Journal of Clinical & Experimental
Cardiology
ISSN: 2155-9880

Sajith, J Clin Exp Cardiolog 2017, 8:5
DOI: 10.4172/2155-9880.1000523

Research Article Open Access

Comparative Study of Two Decellularization Protocols on a Biomaterial for Tissue Engineering

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Received date: April 21, 2017; **Accepted date:** May 22, 2017; **Published date:** May 25, 2017

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Abstract

Cardiovascular disease is a major health risk since past decade. Surgical treatment for major heart diseases has been one of the major challenges since years. Cardiac valves are synthetic or bio prosthetic ones. Mechanical valves are long durable but highly thrombogenic and undergo calcification at higher rates demanding permanent anticoagulation, which increases the risk of bleeding. Biomaterial scaffolds are used in surgical replacements but only after decellularization and processing. Decellularization makes tissue less antigenic; reduce inflammatory response and less tissue degeneration. In this work two decellularization protocols are tried on bovine pericardium (BP) to find out the effect of each of them on the scaffold's integrity. BP undergone protocol 1(0.25% Trypsin-EDTA, TritonX-100, Deoxycholic acid, Peracetic acid/Ethanol) treatment was seen to have highly distorted and damaged collagen matrix. Decellularization with protocol 2 (Deoxycholic acid, DNase, RNase, Ethanol) resulted in a completely decellularised bovine pericardium. The extracellular matrix was intact as native one with collagen bundles. This will help cell attachment to the decellularised matrix.

Keywords: Decellularization; Bovine pericardium; Bioprosthetic; Trypsin-EDTA; Triton X; DNase; RNase

Introduction

Cardiovascular disease is a major health risk since past decade. CVDs are responsible for 17.3 million deaths per year [1]. Surgical treatment for major heart diseases has been one of the major challenges since years. Native valve replacement by prosthesis has been the most important advance in the treatment of patients with this disease, even though this is not without complications [2]. Cardiac valves are synthetic or bio prosthetic ones. Mechanical prostheses offer satisfactory hemodynamic function and excellent durability over the long term, but as they are thrombogenic, they require permanent anticoagulation, which increases the risk of bleeding. Mechanical valves are long durable but highly thrombogenic and undergo calcification at higher rates. This led to the need for bio prosthetic valves which are largely biocompatible. Biomaterial scaffolds are used in surgical replacements only after decellularization and processing.

Decellularization makes tissue less antigenic, reduce inflammatory response and less tissue degeneration. The ultimate goal of organ decellularization is the removal of all cellular material without adversely affecting the composition, biologic activity, or mechanical integrity of the remaining three-dimensional matrix. Commonly used methods of decellularization include the perfusion of chemical or enzymatic agents and physical methods such as sonication, freezing, and thawing, with agitation to disrupt cell membranes, release cell contents, and facilitate the rinsing and removal of cell remnants from the ECM [3]. But it is still not possible to truly predict the biocompatibility of one material over another due to the immunological rejection. Bovine pericardium has come to common clinical use in past few decades. It has many surgical advantages too. Bovine pericardium has reliable consistency, able to be manufactured and processed to a consistent nominal 0.5 mm thickness providing dependable suture retention and ideal operative handling characters [4]. In this work several decellularization protocols are tried on bovine pericardium to find out the effect of each of them on the scaffold's integrity.

Materials and Methods

Bovine pericardium of a 36 months old animal was obtained from nearby abattoir. This was packed in plastic container and transported to laboratory immediately. Pericardium was cleaned thoroughly removing all fat and other adherences. It was then washed in NaCl and finally in PBS. The experiment was conducted in two sets with two different decellularisation protocols.

