의학박사 학위논문

자가 확장식 스텐트에 장착된 이종 판막을 이용한 경심실 폐동맥 판막 치환술

– 변형 항석회화 처리한 돼지 심낭 및 대동맥판막의 비교–

Perventricular Pulmonic Valve Implantation using Xenograft Valves Mounted on Self-Expandable Stent

- Comparison of Porcine Pericardial Versus Aortic Valve Treated by Modified Anticalcification Process -

2016 년 8 월

서울대학교 대학원

의학과 흉부외과학 전공

김 민 석

A Thesis of the Degree of Doctor of Philosophy

자가 확장식 스텐트에 장착된 이종 판막을 이용한 경심실 폐동맥 판막 치환술

– 변형 항석회화 처리한 돼지 심낭 및 대동맥판막의 비교–

Perventricular Pulmonic Valve Implantation using Xenograft Valves Mounted on Self-Expandable Stent

- Comparison of Porcine Pericardial Versus Aortic Valve Treated by Modified Anticalcification Process -

August 2016

Department of Thoracic and Cardiovascular Surgery,
Seoul National University Graduate School
College of Medicine

Min-Seok Kim

자가 확장식 스텐트에 장착된 이종 판막을 이용한 경심실 폐동맥 판막 치환술

– 변형 항석회화 처리한 돼지 심낭 및 대동맥판막의 비교–

지도교수 김 용 진

이 논문을 의학박사 학위논문으로 제출함
2016 년 4 월

서울대학교 대학원
의학과 흉부외과학 전공
김 민 석

김민석의 의학박사 학위논문을 인준함
2016 년 7 월

위원장	노 정 일	(인)
부위원장	김 용 진	(인)
위원	이 정 렬	(인)
위원	윤 태 진	(인)
위원	임 홍 국	(인)

Perventricular Pulmonic Valve Implantation using Xenograft Valves Mounted on Self-Expandable Stent

- Comparison of Porcine Pericardial Versus Aortic Valve Treated by Modified Anticalcification Process -

by

Min-Seok Kim, M.D.

A Thesis Submitted
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Medicine
at Seoul National University College of Medicine

July 2016

Approved by Thesis Committee:

Professor _____ Chairman

Professor _____ Vice Chairman

Professor _____

Professor _____

Professor _____

Abstract

Perventricular Pulmonic Valve Implantation using Xenograft Valves Mounted on Self-Expandable Stent

- Comparison of Porcine Pericardial Versus Aortic Valve Treated by Modified Anticalcification Process -

Min-Seok Kim

Department of Thoracic and Cardiovascular Surgery

Seoul National University College of Medicine

Background: The aims of this study were (1) to compare the hemodynamics, durability, and degenerative changes between the two different xenogenic valves (porcine pericardial versus aortic valve xenografts) implanted into the pulmonary valve position using a perventricular implantation technique in sheep, and (2) to evaluate the safety and efficacy of perventricular implantation of stented pulmonic valve as a preclinical study.

Materials and Methods: Porcine pericardial and aortic valve tissues were treated by our tissue treatment technique in order to improve durability; they were decellularized, alpha-galactosidase treated, treated with space-fillers and glutaraldehyde, and detoxified. A xenograft valve made of porcine pericardial or

aortic valve tissue was manufactured as a stented valve mounted on Nitinol wire that has shape-memory and self-expanding properties. The stented porcine pericardial and aortic valves were implanted into the pulmonary valve position using a perventricular implantation technique under echocardiographic and angiographic guidance in 18 sheep (group PP, pericardial xenograft implantation, n=9; group PAV, aortic valve xenograft implantation, n=9). Hemodynamic and immunohistochemical studies were performed after the implantation, and radiologic, histologic, and calcium quantification studies were performed after a terminal procedure.

