

## Valved Conduit with Glutaraldehyde-Fixed Bovine Pericardium Treated by Anticalcification Protocol

Hong-Gook Lim, M.D., Ph.D.<sup>1,2</sup>, Gi Beom Kim, M.D., Ph.D.<sup>1,3</sup>,  
Saeromi Jeong, M.S.<sup>1</sup>, Yong Jin Kim, M.D., Ph.D.<sup>1,2</sup>

**Background:** A preclinical study was conducted for evaluating a valved conduit manufactured with a glutaraldehyde (GA)-fixed bovine pericardium treated using an anticalcification protocol. **Methods:** Bovine pericardia were decellularized, fixed with GA in an organic solvent, and detoxified. We prepared a valved conduit using these bovine pericardia and a specially designed mold. The valved conduit was placed under *in vitro* circulation by using a mock circulation model, and the durability under mechanical stress was evaluated for 2 months. The valved conduit was implanted into the right ventricular outflow tract of a goat, and the hemodynamic, radiologic, histopathologic, and biochemical results were obtained for 6 months after the implantation. **Results:** The *in vitro* mock circulation demonstrated that valve motion was good and that the valved conduit had good gross and microscopic findings. The evaluation of echocardiography and cardiac catheterization demonstrated the good hemodynamic status and function of the pulmonary xenograft valve 6 months after the implantation. According to specimen radiography and a histopathologic examination, the durability of the xenografts was well preserved without calcification at 6 months after the implantation. The calcium and inorganic phosphorus concentrations of the explanted xenografts were low at 6 months after the implantation. **Conclusion:** This study demonstrated that our synergistic employment of multiple anticalcification therapies has promising safety and efficacy in the future clinical study.

Key words: 1. Xenograft  
2. Heart valves  
3. Bioprosthesis  
4. Bioengineering  
5. Biomaterials  
6. Calcification

### INTRODUCTION

Heart valve substitutes are of two principal types, namely mechanical prosthetic valves and tissue valves [1,2]. Mechanical prosthetic valves last long but have a high risk of thrombotic and hemorrhagic complications. Life-long anticoagulation ther-

apy is also inevitable. In contrast, tissue valves composed of animal or human tissue have a low risk of these complications without anticoagulation. The pulmonary autograft valves and the human allograft valves show good durability, but are not widely used with limited availability [3]. Glutaraldehyde (GA)-preserved porcine aortic valves and bovine pericardial bio-

<sup>1</sup>Xenotransplantation Research Center, Seoul National University Hospital Clinical Research Institute, Departments of <sup>2</sup>Thoracic and Cardiovascular Surgery and <sup>3</sup>Pediatrics, Seoul National University Hospital, Seoul National University College of Medicine

Received: April 7, 2014, Revised: June 10, 2014, Accepted: June 16, 2014, Published online: August 5, 2014

Corresponding author: Yong Jin Kim, Department of Thoracic and Cardiovascular Surgery, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea

(Tel) 82-2-2072-3638 (Fax) 82-2-745-5209 (E-mail) [kyj@plaza.snu.ac.kr](mailto:kyj@plaza.snu.ac.kr)

© The Korean Society for Thoracic and Cardiovascular Surgery. 2014. All right reserved.

© This is an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

prosthetic valves are currently used as cardiac xenografts, but their durability is limited because they are highly prone to progressive tissue calcification with structural deterioration [2,4]. The principal underlying pathologic process for valve failure is calcification, which is also markedly accelerated by young recipient age. In particular, the long-term results of creating various right ventricle (RV)-to-pulmonary artery (PA) conduits for treating complex congenital heart diseases are disappointing. A smaller size of conduit showed the highest reoperation rate. We reported that the reoperation rate for the RV-PA conduit was about 35% at 5 years, so it is mandatory to develop a more durable conduit for the RV outflow [3]. We also studied Shelhigh porcine pulmonic valve conduits, which are not satisfactory according to our short-term results. Small conduits ( $\leq 16$  mm) fail relatively early, and large conduits ( $\geq 18$  mm) fail after 2 years of implantation due to intimal peel formation at the distal segment [5].

The crosslinking with GA renders the cardiac xenografts inert, non-biodegradable, and non-antigenic but, paradoxically, encourages tissue calcification [2,4,6]. Although the mechanisms of GA-crosslinked xenograft calcification are not fully understood, the major determinants are phospholipids, free aldehyde groups, and residual antigenicity. We demonstrated that GA-crosslinked cardiac xenografts resulted in severe calcification more than 300 days after a pig-to-goat pulmonary root xenotransplantation and should be managed using an appropriate anticalcification treatment and novel preservation methods [7].

Our treatment targeted at the prevention of calcification includes the extraction of phospholipids [6,8,9], neutralization of residual unbound aldehyde groups of GA [6,8,9], and decellularization [6,10,11]. We also developed a specially designed mold for the manufacture of the valved conduit [12] and produced a valved conduit with the GA-fixed bovine pericardia treated with our combined anticalcification protocol, which had been proven effective in small-animal experiments [6]. In this study, preclinical safety and efficacy with *in vitro* mock circulation and *in vivo* large-animal circulatory models were evaluated for this valved conduit.

## METHODS

### 1) Tissue preparation

Fresh bovine pericardia were obtained from the local slaughterhouse, placed in phosphate-buffered saline (PBS, 0.1 M, pH 7.4), and immediately transported to our laboratory of Xenotransplantation Research Center. On arrival, they were rinsed with normal saline and were freed from adherent fat.

### 2) Decellularization

Bovine pericardial tissues were washed with 0.9% normal saline and then, 0.1% peracetic acid with 4% ethanol in distilled water for 1 hour and washed for 30 minutes with distilled water. These tissues were treated with a hypotonic buffered solution for 14 hours at 4°C, and treated with a hypotonic buffered solution with 0.1% sodium dodecyl sulfate (SDS) for 24 hours at 4°C. The tissues were then treated with a hypertonic buffered solution (II) for 8 hours at 4°C, and with an isotonic solution for 24 hours at 4°C.

