pathology is an area of interest due to the severity of symptoms, the impact on quality of life and it is usually fatal if neglected [1]. Valvular substitutes, represented by mechanical or biological valves proved their importance by increasing life expectancy and providing patients a better quality of life [2]. Although history reveals a constant improvement of mechanical valves design and sourcing and reduced immunogenicity of the biological ones, an entire panel of risks remains associated with each of them. Mechanical valves require lifelong anticoagulation therapy, which associates specific therapy risks, i.e. patient compliance and under and over dosage of anticoagulant drugs [3]. Biologic replacements are known to last only 10 to 15 years until degeneration and calcification occur [4]. These shortcomings highlight the need to develop a brand new valvular substitute that comes closer to a healthy heart valve, appropriately adapted to the physiological parameters and requirements.

Regenerative medicine, a new medical research field, aims to produce the ideal valve prosthesis, a living and functional heart valve using scaffolds, stem cells and bioengineering [5,6]. Scaffolds are biocompatible and biodegradable tridimensional structures that provide mechanical support to stem cells and adequate stimuli for appropriate cell function [7,8]. Numerous papers report on decellularization of aortic valves, but rarely give account on details regarding optimal dissection protocols to create intact roots for tissue engineering [9-12].

In this paper, we propose and describe a standard method for the dissection of intact porcine aortic root used for further processing as biologic scaffolds.

Methods

Procurement of porcine hearts

This work is part of a grant which has the approval of the Ethics Committee of the University of Medicine and Pharmacy Targu Mures.

Fresh porcine hearts provided by a local abattoir immediately after harvesting were brought to the Tissue Engineering and Regenerative Medicine Laboratory, University of Medicine and Pharmacy Targu Mures.

Preparation for aortic root harvesting

As a first step, all hearts underwent screening and selection. Only those with undamaged ascending aorta and aortic arch were kept. At the beginning of the procedure, the entire valvular complex was carefully analyzed to identify potential congenital malformations of the valve and degenerative or mechanical lesions. Then, the porcine hearts were placed in ice water and the aortic valve roots were harvested using basic surgical instrument: two curved blunt dissecting scissors, two anatomical blunt forceps, one of each being a delicate one, suited for fragile tissues, one regular No. 10 scalpel blade.

Identification of main anatomical components

A "Y" incision was performed in the pericardium by using a pair of scissors, revealing the heart. The aortic root,

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continuing the left ventricle outflow tract, provides the supporting structure for the leaflets of the aortic valve, and forms the bridge between the left ventricle and the ascending aorta. Therefore, in order to isolate an intact aortic root, it's necessary to identify the left ventricle and the aorta. Then, the proximal segment of the aorta is isolated from the pulmonary trunk using a forceps and a dissection scissor (Figure 1a), ending with a transversal section of the pulmonary artery. Then a transversal cut is applied to remove all tissues above the aortic arch. Caution should be taken at the base of the root to avoid the coronary arteries, as the right emerges from the right sinus of Valsalva, surrounds the right heart and ends frequently as the posterior interventricular artery. The left coronary, emerging from the left sinus of Valsalva, passes an area between the pulmonary artery trunk and the left atrium; it then splits into two branches: left anterior descending artery and left circumflex artery.

The dissection that follows their identification is performed using delicate scissors and forceps. The procedure is completed with a transversal section of the coronary vessels 1 cm distally from their ostia and ligation at the origin (Figure 1b).

Aortic root removal and myocardial processing

The height of the root extends externally from the sinotubular junction to the ventricular-arterial junction and internally from the sinotubular junction to the basal ring defined by the plane passing through the cusps base [13]. To make sure that every root component is maintained intact after the decellularization fixation method, the dissection should be extend beyond these limits. The aorta was further dissected just below the origin of the brachiocephalic trunk. One third of the aortic root base is formed by the anterior mitral leaflet and the other two thirds of the ventricular endocardium and myocardium, 3 cm below the basal ring. Depending on the root mounting type, the thinning of the endocardial tissue might be necessary.

The under- leaflet component is formed by the myocardial muscle, belonging to the muscular part of the interventricular septum, anterior and posterior sides of the left ventricle and anterior mitral leaflet. Myocardial tissue is gently trimmed, avoiding fenestration, with a No. 10

scalpel blade, so that the thickness of the myocardium will be the same as for the mitral cusp. This step is essential in order to balance the forces applied during mounting.

Discussions

The porcine aortic valves are prepared in this manner in order to be placed in a perfusion decellularization system. This preparation protocol represents the first step of the procedure in order to obtain completely decellularized porcine derived aortic valve scaffolds using a transvalvular pressure gradient. The technique offers a large variety of options which can be adapted for any protocol that follows the porcine heart valve isolation, needing only a basic surgical instrument kit, the No. 10 scalpel blade could be replaced with any other model or size. This method requires myocardium thinning which should be performed with great care because the excessive thinning of the myocardium can lead to valve integrity damage (Figure 1c), during the decellularization process. This concept was designed as a solution to earlier report on decellularization failure [14]. Variations of the presented protocol could be applied regarding the ligation of the coronary arteries. It could be replaced with a surgical suture with the downside of damaging the coronary ostia, restricting their use in surgical procedures that require coronary arteries reimplantation, such as the Bentall procedure [15] and varied modified procedures[16-18]. The literature presents various methods and protocols used in order to obtain decellularized aortic valves, a search on PubMed by the key words "aortic" "decellularization" returned 302 results, but minimal and limited information are found in these papers regarding the procedure of aortic valve extraction. To prove this statement, a search using the terms "scaffold", "porcine", "aortic", "decellularization", "surgical", "extraction", returned only one result.

Conclusions

A standardized protocol for aortic root harvesting allows reproducible generation of aortic roots for tissue engineering, also offering high availability, low costs and easy procurement procedures. It also fulfills the need of having a rigorous step by step described protocol. The simplicity and advantage of this particular surgical technique



Fig. 1. General aortic root aspects: a. Anatomic position of great vessels; b. Ligated coronary arteries; c. Myocardial rupture due to over thinning- three days before finishing the protocol

are also underlined by the fact that there is a minimal requirement of tools and does not require highly trained personnel.

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Conflict of interest

The authors have declared that there is no conflict of interest.

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