Protocol 1

Materials required

- PBS (pH 7.4) with 10% Ab
- 0.25% Trypsin-EDTA
- TritonX-100
- Deoxycholic acid
- Peracetic acid/Ethanol

The bovine pericardium, collected, was washed thoroughly in water and sterile PBS with Ab. Tissue was washed in sterile PBS (pH 7.4) before treatment. Washed tissue was treated with 0.25% Trypsin-EDTA solution at room temperature for 2 h on a shaker, Washed in sterile PBS on a shaker for 1 h, placed in 3% Triton X-100 at room temperature for 2 h on a shaker, 4% Deoxycholic acid (DCA) for 2 h at room temperature, given wash in water, further decellularisation and disinfection by treating with 0.1% peracetic acid or 4% ethanol and Stored in 70% ethanol.

Protocol 2

Materials required

- Deoxycholic acid (DCA)
- Sterile PBS
- DNase, RNase
- Ethanol

Pericardial tissue was washed thoroughly in water and PBS with 10% Antibiotics. Tissue was treated with : 1% DCA (in sterile PBS) for 24 h in shaker at 37°C, Washed in PBS like 3 changes in 15 minutes each, 100 µg/ml RNase and 150 IU/ml DNase for 12 h in shaker at 37°C, Washed in PBS (3 changes) and Stored in 70% ethanol.

Results

Histological evaluation of decellularised pericardium using Haematoxylin and eosin staining showed no cell remnants, indicating both the protocols give a completely decellularised xenograft. Bovine pericardium that underwent protocol 1 treatment was seen to have highly distorted and damaged collagen matrix. Decellularization with protocol 2 resulted in a completely decellularised bovine pericardium. The extracellular matrix was intact as native one with collagen bundles. This will enhance the cell attachment to the decellularised matrix (Figures 1 and 2).

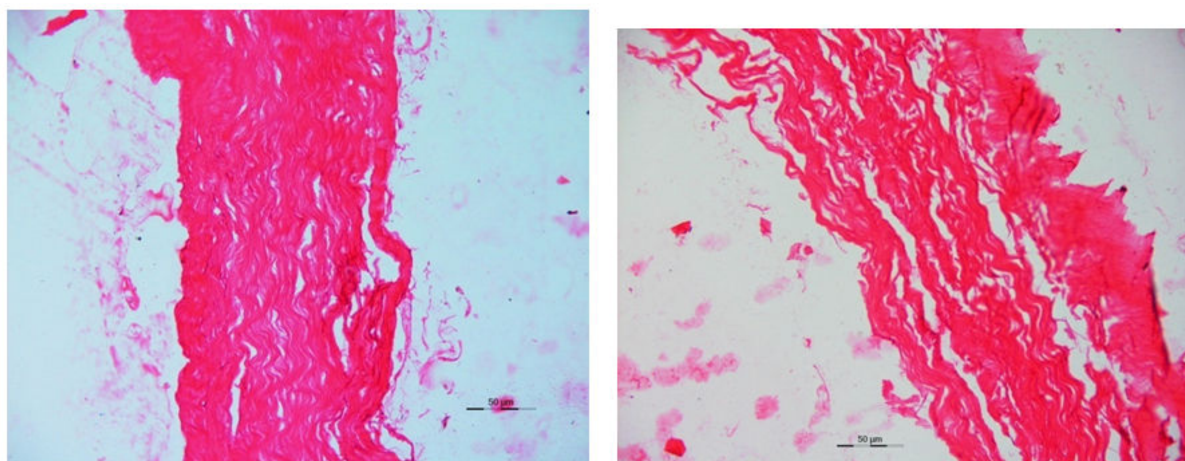

Figure 1: H&E stained bovine pericardium decellularised.