**(1) Hypotonic buffered solution:** distilled water 1,000 mL; tris 10 mmol/L; pH 8.0

**(2) Isotonic buffered solution:** distilled water 1,000 mL; tris 50 mmol/L; NaCl 0.15 mol/L; ethylenediaminetetraacetic acid (EDTA) 0.05%; aprotinin 10 KIU/mL; neomycin trisulfate 50 mg; pH 8.0

**(3) Hypertonic buffered solution II:** distilled water 1,000 mL; tris 200 mmol/L; NaCl 0.6 mol/L; pH 8.0

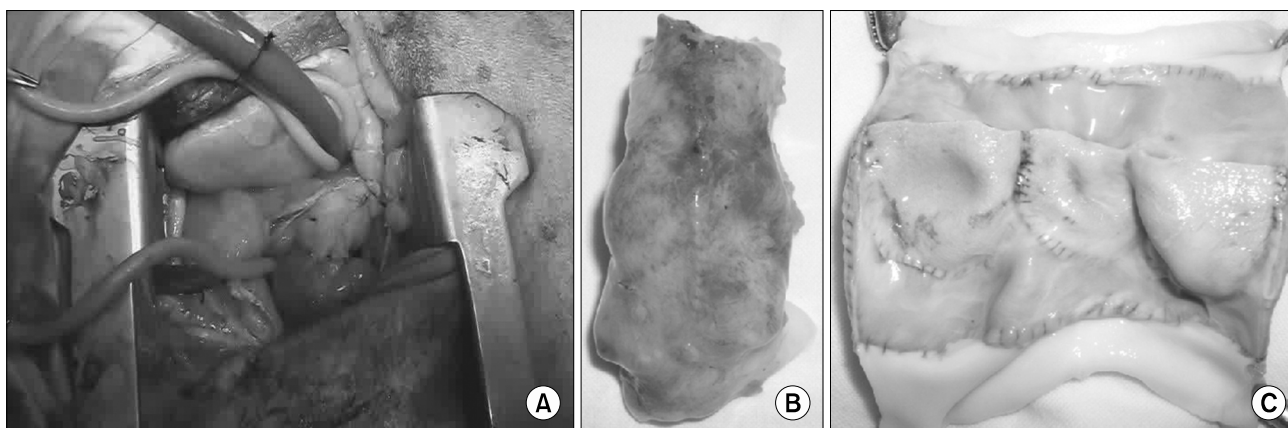
**(4) Distilled water:** distilled water 1,000 mL; EDTA 0.05%; aprotinin 10 KIU/mL

### 3) Glutaraldehyde fixation in an organic solvent

Bovine pericardial tissues were initially fixed with 0.5% GA for 3 days at room temperature, and additionally fixed with 1% GA in an organic solvent of 75% ethanol+5% octanol for 2 days at room temperature, and finally fixed with 0.25% GA for 1 week at room temperature.

### 4) Detoxification

After the completion of fixation, the tissues were treated with a 0.1-M glycine solution (PBS, pH 7.4) at 37°C for 48 hours.



**Fig. 1.** (A) Surgical view of valved conduit implantation in the right ventricular outflow tract. (B) Gross findings of an explanted valved conduit and (C) the same graft unfolded longitudinally 188 days after implantation.

### 5) Manufacture of a pericardial valved conduit

Our molds were specially designed to create various-sized valved conduits with sinuses. Our mold was made to have a four-sinus structure; the bulging sinuses were 1.4 times the mold radius, and the sinus height was 1.45 times the mold radius. The wall and the leaflet of the pericardial valved conduit were made of the bovine pericardia treated with our anti-calcification protocols. Bovine pericardia were furled and fixed around the mold. The pericardia fixed around the lower three part of the mold ( $85^\circ$ ) were used, and the pericardia fixed around the upper part of the mold ( $105^\circ$ ) were not used. One bovine pericardium with three bulging sinuses was used as the conduit wall, and three valve leaflets were made by anastomosing the sinus-shaped valves made of another bovine pericardium to the edge of the bulging sinuses. In this study, bulging sinuses were formed on a pericardial valved conduit having a diameter of 15 mm by using a specially designed mold [12].

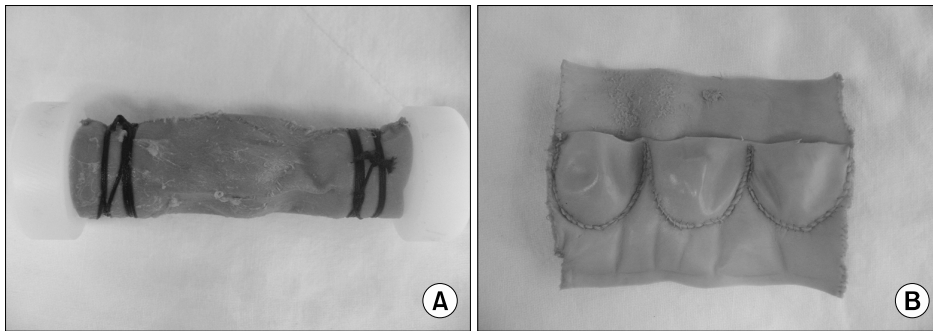
### 6) *In vitro* mock circulation

We developed a specially designed mock circulation model to evaluate the mechanical stress exerted on and the durability of the valved conduit *in vitro*. The valved conduit was fixed to the circuit and placed under *in vitro* circulation. The circulation solutions contained 0.5% benzalkonium chloride (PBS, 0.1 M, pH 7.4). To reproduce the *in vivo* circulation, a pulsatile pressure of 140/80 mmHg was repetitively applied

to the valved conduit at a constant interval of 80 rpm in one direction for 2 months.