Results: All stented valves were positioned properly so a low-pressure gradient between the right ventricle and pulmonary artery was seen immediately after the implantation (median peak pressure gradient: groups PP versus PAV, 4.0 mmHg versus 5.0 mmHg). Of 18 animals, 8 (group PP, n=5; group PAV, n=3) survived more than 300 days. Median survivals of animals were 352 days [87 days, 402 days] and 173 days [98 days, 376 days] in groups PP and PAV, respectively. Echocardiographic and cardiac catheterization studies were performed prior to a scheduled terminal procedure (median follow-up duration 437 days [389 days, 504 days]) In 7 of 8 animals (group PP, n=4; group PAV, n=3). Good hemodynamic state and function of the bioprosthetic valve were demonstrated in all 7 animals. None of the animals showed significant pulmonary regurgitation or stenosis, demonstrating a low-pressure gradient between right ventricle and

pulmonary artery (median peak pressure gradient; groups PP vs. PAV, 5.0 mmHg versus 6.0 mmHg). All the anti-α-Gal IgM and IgG titers measured by enzyme-linked immunosorbent assay were below 0.3 OD and median calcium contents were 0.95μg/mg [0.67μg/mg, 1.13μg/mg] and 2.13 μg/mg [1.18μg/mg, 2.6μg/mg] in groups PP and PAV, respectively. Gross examination of extracted valves showed no calcification or RVOT obstructions, and microscopic findings revealed a minimal amount of calcification in both groups. Regardless of the implantation duration, no significant infiltration of macrophage or T-cell was observed in both groups, by immunofluorescence study. Computed tomography study of the extracted valves performed in 11 animals (group PP, n=4; group PAV, n=7) showed no significant differences between the 2 groups (p = 0.927). The quantity of calcifications analyzed using hydrolysate showed higher calcium contents in group PP tissues than in group PAV tissues (group PP, n=9, 2.13μg/mg [1.18μg/mg, 2.6μg/mg]; group PAV, n=9, 0.95μg/mg [0.67μg/mg, 1.13μg/mg]; p = 0.019).

Conclusion: Perventricular pulmonic valve implantation is a feasible technique, which has a high success rate and produces a good hemodynamic profile. The tissue treatment technique of glutaraldehyde fixation with simultaneous use of multiple anti-calcification methods, including decellularization, immunological modification with α-galactosidase, space-filler treatment, organic solvent treatment, and detoxification, was shown to be an applicable method in treatment

of bioprostheses. Both stented valves made of porcine pericardial and aortic valve tissues showed no significant differences in hemodynamic profile, midterm durability and degree of degenerative dystrophic calcification.

.

Keywords: Xenograft, Bioprosthesis, Heart valve, Bioengineering, Biomaterials, Calcification

Student Number: 2012-21673

Contents

List of Tables and Figures

List of Abbreviations

α-Gal	Galα1,3-Galβ1-4GlcNAc-R
ANOVA	Analysis of variance
BSA	Bovine Serum Albumin
CT	Computed Tomography
DAPI II	4',6'-diamino-2-phenylindole
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FITC	Fluorescein isothiocyanate
GA	Glutaraldehyde
H&E	Hematoxylin-eosin
IgM	Immunoglobulin M
IgG	Immunoglobulin G
OD	Optical density
PA	Pulmonary Artery
PG	Pressure Gradient
PBS	Phosphate Buffered Saline
RV	Right Ventricle
RVOT	Right Ventricular Outflow Tract
SDS	Sodium Dodecyl Sulfate

1. Introduction

Many patients with congenital heart disease require surgical reconstruction for right ventricular outflow tract (RVOT). These patients may gradually develop malfunction of the pulmonary valve or stenosis of the RVOT, which leads to right ventricular failure and eventually require repetitive surgical interventions. To date, surgical approach under cardiopulmonary bypass has been regarded as a standard treatment for a dysfunctional pulmonary valve; however, less invasive strategies for the treatment of RVOT dysfunction are being increasingly proposed since the first report by Bonhoeffer and colleagues on percutaneous pulmonary valve implantation in a 12-year-old boy in 2000 [1]. Percutaneous pulmonic valve implantation is currently accepted as a treatment of RVOT dysfunction in selected patients, and previous studies showed a maintenance of effective hemodynamic profiles on follow-up [2, 3]. Percutaneous pulmonic valve implantation has several advantages over surgical interventions because it is a less invasive technique that avoids cardiopulmonary bypass; however, it is not always technically feasible. Percutaneous pulmonic valve implantation has limitations in patients with poor peripheral vascular access due to repeated interventions or small peripheral vessels, with anatomical variations, and with previous prosthetic valve implantation in the tricuspid or pulmonary position [4].

