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Thrombogenicity studies of three different variants of processed bovine pericardium

Études de thrombogénécité de trois différents variants des péricardes de bovins traités

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Abstract

Versatile use of bovine pericardium in clinical cardiovascular surgery requires processing, especially cross-linking, to make the tissue non antigenic and mechanically strong. Forty-nine bovine pericardia were made acellular and then cross-linked by two different methods, group A (20) with formalin and group B (29) with heparin and subsequently, as a final measure, five of group B pericardia were gamma-ray sterilized with stipulated dose of 25 kGy and classified as group C. The surface property of thrombogenesis and haemolysis were compared in these three groups to identify a suitable method of processing so that it could be used in the vascular system without any thromboembolic complication. Extensive microscopical examination, mechanical testing and other physical property for biocompatibility studies were conducted on these three different groups with key focus on in vitro thrombogenicity studies. Heparin treated group B bovine pericardium appeared to be the best method of processing among these three by the above studies and was confirmed by laser confocal microscopy. Heparin cross-linked and heparin sodium treated processing had gained the higher tensile strength, and appeared to be nonthrombogenic, noncalcifiable (by animal experiments), biocompatible biomaterial, which can be used clinically.

Résumé

L'utilisation du péricarde bovin en chirurgie cardiovasculaire nécessite en particulier, un traitement par réticulation, afin de rendre le tissu non antigénique et de le renforcer mécaniquement. Quarante péricardes bovins ont été rendus acellulaires et ont été réticulés par deux méthodes différentes : le groupe A avec du formol (20), et le group B (29) avec de l'héparine. Cinq péricardes du groupe B ont été stérilisés par rayonnement gamma (25 kGy) et classés dans le groupe C. Les propriétés de la surface des péricardes traités vis-à-vis de la thrombogénèse et de l'hémolyse ont été comparées pour les trois groupes afin d'identifier la méthode de traitement appropriée en vue d'une utilisation clinique pour le système vasculaire sans risque de complications thromboemboliques.

Keywords: Bovine pericardium; Confocal microscopy; Decellurization; Thrombogenic; Transmission electron microscopyPéricarde bovin; Microscopie confocale; Acellurisation; Thromboembolie; Microscopie électronique

1. Introduction

Bovine pericardium (BP) processing towards a clinical biomaterial is a common practice. Bovine pericardium tissue patches have been used for pericardial sac closure after open-

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heart surgery [2], vascular graft repair [1,18] and in making a heart valve [12,24].

It basically comprises of collagen type I, which has got low antigenicity, but high thrombogenicity [14]. It is prepared acellular and cross-linked by chemical or physical methods to reduce antigenicity [8], thereby preventing calcification this enhances the mechanical strength and minimizes xenogenic tissue solubility [3,17]. Standard practice of glutaraldehyde cross-linking carries a high-risk of tissue fatigue and calcific degeneration [7], partly due to cytotoxicity and inflammatory changes [11], and fragmentation of collagen because of continuous wear and tear. To address the issue of calcification, Lee et al. developed a novel methodology for the chemical modification of biological tissues by directly coupling heparin to bovine pericardium (BP). Their heparinization involved pretreatment of BP using glutaraldehyde (GA) and was followed by grafting heparin to BP by the reaction of residual aldehyde with amine group of heparin. They evaluated the effect of heparin coupling on calcification by in vitro as well as in vivo studies. Their results revealed that heparinized BP exhibit greater resistance to collagenase digestion than control tissue [25].

The aim of this study was to find a better processing technique with effective cross-linking to produce a durable biomaterial, which remains flexible, biocompatible and nonthrombogenic. While analyzing the various methods for processing of the biomaterial, our focus was on thrombogenic studies. In this study, we made the tissue acellular, followed by cross-linking with heparin and subsequent treatment via anticalcification and then analysed for major thrombogenic parameters.

2. Materials and methods

Bovine pericardium was harvested and procured from an inspected abattoir in sterile environment. The specimens were stored in Hanks balanced salt solution (HBSS) having an antibiotic cocktail of cifran, gentamycin, streptomycin, cephalosporin and amphotericin B, and later brought into a current Good Manufacturing Practice (cGMP) laboratory for further processing.

Forty-nine bovine pericardial pieces were taken for study, where 20 numbers identified as group A processing were treated with formaldehyde as a cross-linking agent after decellularisation, and subsequently they were preserved in 70% ethanol. Rest 29 BP were treated differently and cross-linked with heparin sodium in buffer solution with a pH of 6.5, at a temperature of 20 °C, followed by formaldehyde treatment in stages for antimicrobial activity, and subsequently they were treated with glutamic acid in buffer solution followed by heparin sodium again in buffer solution at a Ph of 6.5 for long duration. Finally, they all went for preservation in ethanol. This twenty-nine BP were identified as group B processed BP [10].

Five of the group B processed pericardium was subjected to gamma irradiation of 25 kGy, according to the international atomic energy commission stipulation. This later group had been identified as group B + gamma irradiated ones (group C). Twenty-four remained in group B. Microscopical evaluation of the tissues before and after processing were done to assess efficiency of decellularisation without disturbing the collagen architecture, apart from that, a comparative data could be acquired between, group A, B, C processing. Other than ordinary light microscopy after haematoxylin and eosin staining (H&E), and von Geison staining (VG), confocal laser scanning microscopy (CM), high-resolution optical microscopy, transmission electron microscopy (TEM), and environmental scanning electron microscopy (ESEM) studies for evaluation of the effective procedure were conducted. Simultaneously, mechanical testing of those biomaterials was performed with other biological tissue biocompatibility studies.

2.1. Tensile test details

Instron Corporation, series IX automated materials testing system. Tensile test, crosshead speed 10 mm/min. Dum-bell shaped samples were cut from the tissue using ISO 527-2:1993(E) specimen type 5B die and immersed in water until the time of test. Interface type – TT. Sample rate (pts/s): 5.0000, full-scale load range: 0.1000 kN; humidity %: 60; temperature: 23 °C. Thrombogenicity studies as a comparative investigation among the procedures were highlighted in this experimental investigation.

2.2. Thrombogenic studies

Reference ISO 10993-4:2002 (E). (Selection of tests for interaction of materials with blood).

2.2.1. Exposure of materials with blood

Blood from human volunteer was collected into the anticoagulant, sodium citrate. The test materials were placed in polystyrene culture plates and agitated with phosphate buffered saline before they were exposed to blood. To each plate, 7.0 ml blood was added and a 1.0 ml sample was collected immediately for analysis and the remaining 6.0 ml was exposed to the materials for 30 min under agitation at 75 ± 5 rpm, using an Environ shaker thermo stated at 35 ± 2 °C. Two empty polystyrene culture dishes were exposed with blood as reference.

2.2.2. Consumption of platelets and leukocytes (WBC) by cell counts

The count reduction was analyzed by detecting the counts in initial and 30 min samples, using Haematology Analyzer Cobas minos vet (Roche, France). The equipment calibration was verified using traceable standard reference Control.

2.2.3. Data

Counts detected initially and on termination of exposure are given in the table format. Total consumption from the exposed blood as the percentage reduction is calculated for each sample and is given in Table 2.

2.3. Plasma coagulation (fibrinogen and partial thromboplastin time assay)

The blood samples (initial and 30 min) were centrifuged at 4000 rpm for 15 min and platelet poor plasma (PPP) was aspi-