### 7) Open heart surgery for the implantation of a xenograft valved conduit to the right ventricle outflow tract of a goat using cardiopulmonary bypass

This study was approved by Institutional Animal Care and Use Committee of Clinical Research Institute, Seoul National University Hospital (IACUC no. 10-0057). This facility was accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. The body weight of the six goats (*Capra aegagrus hircus*) was 38, 35, 31, 29, 31.5, and 30 kg, respectively (Fig. 1). The goats were injected intramuscularly at doses of 5 mg/kg for tiletamine-zolazepam (Zoletil, Virbac, Luxembourg) and 0.25 mg/kg for xylazine hydrochloride (Rompun, Bayer AG, Leverkusen), which were used as a preanesthetic for surgical procedures and for short-term restraint. Electrocardiogram and SpO<sub>2</sub> were continuously monitored using the tongue for SpO<sub>2</sub> sensor placement. The goats were endotracheally intubated and anesthetized with 1.3% to 2.0% enflurane in O<sub>2</sub> and instrumented for hemodynamic monitoring. A femoral artery and an internal jugular vein were catheterized for the measurement of blood pressure and central venous pressure; the withdrawal of blood for the determination of the partial pressure of carbon dioxide, oxygen, and arterial pH; and the administration of drugs and fluids. Respiration was controlled throughout the study. The prophylactic antibiotics were intra-



**Fig. 2.** (A) Gross findings taken from an explanted glutaraldehyde-fixed xenograft treated with our anticalcification protocols after mock circulation for 2 months and (B) from the same graft unfolded longitudinally.

venously administered at doses of 1.0-g cefazolin. Under general anesthesia, the goats were placed in the right decubitus position, and left thoracotomy was performed through the 4th intercostal space under sterile conditions. The descending aorta was dissected and mobilized for aortic cannulation. The pericardium was opened and tented, and the heart was exposed. Three hundred IU/kg of heparin was injected intravenously. Aortic and venous cannulation was performed through the descending aorta and the right atrial auricle. Partial cardiopulmonary bypass without cardiac arrest was performed using a membrane oxygenator under normal temperature conditions. The distal main PA was transected, and distal anastomosis was performed using 5-0 Prolene (Ethicon, Somerville, NJ, USA) continuously between the distal main PA and the xenograft valved conduit (internal diameter: 15 mm). The main PA was excised, and the native pulmonary valve was also completely excised. Proximal anastomosis was performed in the same manner between the xenograft valved conduit and the proximal main PA. The patient was weaned off cardiopulmonary bypass, and modified ultrafiltration was performed. Three mg/kg of protamine was injected intravenously. After a chest drain was placed in the thoracic cavity, the thoracotomy incision was closed in layers. After the operation, prophylactic antibiotics were intravenously administered twice in doses of 1.0-g cefazolin.

#### **8) Echocardiography and cardiac catheterization**

Transthoracic echocardiography was performed at 6 months post-transplantation to evaluate hemodynamic changes. The morphologies and competences of a leaflet were investigated. Cardiac catheterization was also performed to confirm the hemodynamics just before sacrificing the goats.

#### **9) Radiologic confirmations with the quantization of calcification**

After sacrificing the goat, grafts were tested for radiologic confirmation with a simple X-ray.

#### **10) Microscopic examination**

Representative tissue samples were examined with light microscopy. Tissue samples were fixed in 10% formalin, embedded in paraffin wax and 2- to 4- $\mu$ m-thick sections were stained with hematoxylin-eosin (H&E) and Masson's trichrome.

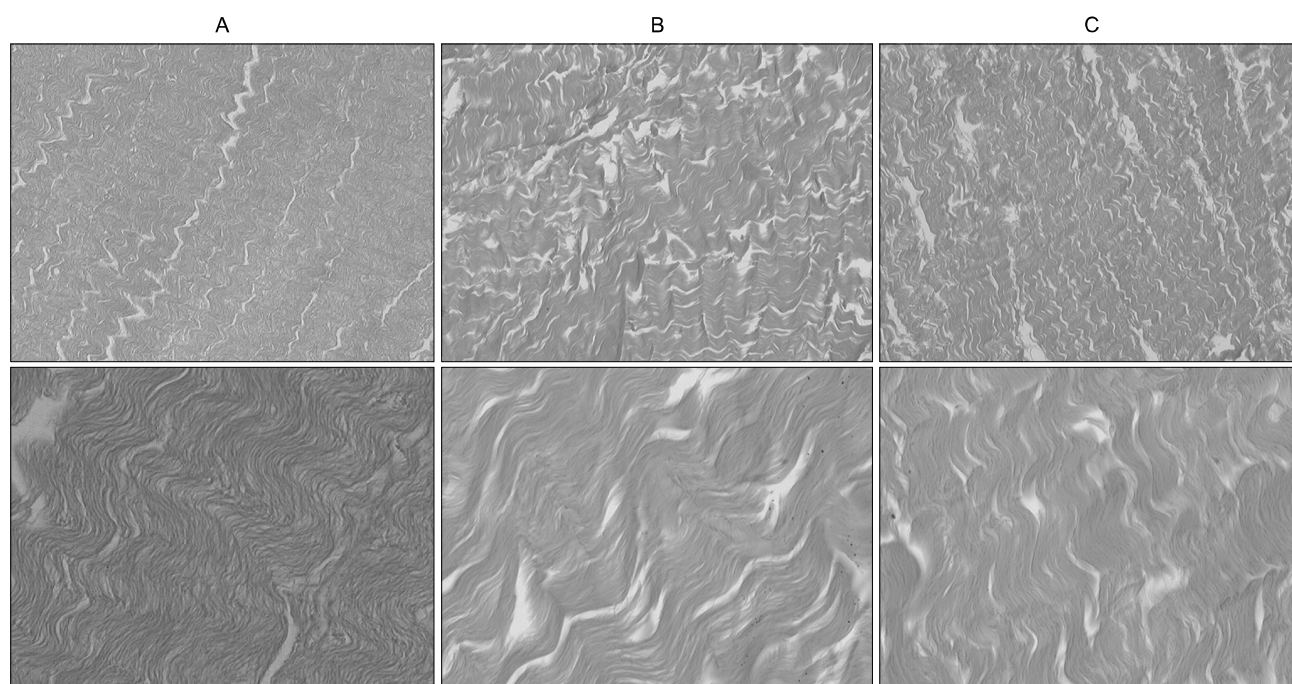
#### **11) Calcium analysis**

Harvested tissue samples were washed with normal saline, dried at 70°C for 24 hours, and weighed. Samples were then hydrolyzed with a 5.0-N HCl solution. The calcium content of the hydrolysate was measured colorimetrically by the o-cresolphthalein complexone method, as previously described [13], using an automatic chemistry analyzer (Hitachi 7070; Hitachi, Tokyo, Japan). Calcium contents were expressed in the unit of micrograms per milligram (dry weight).