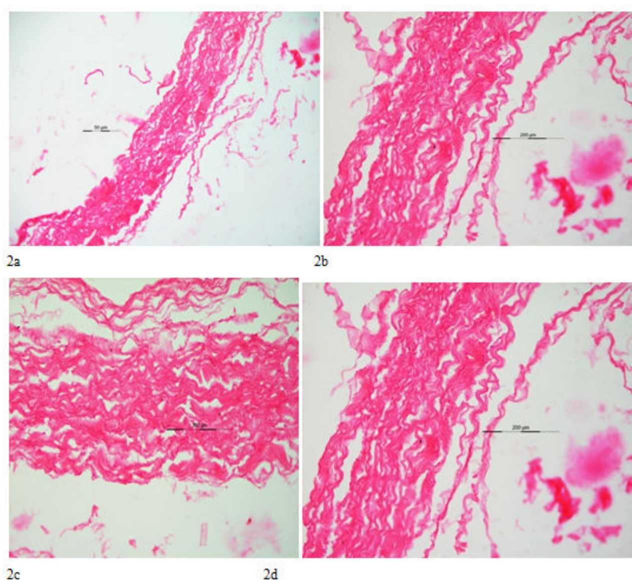

Figure 2: H&E stained bovine pericardium decellularised under protocol.

Discussion

Currently, the optimal treatment for valvular heart diseases is surgical repair and replacement [4]. Bioprosthetic scaffolds or valves are mostly used. They are supported by a stent or a Dacron support. Bovine pericardium is the widely used xenogenic scaffold in tissue engineering studies because it provide ground for cell-cell interactions, retain mechanical properties even after decellularization and can be modelled to desired ways. An engineered graft must not only remain biocompatible, but it must also exhibit mechanical properties similar to the native vasculature which it will replace [5].

The native DNA and nuclei present can elicit serious inflammatory reactions, thus causing ejection of the implant. Decellularization of the implants removes all cell remnants of the native tissue, which can otherwise elicit immune response and damage the tissue and reject the same. So, choosing an appropriate decellularization technique is inevitable. In this study we have compared the effect of different chemical decellularization protocols on ECM. The methods employed were TritonX-100, Trypsin EDTA and enzymatic method. The xenograft that underwent the first protocol showed complete decellularization but with a much distorted ECM. Valves treated with Triton X-100 showed a completely cell-free structure across the complete thickness of the valve leaflet, which is consistent with earlier results by Bader et al. [6] Triton X-100 disrupts lipid-lipid and lipid-protein interactions while leaving protein-protein interactions intact.

Trypsin treatment has previously been reported to be a successful method for decellularization of ovine and human heart valves. Trypsin is a highly specific enzyme that cleaves the peptide bonds on the carbon side of Arg and Lys if the next residue is not Proline [7]. The maximum enzymatic activity of trypsin occurs at a maximum temperature of 37c and a pH of 8. Prololnged exposure of the tissue to trypsin can cause disruption of ECM, removes laminin, Fibronectin, elastin and GAGs. So, in the first protocol tissue was treated with trypsin only for a short time.

Nucleases such as Endonucleases catalyze the hydrolysis of the interior bonds of ribonucleotides or deoxyribonucleotide chains [8]. Deoxycholic acid not only decellularises the tissue but also acts as a mucolytic antibacterial agent. The RNase and DNase enzymes are able to digest the nucleic acids in the graft [9]. This method is seen to create negligible damage to collagen framework of the tissue. Also, destroys the Gal-epitopes. Storage of the processed tissue in alcohol gives anticalcifying and antimicrobial effects.

After decellularization, changes in the ECM were examined by H&E staining for both protocols. We could observe the arrangement of collagen matrix by routine staining. This is the first criteria to find the better technique. Considerable amounts of collagen fibres were lost when the scaffold was treated with Trypsin-TritonX and DCA for short time. Trypsin-Triton X cell extraction resulted in a decrease of collagen density with widening of interfibrillar spaces, which is consistent with earlier finding by Badylak.

Conclusion

Thus, we are able to conclude from the results that decellularising the bovine pericardium using DCA, DNase and RNase give a completely decellularised and stable bioprosthetic scaffold that can be used for tissue engineering, whereas the technique employing Trypsin EDTA-Triton X rendered a highly damaged biomaterial.

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