The percutaneous method has an additional limitation in the size of the prosthetic valve because a relatively small-sized valve (i.e. equal to or smaller than 22 mm in diameter) is currently available. In contrast, perventricular pulmonic valve implantation may have advantages over a traditional surgical approach. In addition to the advantage as a less invasive technique avoiding cardiopulmonary bypass. It is easier and more straightforward to perform. It has broader indications than a percutaneous approach, even in small patients with difficulties in vascular access or in patients with a relatively large pulmonary annulus who may require large-sized prosthetic valves.

Of the types of prosthetic valves used for valve replacement, bioprosthetic valves have been reported to have limitations in long-term durability although they have advantages of lower thrombogenicity and no need for lifelong anticoagulation that is needed after mechanical valve replacement [5]. The limited long-term durability of bioprosthetic valves is attributed to dystrophic calcification resulting from multiple mechanisms such as immunological, chemical, hemodynamic and mechanical factors [6]. Bioprosthetic valves are commonly fixed with glutaraldehyde (GA) to improve tissue stability and to reduce antigenicity; however, this fixation process may cause the valve tissue to be more prone to calcification [7]. We have previously reported on novel tissue treatment techniques, including glutaraldehyde fixation with simultaneous use of multiple anticalcification methods, decellularization, immunological modification

with α-galactosidase, organic solvent treatment and detoxification, and demonstrated that the modified valve preservation process was effective in preventing calcification of bioprostheses [8-10]. In the present preclinical study using a sheep experiment model, we treated porcine pericardial and aortic valve tissue using the aforementioned tissue preservation technique, and developed a stented valve with a Nitinol wire backbone that has a shape-memory property and self-expandable nature. A preclinical study using these self-expandable stented valves was performed in sheep.

The aims of the study were: (1) to compare hemodynamic profiles, durability, and degenerative changes by histology between the two different xenogenic valves (porcine pericardial versus porcine aortic valve xenografts) which were implanted into the pulmonary valve position using a perventricular implantation technique in sheep, and (2) to evaluate the safety and efficacy of perventricular implantation of stented pulmonic valve as a preclinical study.

2. Materials and Methods

Tissue preparation and fixation

Fresh porcine pericardial tissue and aortic valves were obtained from the local slaughterhouse. Tissue preparation including decellularization, α-galactosidase treatment, space-filler treatment, GA fixation, organic solvent treatment, and detoxification was performed as introduced in our previous study [10].

The porcine pericardial tissue was washed in 0.9% normal saline, and treated with 0.1% peracetic acid with 4% (v/v) ethanol in distilled water for 2 hours for bioburden reduction, followed by vigorous washing with distilled water for 1~2 additional hours. After that process, the tissues were incubated in a hypotonic solution with 0.25% sodium dodecyl sulfate (SDS) for 24 hours and washed with distilled water for 1~2 hours. The tissues then were treated with 0.5% Triton X-100 hypotonic solution for 24 hours and washed with distilled water for 1~2 hours, followed by treatment with isotonic solution containing 0.1U/mL α-galactosidase for 48 hours, and washed again with distilled water for 1~2 additional hours. Following that process, the tissues were treated with phosphate-buffered saline (PBS) containing 30% polyethylene glycol 1000 for 48 hours, and washed with 0.9% normal saline for 1~2 hours. Finally, the tissues were treated with hypertonic solution for 3 hours, then washed with PBS solution for 1~2 hour.

All the processes were performed at 4℃ with shaking. After all treatments were completed, the tissues were fixed in 0.5% GA solution for 72 hours, then further fixed in 0.25% GA solution with 75% ethanol and 5% octanol for 48 hours. All the fixation processes were performed at room temperature with shaking. After the fixation process, the tissues again were incubated in 0.25% GA solution for 1 week, then in 0.2M glycine solution for 48 hours.

The porcine aortic valves were washed in 0.9% normal saline, treated with 0.1% peracetic acid with 4% (v/v) ethanol in distilled water for 2 hours, and vigorously washed with distilled water for 1~2 additional hours. After that process, the valves were incubated in a hypotonic solution with 1% SDS for 48 hours, then washed with distilled water for 1~2 hours. The tissues then were treated with hypotonic solution with 1% Triton X-100 and 1% sodium lauroyl sarcosinate for 48 hours and washed with distilled water for 12 hours. These decellularization procedures using detergents were repeated 3 times, followed by treatment with isotonic solution containing 0.1U/mL α-galactosidase for 48 hours, and washing with distilled water for 1~2 additional hours. After that process, the tissues were treated with PBS containing 30% polyethylene glycol 1000 for 48 hours, then washed with 0.9% normal saline for 1~2 hours. Finally, the tissues were treated with hypertonic solution for 3 hours and then washed with PBS solution for 1 hour. All the processes were performed at 4℃ with shaking. After all treatments were completed, the tissues were fixed in 0.5% GA solution for 72 hours, then

further fixed in 0.25% GA solution with 75% ethanol and 5% octanol for 48 hours. All the fixation processes were performed at room temperature with shaking. After the fixation process, the tissues again were incubated in 0.25% GA solution for 1 week, then in 0.2M glycine solution for 48 hours.