## **RESULTS**

#### **1) Gross and microscopic findings after the mock circulation**

Gross findings taken from a GA-fixed xenograft treated with our anticalcification protocols after mock circulation for 2 months and from the same graft unfolded longitudinally, showed that the wall and the leaflet of the valved conduit maintained good mechanical stability without dehiscence



**Fig. 3.** Microscopic findings of a GA-fixed bovine pericardium treated with our anticalcification protocols before and after mock circulation (H&E,  $\times 100$  [upper] and  $\times 400$  [lower]). (A) GA-fixed bovine pericardium treated with our anticalcification protocols before mock circulation. (B) GA-fixed bovine pericardium treated with our anticalcification protocols after mock circulation as a leaflet of the valved conduit for 2 months. (C) GA-fixed bovine pericardium treated with our anticalcification protocols after mock circulation as a wall of the valved conduit for 2 months. GA, glutaraldehyde.

around the suture line, and the leaflet remained mobile (Fig. 2). Microscopic findings (H&E staining) of GA-fixed bovine pericardia treated with our anticalcification protocols before and after the mock circulation demonstrated that the collagen fibers were well preserved with a normally banded structure and that no specific matrix derangement was noticeable. Because of complete decellularization, cellular nuclei were not observed (Fig. 3).

## 2) Echocardiography and cardiac catheterization

The six goats survived to 18, 26, 42, 50, 72, and 188 days, respectively, after pulmonic root xenotransplantation. The reasons that prevented the goats from surviving until the designated period included infections and gastrointestinal problems but not cardiac problems. One hundred and eighty-eight days after the implantation, the evaluation of echocardiography and cardiac catheterization demonstrated a good hemodynamic status and function of the pulmonary xenograft valve. The echocardiography demonstrated good leaflet motion, insignificant pulmonary stenosis of 2 m/sec, and trivial

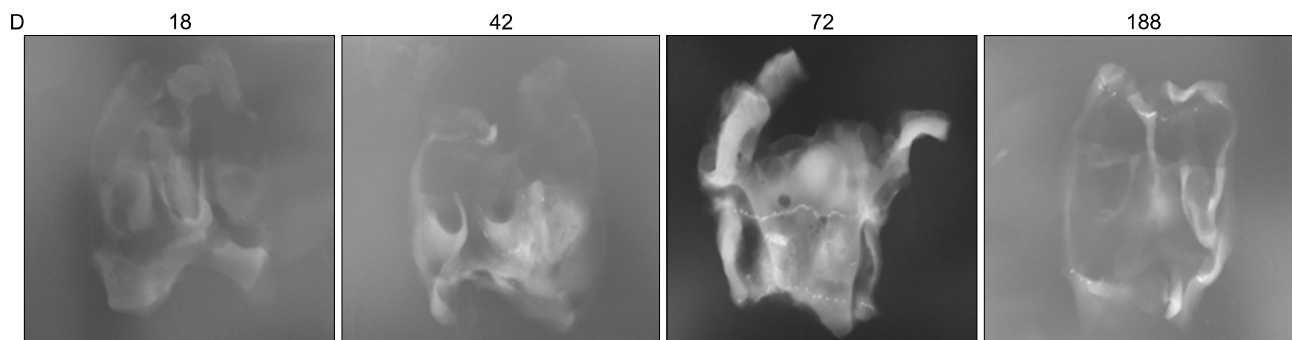
pulmonary regurgitation. Cardiac catheterization demonstrated that the aorta pressure was 63/28/38, 65/29/38, and 63/29/38 mmHg; the PA pressure was 17/9/13, 16/8/12, and 17/9/13 mmHg; the systolic RV pressure was 28, 29, and 30 mmHg; and the mean right atrial pressure was 8, 9, and 8 mmHg. The pressure ratio of the RV to the aorta was  $0.46 \pm 0.02$ , and the pressure gradient between the RV and the PA was  $12.3 \pm 1.2$  mmHg.

## 3) Gross findings

On gross inspection, none of the explanted valved conduits showed calcific deposits or plaque and all remained mobile (Fig. 1).

## 4) Specimen radiography

Specimen radiography was taken unfolded longitudinally from a xenograft, which was explanted 18, 42, 72, and 188 days after the implantation, and demonstrated no calcification (Fig. 4).



**Fig. 4.** Specimen radiographic findings taken unfolded longitudinally from an explanted xenograft. D, days after implantation.

### 5) Microscopic examination taken from an explanted xenograft after transplantation

In the H&E staining, collagen fibers appeared well preserved with a normally banded structure and no specific matrix derangement was noticeable. Because of complete decellularization, cellular nuclei were not observed (Fig. 5). In Masson's trichrome staining, the xenografts still had a compact array of collagen fibers with the structural integrity preserved (Fig. 6).

### 6) Calcium analysis

The calcium concentrations of the explanted pulmonary conduit leaflets made of a GA-fixed bovine pericardium treated with our anticalcification protocol were 0.83, 0.56, 0.39, 2.15, 1.67, and 0.70  $\mu\text{g}/\text{mg}$  18, 26, 42, 50, 72, and 188 days after the transplantation, respectively (Fig. 7). The calcium concentrations of the explanted pulmonary conduit walls made of the GA-fixed bovine pericardium treated with our anticalcification protocol were 0.51, 0.63, 0.34, 0.59, 0.86, and 0.60  $\mu\text{g}/\text{mg}$  18, 26, 42, 50, 72, and 188 days after the transplantation, respectively. In contrast, in our previous study of a GA-fixed xenograft without the anticalcification treatment, the calcium content increased to  $7.93 \pm 5.34$   $\mu\text{g}/\text{mg}$  6 months after the implantation, and was more than 20  $\mu\text{g}/\text{mg}$  1 year after the implantation [7].

