Reduction of Antigenicity and Risk of Infection in Regenerative Tissue Transplantation by Cold Isostatic Pressing

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Abstract
Tissue engineered heart valves based on acellular tissue have been studied to have more durability and bio-functionality with growth potential and less immunogenicity. Whereas they have still several problems to be solved such as complete cell removal and transfer of unknown animal related infectious diseases. In this paper, our novel tissue processing for decellularization using ultrahigh pressure for the safe tissue transplantation was reported. Porcine cardiac tissues were isolated and treated by a cold isostatic pressing for a disruption of donor cells. The cell debris was then washed out by washing solution at 4°C. The tissues treated were completely cell free when they were applied to 980 MPa for 10 min. There was no porcine endogeneous retrovirus detected. There were no significant changes in biomechanical properties of the breaking strength and elastic modulus. The acellular grafts of pulmonary valve were transplanted to allogeneic miniature pigs. The explanted grafts showed remarkable cell infiltration and endothelialization. This processing may provide more durable and safe scaffold for the regenerative tissue transplantation.

Keywords: tissue engineering, tissue transplantation, acellular, scaffold

1. Introduction

The implantable cardiovascular medical devices have been clinically used for more than 30 years as substitution for the patient's deficient tissues. The artificial heart valve is one of the most clinically used medical devices applied to about 300,000 patients per year worldwide. There are two kinds of artificial heart valves currently used. A xenograft heart valve is made of the chemically crosslinked porcine valve or bovine pericardium to reduce antigenicity of the xenogeneic tissue. A mechanical heart valve is made of pyrolytic carbon or titanium. The former has good biocompatibility, hemodynamics, and resistant to infections compared with the latter. However, the durability of the xenograft valve is relatively short especially in pediatric patients for about 5-10 years by the calcification of the glutaraldehyde-fixed animal tissue. Recent establishment of the human tissue bank has made it easy to use allogeneic tissues for the transplantation that are superior to the current artificial devices. However, since they are donated from the cadavers, the supply is very limited and some donated tissues may not be applicable due to infection. In addition to the above issues, all the devices and tissues lack the growth potential and they may be replaced repeatedly through the patients' growth process.

All of the current medical devices remain as foreign bodies even after the implantation. If a device accepts host cell impregnation and is replaced by the host tissue after the implantation,
it may acquire perfect biocompatibility and growth ability. An ideal candidate for such a regenerative scaffold is a decellularized allogeneic or xenogeneic tissue since it does not require tissue fixation for removal of antigenicity. Detergents and/or enzymes such as Triton® X-100, sodium dodecyl sulphate, deoxycholate, trypsin, DNase, and RNase have been commonly used for the cell removal media from the tissue [1-4]. However, the decellularization depends on their permeation in the tissue and may not be achieved completely in large or hard tissues. And furthermore, since the detergents are generally cytotoxic and it takes time for their removal, it may lead denature of biological properties and contamination in the process. Recent BSE (Bovine Spongiform Encephalopathy) and vCJD (variant Creutzfeldt-Jakob disease) issues have been affecting to the tissue transplantation from the point of view of safety. In this paper, a cold isostatic pressing (CIP) was applied for removal of the cells and inactivation of viruses in the cardiovascular tissues to have scaffold for the safe regenerative tissue transplantation.

2. Material and methods

The porcine heart valves were isolated from 4 month-old Clawn miniature pigs (Japan Farm Co. Ltd, Kagoshima, Japan) weighing about 10 kg under the sterile condition. The harvested tissues were packed immediately in sterile bags filled with phosphate buffered saline (PBS) and treated by ultrahigh pressure of 980 MPa for 10 min using a CIP apparatus (Dr. Chef, Kobe Steel Ltd, Kobe, Japan) for cell demolition (Fig. 1). The range of temperature in the process is about 5 to 30°C. They were then rinsed by PBS for 2 weeks under gentle stirring at 4°C for removal of the residues of the broken cells. They were subjected to the histological observation by the light and electron microscopy, DNA and phospholipids assay, detection of porcine endogeneous retrovirus (PERV) by the PCR, and biomechanical study by the tensile strength measurement.

The acellualr tissues were transplanted orthotopically into nine allogeneic miniature pigs. The pulmonary valves were transplanted at right ventricular outflow tract through a median sternotomy with extracorporeal circulation without blood oxygenation [5]. The postoperative anticoagulation or anti-platelet therapy was not instigated. They were explanted 4, 12, and 24 weeks (n=3) after the transplantation and examined histologically and immunohistologically. All animals were carefully reared in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH publication No.85-23, revised in 1985).
3. Results and discussion

The tissues were completely cell free when they were treated by the CIP for 10 min followed by washing for 2 weeks from the H-E staining (Fig. 2). The amount of DNA and phospholipids were lower than 1 µg/ml and 0.5 mg/wet g, respectively and those were less than 10% in the native tissue (Fig. 3).

![Fig. 2 Cross sections of (A) native and (B) treated tissues (H-E staining).](image)

![Fig. 3 Residual amounts of DNA and phospholipids in native and treated tissues.](image)

The collagen and elastin fibers were well maintained in the acellular tissue and there were no significant changes in biomechanical properties of the breaking strength and elastic modulus. We have already found that this process could be successfully applied to cartilage tissues for decellularization (not shown). More effectively, it has been reported that the most of viruses including HIV are inactivated by the CIP only of more than 600 MPa without washing [6]. This means the treatment is able to sterilize the tissue in addition to the decellularization. The Clown miniature pig was chosen as a donor animal since its size adapts human tissues well and its genome has been well studied in order to develop a human gene induced transgenic animal for the organ transplantation. There was no PERV detected in PCR assay from the tissue treated (Fig. 4).

![Fig. 4 PCR products of PERV (arrow) in native and treated tissues.](image)
The animals survived after the transplantation in all cases. The explanted grafts showed no macroscopical abnormality and no dilatation and aneurysmal changes including their anastomosis. The inner surface was completely covered with endothelial cells and the inside was infiltrated by cells from both sides of endothelium and outer tissue after 12 weeks. It was dominant in the latter. Almost of the tissue including cusps were filled by the cells at 24 weeks, mainly by smooth muscle cells (Fig. 5). There was no inflammation and calcification observed in the tissue.

Recently, some groups have reported excellent clinical results of acellular pulmonary heart valve transplantation [7-9]. We are planning a clinical application of the acellular grafts made by this process in the near future.

4. Conclusion

Porcine cells and PERV were removed completely by the CIP treatment without using any detergents. The acellular grafts showed remarkable ability in repopulation after the transplantation. This CIP treatment may have more secure acellular graft for the regenerative tissue transplantation.

5. Acknowledgement

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6. References