Preparation of the self-expandable valved stent

An initial outer stent was knitted using a single-strand Nitinol wire with 0.008-in. thickness (Taewoong Medical Co., Gyeonggi-do, Republic of Korea). The initial valve diameter ranged from 18mm to 26mm. A Dacron membrane was fixed to the Nitinol wire in order to make the stent wall. After preservation with anticalcification treatment, bioprosthetic valves made of porcine pericardial and porcine aortic valve tissues were tightly hand-sewn to the stent wall with 5-0 braided polyester to allow good coaptation.

Preparation of the delivery system

We developed a self-expandable trans-catheter delivery system. The proximal area of the delivery catheter had a valved stent loading zone with a 17.5mm conical tapered tip. The diameter of the outer sheath in the stent loading zone was 18Fr, and the diameter of the catheter shaft was 14Fr. By turning the catheter counterclockwise, the outer sheath could be pulled back to the proximal area of the stent and the self-expandable valved stent could be completely deployed by

pulling the lever. The valved stent was loaded by hand crimping it into the delivery catheter just before catheter exchange. A portion of the valved stent was immersed in saline solution until introduction.

Preparation of animals

Eighteen healthy sheep of median body weight 38.5 kg [36.625 kg, 40.875 kg] were prepared for the study. All animals received routine medical peri-procedural care according to the Guide for the Care and Use of Laboratory Animals from the US National Research Council Committee. This study was approved by the Ethical Committee of Seoul National University Hospital (Protocol approval No.13-2011-003-3).

Implantation of the stented valve and follow-up

The stented porcine pericardial and aortic valve xenografts were implanted into the pulmonary valve position using a perventricular implantation technique in 18 animals (group PP, pericardial xenograft implantation, n=9; group PAV, aortic valve xenograft implantation, n=9).

Under general anesthesia and mechanical ventilation, the animals were placed in a right lateral decubitus position. The left common carotid artery and internal jugular vein were exposed in order to insert a 6-Fr sheath, and routine hemodynamic studies (measurement of the right atrial, right ventricular,

pulmonary arterial and aortic pressure) and angiography were performed. A left thoracotomy was performed, and the thoracic cavity was entered through the fifth intercostal space. After the procedure, purse-string sutures were placed near the right ventricular apex, and perventricular pulmonic valve implantation was performed using the delivery catheter. Single-plane C-arm fluoroscopy and transthoracic echocardiography were used to guide the deployment into a good position within the native pulmonary valve by meticulous control of the catheter handle. After the stented valve was implanted, the right ventricle (RV) and pulmonary artery (PA) pressures were measured again by angiography to check the pressure gradient across the implanted stented valve, and echocardiography was performed to check for the presence of paravalvular leakage.

Each animal was extubated after evaluation by a veterinary anesthesiologist and postoperative care was administered. Animals were survived for up to a maximum of 18 months after implantation, and a bolus injection with 2M KCl was administered in a terminal procedure. Angiography and echocardiography were performed prior to the scheduled terminal procedure of >300 days post-implantation.

Enzyme-linked immunosorbent assay (ELISA)

Anti-α-Gal serum IgM and IgG antibody titers were measured by enzyme-linked immunosorbent assay (ELISA) before implantation, immediately after