### 7) Inorganic phosphorus analysis

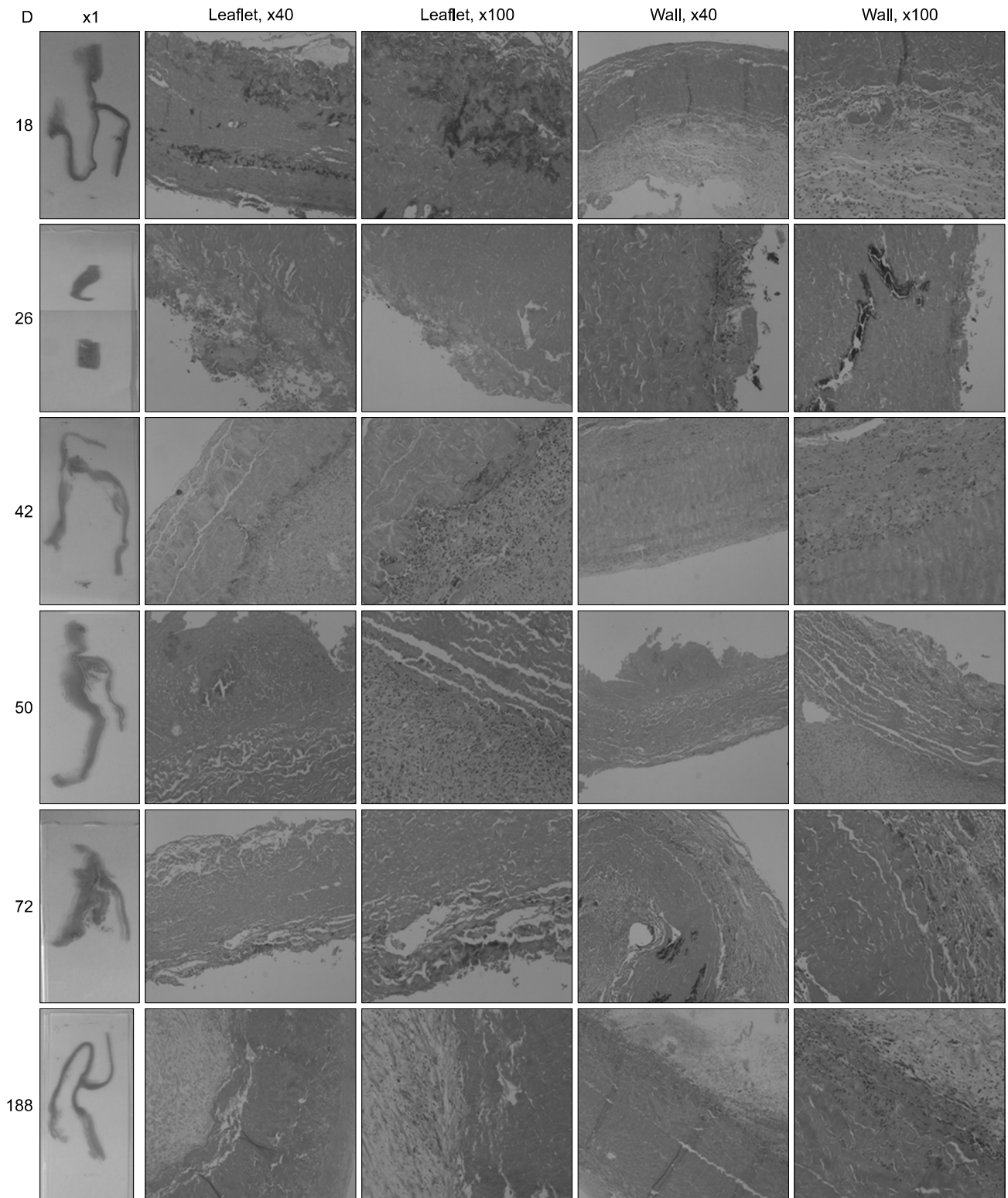
The inorganic phosphorus (IP) concentrations of the explanted pulmonary conduit leaflets made of a GA-fixed bovine pericardium treated with our anticalcification protocol

were 1.02, 2.01, 0.62, 1.42, 1.51, and 0.27  $\mu\text{g}/\text{mg}$  18, 26, 42, 50, 72, and 188 days after transplantation, respectively. The IP concentrations of the explanted pulmonary conduit walls made of a GA-fixed bovine pericardium treated with our anticalcification protocol were 0.87, 2.61, 0.67, 1.15, 1.72, and 0.76  $\mu\text{g}/\text{mg}$  18, 26, 42, 50, 72, and 188 days after the transplantation, respectively (Fig. 8).

