Regenerative Artificial Vascular Graft Using Acellular Scaffold

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Introduction

The artificial synthetic vascular grafts have been clinically used for more than 30 years as substitution for the large diameter blood vessels. Recently, the cryopreserved allograft (homograft) grafts have been clinically applied thanks to the establishment of a tissue banking system worldwide. They are useful especially in the infected region and may be applicable to the smaller blood vessels. However, since they are donated from the cadavers, the supply is limited. The regenerative vascular grafts which may be replaced by the host tissue after the implantation may have growth potential and quite useful for the paediatric patients. Prof. Shin'oka (Tokyo Women's Medical Univ.) has already applied polyglycolide-poly(ε-caprolactone) copolymer (PGCL) vein graft (pulmonary artery) to about 50 paediatric patients. It has been reported of their good clinical results, whereas it may not be easy to substitute the arteries because of difficulty of degradation control of the polymeric scaffolds.

We are developing vascular grafts made of acellular scaffold. The allogeneic or xenogeneic tissue may be digested by infiltrated cells and show a different degradation profile from that of the biodegradable materials which are hydrolyzed even out of the living body. Most of the groups developing acellular scaffolds have been using detergents and/or enzymes as decellularization media such as Triton® X-100, sodium dodecyl sulphate, deoxycholate, trypsine, DNase, and RNase. 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. We have been developing a novel tissue processing for preparation of acellular grafts by ultrahigh pressure treatment named PowerGraft for the safe valvular and vascular tissue transplantation. This process does not include any detergent and may be applicable to large tissues.

Experimental Part

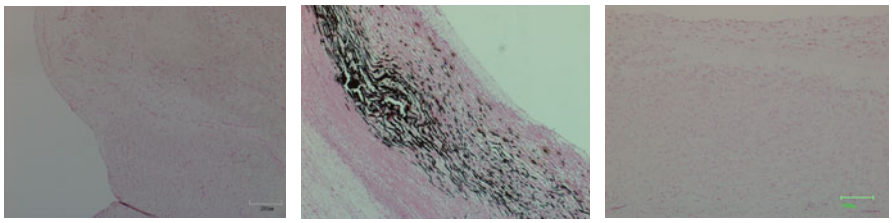
The porcine aortae and pulmonary arteries were isolated from 4 month-old Clawn miniature pigs (Japan Farm Co. Ltd, Kagoshima, Japan) weighing about 10 kg under the sterile condition. The harvested tissues were decellularized by our PowerGraft technology. Briefly, the tissues were packed in sterile bags filled with PBS. The packed tissues were treated by ultrahigh pressure of 980 MPa at 4°C using a cold isostatic pressing (CIP) apparatus (Kobe steel LTD, Kobe, Japan) for cell demolition. They were then rinsed by PBS-based washing solution for 2 weeks and alcohol aqueous solution for 2 days at 4 °C with gentle stirring for removal of the residues of the broken cells. The tissues treated were then crosslinked by glutaraldehyde (GA) or in a vacuum oven followed by elastase digestion. 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 scaffolds were transplanted into the allogeneic miniature pigs. The aortae were transplanted at descending aorta through left thoracotomy in the surgery carried out with single clamp technique. The pulmonary arteries were transplanted at right ventricular outflow tract through a median sternotomy with extracorporeal circulation without blood oxygenation[1)]. In both cases, postoperative anticoagulation or anti-platelet therapy was not instigated. They were explanted 4, 12, and 24 weeks 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).

Results

The tissues were completely cell free after they were applied of the CIP of 970 MPa for 10 min and rinsed by washing solutions in the H-E staining sections. There was no PERV products detected in PCR assay from the tissues applied by the CIP whereas still detected in the tissue treated by Triton® X-100 of 24 hr incubation. There were no significant changes in biomechanical properties of the breaking strength and elastic modulus of the leaflets treated at 970 MPa for 10 min, whereas the both properties of the aortic wall after elastase digestion were lower than those of the native aorta but higher than of the native pulmonary artery. The amounts of DNA and phospholipids were lower than 5% of the native tissue.

The animals survived after the transplantation in the all cases. The explanted grafts showed no macroscopical abnormality and no dilatation and aneurysmal changes including their anastomosis. In the pulmonary artery study, 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 were filled by the cells after 24 weeks, mainly by smooth muscle cells. There was no inflammation and calcification observed in the tissue. In the descending aorta study, the endothelium and cell infiltration was same as the pulmonary artery study after 12 weeks, however calcification was observed along to elastic fibrils in a middle area of the acellular graft without elastase digestion after 24 weeks. Whereas there were few deposits observed in the graft with elastase digestion (Fig. 1).



(a) (b) (c)

Figure 1. Explanted acellular grafts 24 weeks after the transplantation in von-Kossa staining. (a: pulmonary artery, b: descending aorta without elastase digestion, c: descending aorta with elastase digestion)

Discussion

Most of the groups have been using detergents and/or enzymes for decellularization. Since the decellularization depends on the permeation of the agent through the tissue, it may not be applicable to large and hard tissues like cartilage. We have already found that this technology could be successfully

applied to cartilage tissues, lung, skin, and cornea for decellularization. More effectively, it has been reported that the most of viruses including HIV are inactivated by the CIP more than 600 MPa[2]. This means the treatment is able to sterilize the tissue in addition to the decellularization.

We have chosen the Clawn miniature pig 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 xenogeneic organ transplantation. The explanted grafts showed host cell infiltration especially in the pulmonary arteries. There were several calcium deposits observed in the graft without elastase digestion, however there were few deposits observed with elastase treatment. This treatment for elimination of elastin may be useful for having regenerative scaffolds especially for the aortic tissue[3]. Recently, Prof. Konertz and his group in Germany have reported excellent clinical results of acellular porcine pulmpnary heart valves[4]. We are planing a clinical application of the acellular grafts in the near future.

Acknowledgements

This study was supported by the Research Grants from the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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