implantation, 2 weeks after implantation, and after the terminal procedure. Blood samples of 4 mL per animal were collected. Synthetic α-Gal epitopes linked to bovine serum albumin (α-Gal BSA; Dextra, Reading, UK) were used as solid-phase antigens. The microtiter plates (Nunc, Roskilde, Denmark) were coated with 100μL of α-Gal BSA (1μg/mL in PBS) per well for 1 hour at 37℃. After incubation, excess α-Gal BSA was removed and each well was washed 5 times with distilled water. The plates were blocked with 100μL of 5% (w/v) BSA (Gibco, Gaithersburg, MD, USA) in PBS per well. After 1 hour, excess BSA solution was removed and each well was washed 5 times with distilled water. The serum samples were diluted 1:10 with PBS containing 0.01% Tween 20 (Biorad, Richmond, CA, USA) and 3% BSA. Diluted 100μL serum samples per well were incubated for 1 hour at 37℃. The serum samples were removed and washed 5 times with distilled water. The secondary antibodies used to detect anti-α-Gal serum IgM and IgG antibodies were horseradish peroxidase-conjugated rabbit anti-sheep IgM and IgG (Abcam, Cambridge, UK), respectively, diluted 1:10,000 in PBS containing 0.01% Tween 20 and 3% BSA. Secondary antibodies (100μL) per well were incubated for 1 hour, removed, and washed 5 times with distilled water. The color reaction was developed with 100μL of tetraethylbenzidine solution (BD Bioscience, San Diego, CA, USA) per well for 15 minutes at room temperature. After that process, 50μL of H_2SO_4 were added to each well to stop the reaction. The absorbance was measured in a microplate reader (Labsystems,

Vienna, VA, USA) at 450nm. The titer of anti-α-Gal antibody was defined as the reciprocal of serum dilution, which yielded a 1.0 optical density (OD).

Histological study

After the terminal procedure, the heart was explanted and the gross morphology of the heart and pulmonary trunk was inspected. The implanted stented valve was harvested and rinsed. Tissue samples of the bioprostheses were examined with light microscopy. Harvested tissue samples were fixed in 10% formalin, embedded in paraffin wax, sliced into 6μm thick sections, and stained with hematoxylin-eosin (H&E), Masson's trichrome stain, and von Kossa stain.

Immunofluorescence

Paraffin section (6μm thickness) of tissues was deparaffinized by washing 3 times in xylene (for 5 minutes per each washing), 2 times in 100% ethanol (for 10 minutes per each washing), and finally 2 times in 95% ethanol (for 10 minutes per each washing). The tissue section slide was then rehydrated by rinsing 2 times in distilled water (for 5 minutes per each rinsing). The antigen unmasking was performed using 1mM ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.0) for 15 minutes at 100℃. The slide was cooled down, and unspecific signals were blocked by applying a blocking solution of 5% normal horse serum (Vector Laboratories, California, USA) in 0.01M PBS for 30 minutes. Immunostaining

was performed using the following antibodies: FITC(Fluorescein isothiocyanate)-conjugated monoclonal anti-CD11b (#MCA1425F, Bio-rad, Kidlington, UK) and FITC-conjugated monoclonal anti-CD25 #MCA2218F, Bio-rad, Kidlington, UK). After the immunostaining procedure, the slide was rinsed 3 times with PBS (for 5 minutes per each rinsing). The slide was counterstained with a drop of mounting medium with 4',6'-diamino-2-phenylindole (DAPI II) counterstain (Abott Molecular, Illinois, USA), and was examined using a digital microscopy (Axiocam HRc, Zeiss, Goettingen, Germany).

Qualitative and quantitative calcification analysis

The degree of calcification of the extracted stented valves was measured using a high-definition 64-slice computed tomography (HDCT) scanner (Discovery CT750 HD, GE Healthcare, UK). The scanning parameters included 64 x 0.625mm collimation, 0.5 sec rotation time, 120kV tube voltage, and 300mA tube current. Based on the length of the stent, 64 to 128 slices were obtained for each stent. For each image series, the calcium score was analyzed using semi-automatic software (Rapidia, Infinitt, Seoul, Korea). All pixels with a density above 130 Hounsfield units and conjugated along at least two pixels were considered calcification. Each cluster of calcification was marked automatically, and lesions were selected manually in each slice. The software calculated the area (mm^2) of calcification from the selected areas, and total volume (mm^3) of

calcification was calculated from the integral of the product of area and slice thickness.

In addition, the leaflets harvested from the extracted stented valves were washed with normal saline and dried at 70℃ for 24h. The dried tissues were weighed and hydrolyzed in 5.0N HCl solution at 70℃. After the HCl evaporated, the precipitates were dissolved in distilled water and filtered through syringe filters (Sartorius AG, Goettingen, Germany). The calcium content of the hydrolysate was analyzed using an automatic chemistry analyzer (Hitachi 7070, Hitachi Ltd., Tokyo, Japan), and expressed as μg of calcium per mg of leaflet dry weight.