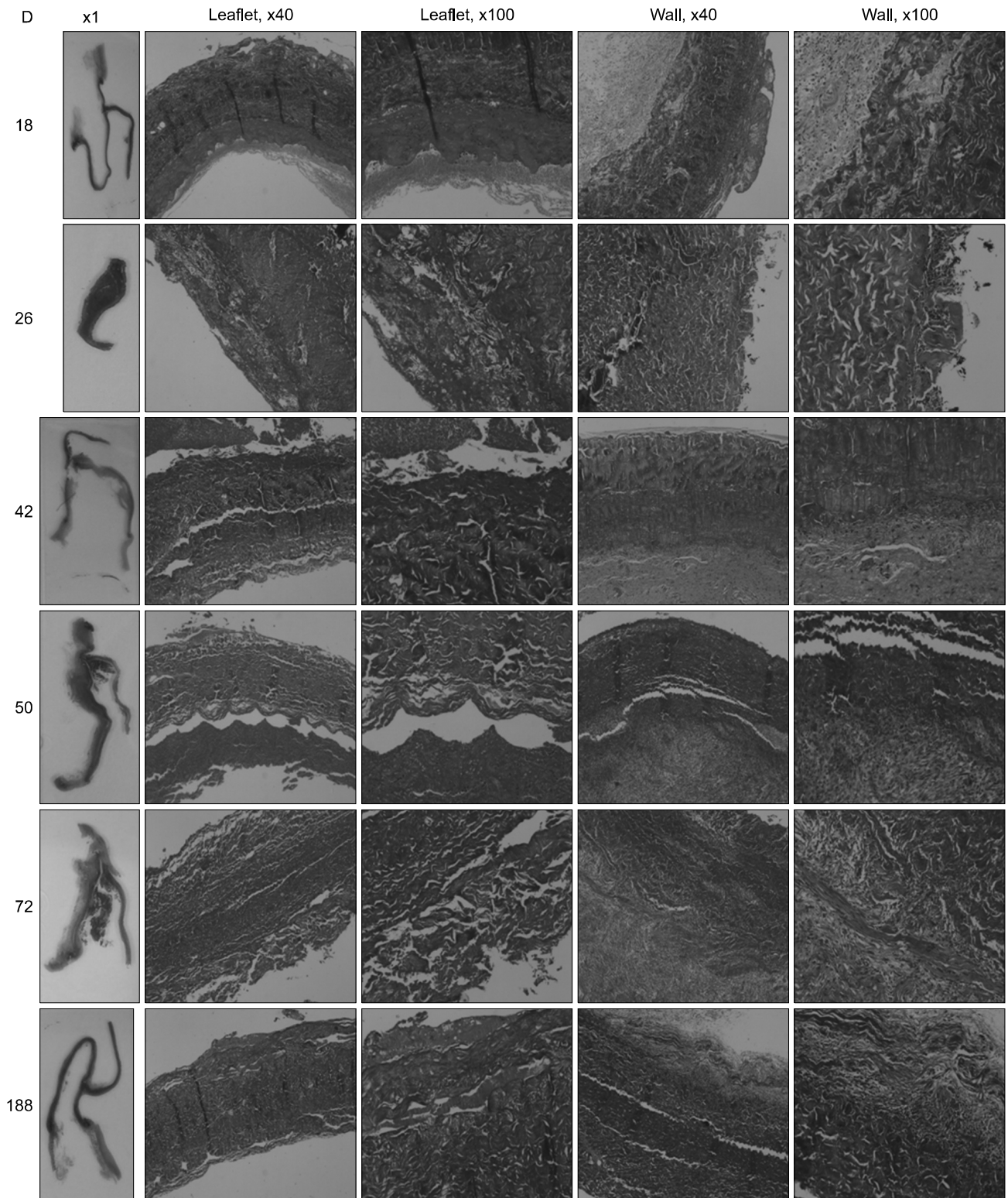
## DISCUSSION

Previously, we proved the efficacy of our combined anticalcification treatments for GA-fixed cardiac xenografts in small-animal experiments [6,8,9]. However, these anticalcification treatments have not always been safe and efficacious if used on valves placed under *in vitro* mock circulation or large-animal *in vivo* circulation [2]. The aim of this study was to evaluate the preclinical safety and efficacy of our combined anticalcification strategies for a GA-fixed bovine pericardium.

Calcification of a cardiac xenograft was initiated primarily within residual cells that were devitalized, and a decellularization approach prevented the formation of the nidus for the calcification [2,6,10,11,14-16]. Various decellularization methods have been studied in order to develop a less immunogenic and more durable tissue graft [17]. Considering the number and the amount of chemicals that were used, the incubation time, and the degree of damage to the extracellular matrix, we concluded that a multi-step method with a hypotonic solution followed by SDS is a relatively optimal method for decellularization in our previous study [18]. We also investigated the effect of appropriate environmental conditions such as temperature, treatment

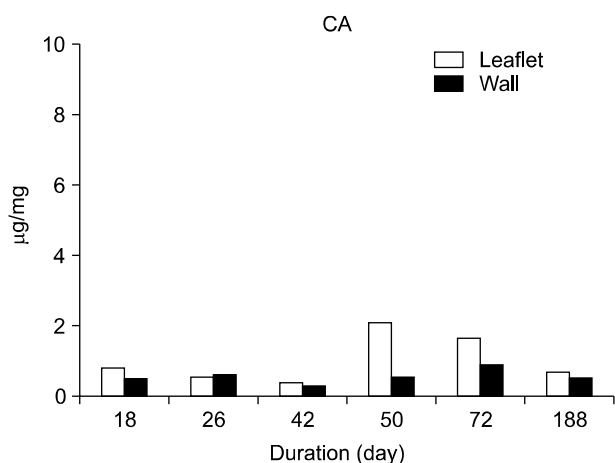


**Fig. 5.** Microscopic findings taken from an explanted xenograft after implantation (H&E,  $\times 40$  and  $\times 100$ ). D, days after implantation.



**Fig. 6.** Microscopic findings taken from an explanted xenograft after implantation (Masson's trichrome staining,  $\times 40$  and  $\times 100$ ). D, days after implantation.

