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Enhanced vascular regeneration with chemically/physically treated bovine/human pericardium in rodent

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ABSTRACT

Background: Glutaraldehyde-treated pericardia for cardiovascular applications have poor long-term clinical results. The efficacy of a combined physical/chemical treatment to improve pericardium biocompatibility and vascular regeneration was assessed and compared with detergent treatment and two commercial bovine pericardia: PeriGuard (DGBP) and Edwards pericardium (nDGBP). The physical and chemical process was applied to bovine and human pericardia (DBP-DHP), and the detergent process was applied to bovine (DDBP).

Material and methods: Native (NBP) and treated bovine tissues were assessed for decellularization (HE/DAPI/DNA/α-Gal and MHC-1 staining) and mechanical integrity *ex vivo*. Twenty Wistar rats received subcutaneous patches of each bovine tissue to assess immunogenic response up to 4 months (flow cytometry). Ten additional rats received four subcutaneous bovine-treated patches (one/condition) to evaluate the inflammatory reaction (CD3/CD68 immunostaining), calcification (von Kossa staining/calcium quantification), and integration assessment (Hematoxylin and eosin staining). Finally, 15 rodents received a patch on the aorta (DBP $n = 5$, DHP $n = 5$, and DGBP $n = 5$), and vascular biocompatibility and arterial wall regeneration were assessed after 4 months (CD3/CD68/CD31/ASMA and Miller staining).

Results: DBP reached the higher level of decellularization, no immunogenic response whereas maintaining mechanical properties. DBP induced the lowest level grade of inflammation after 2 months ($P < 0.05$) concomitantly for better remodeling. No complications occurred with DBP and DHP where vascular regeneration was confirmed. Moreover, they induced a low level of CD3/CD68 infiltrations.

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Conclusions: This process significantly reduces immunogenicity and improves biocompatibility of bovine and human pericardia for better vascular regeneration.

Introduction

Bovine and human pericardia (BP-HP) are largely used in cardiovascular surgery to repair valves or congenital heart defects and for a large spectrum of other reconstructive procedures.

The standard treatment of pericardium for clinical use is glutaraldehyde fixation (G)[1] to produce cross-links in the cellular and extracellular matrix (ECM) improving mechanical resistance and to reduce graft immunogenicity, especially for xenogeneic tissue. Aldehyde treatment is, however, also cytotoxic and does not completely suppress the immunological reaction against xenogeneic graft.[2] In fact, the G-treatment leaves dead cells/cell debris in the tissue and does not completely remove phospholipids, thereby leading to chronic inflammation and calcification of grafted tissues.[3] The consequences are subsequent function and structural deterioration of prostheses.[4] This deterioration occurs earlier in younger patients because of their strong immunity and high phosphocalcic metabolism.[5]

Decellularization was therefore proposed to improve long-term efficacy of these prostheses. It enhances *in situ* graft biocompatibility and improves recellularization toward host-tissue regeneration with similar native properties. The tissue decellularization process must remove cells/antigens and preserve extracellular matrix and mechanical properties. Different decellularization treatments have been used, and the most popular are those using detergents and/or enzymes.[6] New products (ECM) based on those protocols are already commercialized for cardiovascular applications. However, short-term results with some of those implants in clinical practice did not demonstrate convincing results in the pediatric population.[7] Better results regarding the absence of calcification and/or aneurysm formation were achieved at a short follow-up with some others for pediatric cardiac reconstructions.[8,9]

We recently studied a tissue process based on non-detergent methods on different xeno/allogeneic tissues and, in particular, on human pericardium that can be banked and proposed as a decellularized homograft.[10,11] Our *in vitro* and *in vivo* results in a rodent vascular model showed that this decellularization method offers excellent short-term results in terms of biocompatibility. Moreover, our processed implants and more precisely the human pericardium demonstrated significantly better results in terms of biocompatibility when compared to a conventional glutaraldehyde-fixed pericardium.[11]

Since the availability of human pericardium is limited, the source of xenogeneic tissue must be considered as an alternative although it carries noncellular xenogeneic antigens responsible for higher immunogenic host response, and it is difficult to remove without ECM damage.[12,13]

Therefore, the present study compared the *in vitro* and *in vivo* (subcutaneous), the efficacy of a physical, chemical nondetergent/enzymatic and glutaraldehyde-free process to decellularize and improve the biocompatibility of bovine pericardium (DBP) with (1) a conventional detergent/glutaraldehyde-free process (DDBP) as well as with two commercially treated available bovine pericardia currently used in clinical setup, (2) the supple PeriGuard bovine pericardium (DGBP) similarly treated as DBP, and (3) the Edwards Lifesciences bovine pericardium (nDGBP), which is not decellularized and is considered a standard in cardiovascular surgery.

In vitro studies compared these tissues with native tissue regarding cellularity (HE-DAPI), DNA content, antigenicity (MHC-class I and Gal1,3-Galβ1,4GlcNAc-R [α-Gal] antigens immunostaining), and biomechanical properties (elongation stress test).

In vivo, the rodent systemic immune response, local inflammatory reaction, remodeling of the implant, and calcification occurrences were assessed after subcutaneous implantation and compared between each bovine pericardium tissue conditions.

In a supplementary *in vivo* model, a vascular patch was achieved on rat aorta: both DBP and DHP treated by combined physical/chemical decellularization process were compared with the PeriGuard (DGBP) regarding vascular biocompatibility and vascular regeneration after 4 months of follow-up.

Material and methods

Sources of matrices

Bovine pericardium (BP) was procured from a local slaughterhouse (Eurovlees, Zele, Belgium). Human pericardium (HP) was procured according to the common standards of the European Association of Musculoskeletal Transplantation (EAMST, Vienna, 1997). Human pericardium was harvested in agreement with the Ethic Committee of the Université catholique de Louvain.

The tissues were rinsed with sterile ringer solution, mechanically stripped of loose adipose tissue and frozen at −80°C.

The two commercialized pericardia purchased were the following: Edwards Lifesciences bovine pericardium (Edwards Lifesciences Corporation, Irvine, CA), nondecellularized and preserved in glutaraldehyde (nDGBP) and Supple PeriGuard bovine pericardium (Baxter International, Inc, Deerfield, IL), treated with NaOH 1N before glutaraldehyde preservation (DGBP).

Matrix preparation

Before processing, tissues were thawed and washed in sterile ringer solution.

The pericardium was cut into pieces of 15 cm by 10 cm. Two treatment protocols were conducted. One process was