Statistical analysis

Statistical analysis was performed with the SPSS software package (version 20.0; SPSS, Inc., Chicago, IL, USA). Data were expressed as median [interquartile range]. A p-value of < 0.05 was considered statistically significant. Comparisons between groups were performed using the Mann-Whitney U-test. The repeated measured analysis of variance (ANOVA) was used to assess the significance of differences at each time interval within the groups and between groups.

3. Results

Overall outcomes

Terminal procedures were scheduled after 10 months postoperatively (> 300 days), and efforts were made to keep the follow-up duration of the two groups the same. However, only 5 group PP animals and 3 group PAV animals underwent scheduled terminal procedures. In group PP, 4 animals died before reaching 300 days post-implantation and 5 animals survived >300 days (median 352 days [87 days, 402 days]). Three animals died from infection and one (animal #4) died of low cardiac output syndrome immediately postoperatively. The autopsy of that animal revealed anomalous coronary artery anatomy, which may have been the cause of coronary artery compression by the stented valve (procedure-related complication). In group PAV, 6 animals died before reaching 300 days post-implantation and 3 animals survived >300 days (median 173 days [98 days, 376 days]). Five animals died from infection and 1 animal died during a fight with another animal (Table 1). The deaths from infection were caused by enterocolitis in all cases, and the autopsy findings of those animals revealed no inflammatory findings or abnormalities in the heart.

Echocardiographic and cardiac catheterization studies were performed before implantation (group PP, n=9; group PAV, n=9), immediately after implantation

(group PP, n=9; group PAV, n=9), and prior to a scheduled terminal procedure (group PP, n=4; group PAV, n=3). Immediately after implantation, all the stented valves were placed in a position to ensure a low-pressure gradient between the RV and PA and no paravalvular leakage was observed in both groups. Echocardiographic examinations performed immediately after implantation and before the terminal procedure did not demonstrate any significant pulmonary regurgitation or stenosis. Cardiac catheterization studies demonstrated that preoperative median pressure gradients between the RV and PA were 4.0 mmHg and 2.0 mmHg in groups PP and PAV, respectively, and showed no significant difference ($p = 0.297$). Immediately after implantation, median pressure gradients between the RV and PA were 4.0 mmHg and 5.0 mmHg in groups PP and PAV, respectively ($p = 0.863$). The median pressure gradients measured before the terminal procedure were 5.0 mmHg and 6.0 mmHg in groups PP and PAV, respectively ($p = 1.000$) (Table 2).

Table 1. Overall outcomes of the perventricular pulmonic valve implantation in group PP (porcine pericardial xenograft implantation, n=9) and group PAV (porcine aortic valve xenograft implantation, n=9). Body weight, implanted stent diameter, follow-up duration, peak pressure gradient between RV and PA before and immediately after implantation, and the result of the animal (died unexpectedly or underwent scheduled terminal procedure) are described.

Animals	Body weight (kg)	Stent diameter (mm)	Type of tissue	F/U duration (d)	Preop. peak PG (RV-PA) (mmHg)	Immediate postop. peak PG (RV-PA) (mmHg)	Result
# 4	37.5	24	PP	0	5	7	Died
# 3	40.5	26	PP	43	0	0	Died
# 18	47.5	26	PP	87	2	2	Died
# 17	45.5	24	PP	136	2	2	Died
# 16	41.0	26	PP	352	4	4	Sacrificed
# 6	37.5	24	PP	360	4	5	Sacrificed
# 5	38.0	26	PP	402	8	9	Sacrificed
# 2	36.0	26	PP	437	4	4	Sacrificed
# 1	39.0	26	PP	478	5	5	Sacrificed
# 7	36.5	26	PAV	7	3	6	Died
# 13	37.0	22	PAV	48	3	3	Died
# 9	17.5	18	PAV	98	1	1	Died
# 11	39.5	24	PAV	139	2	2	Died
# 8	39.5	26	PAV	173	2	5	Died
# 15	42.5	26	PAV	258	7	5	Died
# 12	33.0	24	PAV	376	1	1	Sacrificed
# 14	50.0	22	PAV	530	5	8	Sacrificed
# 10	19.5	18	PAV	546	1	1	Sacrificed

Table 2. Pressure gradients between the RV and PA before implantation, immediately after implantation, and before the scheduled terminal procedure. Pressure gradients of each group were shown as median values [interquartile range]. There were no significant differences between the 2 groups at each time point.