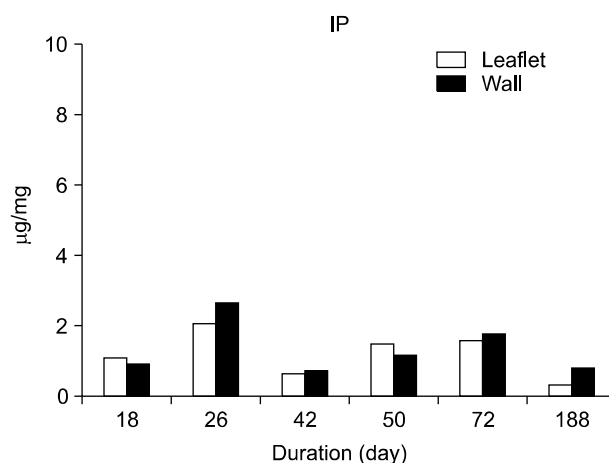




**Fig. 7.** Calcium (CA) concentrations of all the explanted pulmonary conduit walls and leaflets made of a glutaraldehyde-fixed bovine pericardium treated with our anticalcification protocol. Duration, days after implantation.

duration, and SDS concentration for achieving proper decellularization. The exposure of cardiac xenografts to a hypotonic solution prior to the SDS treatment was highly effective in achieving decellularization [19]. Since a high concentration of the detergent (higher than 0.25% SDS) resulted in significant matrix derangement, the use of a low concentration of detergent and treatment under a hypertonic solution have better mechanical characteristics [20]. Our decellularization has *in vivo* anticalcification effects although decellularization may cause mechanical instability [21]. We demonstrated the safety of our decellularization protocol in this mock circulation, since valve functioning was well maintained and structural integrity was well preserved in the gross and microscopic findings recorded at systemic pressure for 2 months. In this large-animal study, a multi-step method with SDS under a hypotonic solution was applied for optimal decellularization with the preservation of an extracellular matrix, which resulted in complete decellularization with the preservation of the collagenous structural integrity and little *in vivo* calcification, which was compared with GA fixation in our previous study [7].

The reaction of a calcium-containing extracellular fluid with the cell membrane-associated phosphorus yields the calcium phosphate mineral deposits after GA fixation [2]. Treatment with organic solvents reduces calcification in the experimental models [22]. This anticalcification mechanism is related to the extraction of cholesterol and phospholipids, per-



**Fig. 8.** Inorganic phosphorus (IP) concentrations of all the explanted pulmonary conduit walls and leaflets made of a glutaraldehyde-fixed bovine pericardium treated with our anticalcification protocol. Duration, days after implantation.

manent alterations in collagen conformation, and binding to hydrophobic residues within collagen and elastin [4]. In our previous study, a mixture of GA and an organic solvent treatment showed better mechanical durability than did the single GA treatment [23]. When fixing xenograft prosthetic devices with GA, the addition of an organic solvent did not cause a loss in the pressure tension, tension elasticity, and thermostability [24]. Organic solvent treatment prevented the *in vivo* calcification of a cardiac xenograft fixed with GA [6,8,9,21,25,26]. In this study, we applied combinations of short-chain alcohols at a high concentration and long-chain alcohols, which are similar to phospholipids in terms of structure, instead of using a pure organic solvent, and demonstrated the safety of our organic solvent protocol in this mock circulation, since valve functioning was well maintained and structural integrity was well preserved in the gross and microscopic findings recorded at systemic pressure for 2 months. In this large-animal study, our organic solvent protocol efficiently mitigated the *in vivo* calcification of GA-fixed cardiac xenografts in pulmonary circulation without the alteration of the microscopic structure, which was compared with the GA fixation in our previous study [7].

The action of toxic aldehyde group residuals from GA cross-linking promotes calcification. Amino groups in the organic molecules, such as amino acids, can bond aldehyde groups and neutralize toxicity [27]. In our previous study, de-

toxification with the diamine bridges using L-lysine decreased the calcification of cardiac xenografts fixed with GA [25,28]. Additionally, it seemed to enhance the tensile strength. A post-fixation treatment with glutamate, urazole, glycine, L-glutamic acid, and sodium bisulfite mitigated *in vivo* calcifications without worsening the physical properties [6,8,9]. We demonstrated the safety of the detoxification process with glycine in this mock circulation, since valve functioning was well maintained and structural integrity was well preserved in the gross and microscopic findings recorded at systemic pressure for 2 months. In this large-animal study, the detoxification process with glycine efficiently mitigated the *in vivo* calcification of GA-fixed cardiac xenografts in pulmonary circulation without the alteration of the microscopic structure, which was compared with GA fixation in our previous study [7].

This mock circulation model is *in vitro* evaluation for the mechanical stress of pressure and durability, but, has a limitation of the risk for infection, and unsuitability for long-term evaluation. This study confirms the *in vitro* durability of our anticalcification and decellularization protocol for the cardiac xenograft, and the feasibility of an *in vivo* long-term circulation study. This promising result can also be used for an *in vivo* circulation model of the aortic valve and mitral valve replacement at systemic pressure. In the future, long-term *in vivo* circulatory experiments using a large animal are needed for various cardiac xenografts such as porcine pericardium and porcine aortic valve, and clinical research is warranted on the basis of these promising preclinical results.

Our preclinical approaches demonstrated that our synergistic and simultaneous employment of multiple anticalcification therapies or novel tissue treatments such as organic solvent, decellularization, and detoxification using an *in vitro* mock circulation model and a large-animal *in vivo* circulatory model is promising in terms of safety and efficacy for future clinical studies.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This study was supported by a grant of the Korea Health 21 Research and Development Project, Ministry for Health, Welfare, and Family Affairs, Republic of Korea (Project no. A040004-006). We would also like to thank TaeWoong Medical Co., Ltd. for technical support.