Group	**Preop. peak PG (RV-PA) (mmHg)**	**Immediate postop. peak PG (RV-PA) (mmHg)**	**peak PG (RV-PA) at sacrifice (mmHg)**
Porcine pericardium	4.0 [2.0, 6.0]	4.0 [3.0, 5.0]	5.0 [4.0, 6.0]
	n=9	*n=9*	*n=4*
Porcine aortic valve	2.0 [1.0, 3.0]	5.0 [2.0, 8.0]	6.0 [4.0, 8.0]
	n=9	*n=9*	*n=3*
p-value	*0.297*	*0.863*	*1.000*

Enzyme-linked immunosorbent assay (ELISA)

Anti-α-Gal serum IgM and IgG antibody titers were <0.3 OD (dilution ratio of 1:10) in all animals, regardless of the time point (Figure 1-1). There were no differences in anti-α-Gal serum IgM titers between the 2 groups. However, anti-α-Gal IgG titers were higher in group PP than in group PAV at 2 weeks after implantation (p=0.006). Anti-α-Gal IgM antibody titers showed no significant differences between the 2 groups based on the time sequence (p=0.152); however, anti-α-Gal IgG antibody titers showed differences between the 2 groups based on the time sequence (p=0.003) (Table 3). Although anti-α-Gal IgG antibody titers were lower in group PAV than in group PP and showed differences between the 2 groups based on the time sequence, they have little clinical implications because the absolute values of the titers were low in both groups.

In order to examine the changes in antibody titers, anti-α-Gal serum antibody titers measured before the terminal procedure were grouped by the time point because the time points of performing the terminal procedure were different between animals. Time points were grouped as pre-implantation, immediately post-implantation; and 2 weeks, 1~3 months, 3~6 months, 6~12 months, and 12~24 months post-implantation. When the antibody titers were calculated arithmetically, the average anti-α-Gal IgM and IgG titers were low throughout the observed time points, regardless of the group (Figure 1-2).

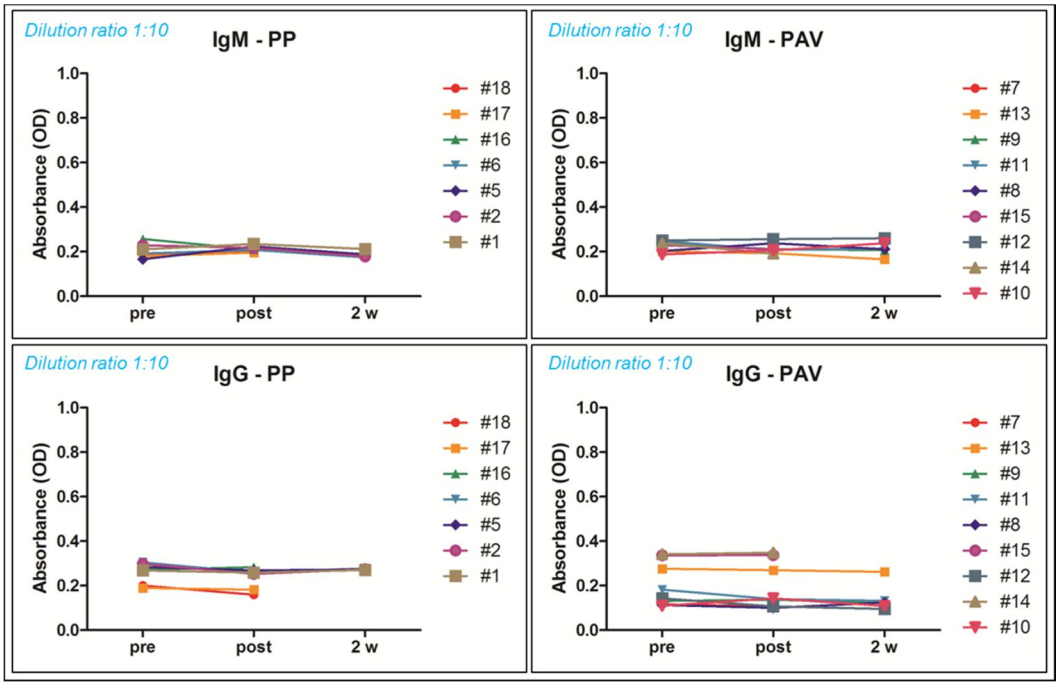

Figure 1-1. Anti-α-Gal serum IgM and IgG antibody titers measured at pre-implantation, immediately post-implantation, and 2 weeks post-implantation (IgM-PP, anti-α-Gal IgM titers of porcine pericardial xenograft group; IgM-PAV, anti-α-Gal IgM titers of porcine aortic valve xenograft group; IgG-PP, anti-α-Gal IgG titers of porcine pericardial xenograft group; IgG-PAV, anti-α-Gal IgG titers of porcine aortic valve xenograft group).