## REFERENCES

1. Vongpatanasin W, Hillis LD, Lange RA. *Prosthetic heart valves*. N Engl J Med 1996;335:407-16.
2. Schoen FJ, Levy RJ. *Calcification of tissue heart valve substitutes: progress toward understanding and prevention*. Ann Thorac Surg 2005;79:1072-80.
3. Kwak JG, Yoo JS, Kim YJ, Kim WH, Lee JR. *Twenty-one year experience with right ventricle to pulmonary artery conduit interposition*. Korean J Thorac Cardiovasc Surg 2008; 41:417-22.
4. Simionescu DT. *Prevention of calcification in bioprosthetic heart valves: challenges and perspectives*. Expert Opin Biol Ther 2004;4:1971-85.
5. Kim WH, Min SK, Choi CH, et al. *Follow-up of Shelhigh porcine pulmonic valve conduits*. Ann Thorac Surg 2007;84: 2047-50.
6. Lim HG, Kim SH, Choi SY, Kim YJ. *Anticalcification effects of decellularization, solvent, and detoxification treatment for genipin and glutaraldehyde fixation of bovine pericardium*. Eur J Cardiothorac Surg 2012;41:383-90.
7. Kim DJ, Kim YJ, Kim WH, Kim SH. *Xenograft failure of pulmonary valved conduit cross-linked with glutaraldehyde or not cross-linked in a pig to goat implantation model*. Korean J Thorac Cardiovasc Surg 2012;45:287-94.
8. Chang HW, Kim SH, Kim KH, Kim YJ. *Combined anti-calcification treatment of bovine pericardium with amino compounds and solvents*. Interact Cardiovasc Thorac Surg 2011; 12:903-7.
9. Lee C, Kim SH, Choi SH, Kim YJ. *High-concentration glutaraldehyde fixation of bovine pericardium in organic solvent and post-fixation glycine treatment: in vitro material assessment and in vivo anticalcification effect*. Eur J Cardiothorac Surg 2011;39:381-7.
10. Nam J, Choi SY, Sung SC, et al. *Changes of the structural and biomechanical properties of the bovine pericardium after the removal of  $\alpha$ -gal epitopes by decellularization and  $\alpha$ -galactosidase treatment*. Korean J Thorac Cardiovasc Surg 2012;45:380-9.
11. Min BJ, Kim YJ, Choi JW, Choi SY, Kim SH, Lim HG. *Histologic characteristics and mechanical properties of bovine*

- pericardium treated with decellularization and  $\alpha$ -galactosidase: a comparative study. *Korean J Thorac Cardiovasc Surg* 2012; 45:368-79.
12. Park SJ, Kim YJ, Nam J, Kim SH, Lee CH, Lim HG. Measurement of porcine aortic and pulmonary valve geometry and design for implantable tissue valve. *Korean J Thorac Cardiovasc Surg* 2010;43:602-13.
  13. Steitz SA, Speer MY, McKee MD, et al. Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. *Am J Pathol* 2002;161:2035-46.
  14. Hulsmann J, Grun K, El Amouri S, et al. Transplantation material bovine pericardium: biomechanical and immunogenic characteristics after decellularization vs. glutaraldehyde-fixing. *Xenotransplantation* 2012;19:286-97.
  15. Bloch O, Golde P, Dohmen PM, Posner S, Konertz W, Erdbrugger W. Immune response in patients receiving a bioprosthetic heart valve: lack of response with decellularized valves. *Tissue Eng Part A* 2011;17:2399-405.
  16. Pagoulatou E, Triantaphyllidou IE, Vynios DH, et al. Biomechanical and structural changes following the decellularization of bovine pericardial tissues for use as a tissue engineering scaffold. *J Mater Sci Mater Med* 2012;23:1387-96.
  17. Seong YW, Kim YJ, Kim SH, Min BJ, Lee YO, Lim HG. Effect of trypsin on physico-dynamic and histological changes after decellularization of bovine pericardium. *Korean J Thorac Cardiovasc Surg* 2010;43:565-75.
  18. Park CS, Kim YJ, Sung SC, et al. Study on an effective decellularization technique for cardiac valve, arterial wall and pericardium xenographs: optimization of decellularization. *Korean J Thorac Cardiovasc Surg* 2008;41:550-62.
  19. Sung SC, Kim YJ, Choi SY, Park JE, Kim KH, Kim WH. A study on an effective decellularization technique for a xenograft cardiac valve: the effect of osmotic treatment with hypotonic solution. *Korean J Thorac Cardiovasc Surg* 2008;41: 679-86.
  20. Jang WS, Kim YJ, Kim SH. Effects on tensile strength and elasticity after treatment with glutaraldehyde, solvent, decellularization and detoxification in fresh bovine pericardium. *Korean J Thorac Cardiovasc Surg* 2010;43:1-10.
  21. Hwang SW, Kim YJ, Kim SH, Choi SH. Anti-calcification effects in decellularized and variously fixed bovine pericardium. *Korean J Thorac Cardiovasc Surg* 2010;43:235-45.
  22. Pathak CP, Adams AK, Simpson T, Phillips RE Jr, Moore MA. Treatment of bioprosthetic heart valve tissue with long chain alcohol solution to lower calcification potential. *J Biomed Mater Res A* 2004;69:140-4.
  23. Chang HW, Kim YJ, Kim SH, et al. The dynamic and histologic changes of variously fixed bovine pericardiums specimens after mechanical fatigue stimuli. *Korean J Thorac Cardiovasc Surg* 2009;42:148-56.
  24. Cho S, Kim YJ, Kim SH, Park JE, Kim WH. Comparison of the uniaxial tensile strength, elasticity and thermal stability between glutaraldehyde and glutaraldehyde with solvent fixation in xenograft cardiovascular tissue. *Korean J Thorac Cardiovasc Surg* 2009;42:165-74.
  25. Kim KC, Kim YJ, Kim SH, Choi SH. Synchronized synergism using ethanol, L-lysine and NaBH<sub>4</sub> in glutaraldehyde treated porcine pericardium. *Korean J Thorac Cardiovasc Surg* 2009;42:685-95.
  26. Lee C, Kim YJ, Lee CH, Kim SH, Choi SH. Anticalcification treatment of glutaraldehyde-fixed bovine pericardium with amino acids (the effect of ethanol, glutamic acid and homocysteic acid treatment). *Korean J Thorac Cardiovasc Surg* 2009;42:409-17.
  27. Jorge-Herrero E, Fernandez P, Escudero C, Garcia-Paez JM, Castillo-Olivares JL. Calcification of pericardial tissue pretreated with different amino acids. *Biomaterials* 1996;17:571-5.
  28. Kim KC, Choi YK, Kim SH, Kim YJ. Effect of diamine bridges using L-lysine in glutaraldehyde treated porcine pericardium. *Korean J Thorac Cardiovasc Surg* 2009;42:157-64.