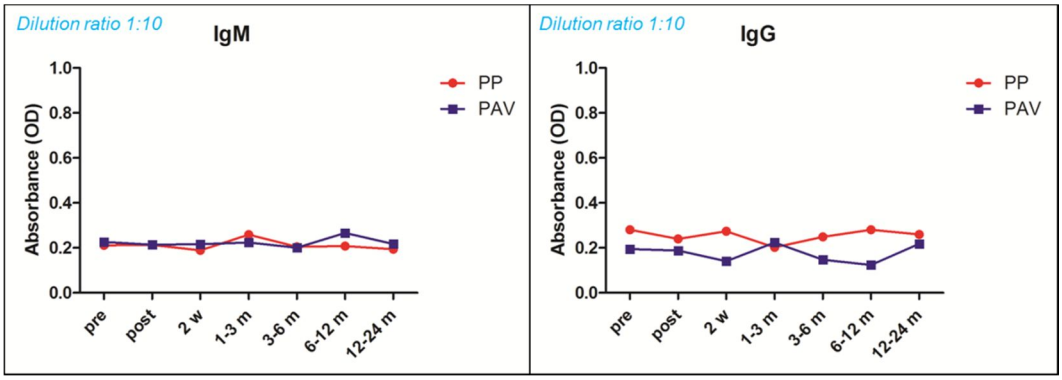

Figure 1-2. Changes in anti-α-Gal serum IgM and IgG antibody titers grouped by time point: pre-implantation, immediately post-implantation; and 2 weeks, 1~3 months, 3~6 months, 6~12 months, and 12~24 months post-implantation. The antibody titers were calculated arithmetically. (group PP, porcine pericardial xenograft implantation; group PAV, porcine aortic valve xenograft implantation).

Table 3. Median values of anti-α-Gal serum IgM and IgG antibody titers measured at pre-implantation, immediately post-implantation, 2 weeks post-implantation, and before death or terminal procedure. **p*-values, comparing the antibody titers of porcine pericardial and porcine aortic valve xenograft groups at specific time point. ***p*-values, comparing the differences of antibody titers based on the time sequence in both groups.

Type of tissue	Pre-implantation	Immediately post-implantation	2 weeks post-implantation	Before terminal procedure
Anti-α-Gal IgM antibody				
Porcine pericardium	0.2104	0.2095	0.1835	0.2023
	n = 5	*n = 7*	*n = 4*	*n = 7*
Porcine aortic valve	0.2370	0.2083	0.2114	0.2108
	n = 9	*n = 9*	*n = 6*	*n = 8*
p-value*	***0.438***	***0.837***	***0.230***	***0.620***
p-value**		***0.152***		
Anti-α-Gal IgG antibody				
Porcine pericardium	0.2834	0.2585	0.2734	0.2490
	n = 5	*n = 7*	*n = 4*	*n = 7*
Porcine aortic valve	0.1432	0.1396	0.1240	0.1518
	n = 9	*n = 9*	*n = 6*	*n = 8*
p-value*	***0.190***	***0.252***	***0.006***	***0.073***
p-value**		***0.003***		

Gross examination & histological study

Gross examination of extracted valves after the terminal procedure showed no gross calcifications or RVOT obstructions in either group, regardless of implantation duration. Typical findings from extracted valves from group PP (animal #6, 360 days post-implantation; animal #2, 437 days post-implantation) and group PAV (animal #15, 258 days post-implantation; animal #14, 530 days post-implantation) are shown in Figure 2. In both groups, H&E staining showed well-decellularized features with a minimal amount of inflammatory cellular infiltrates, and Masson's trichrome and von Kossa staining revealed intact collagen fibers without significant calcific deposits. Light microscopy findings from extracted valves from group PP (animal #6, 360 days post-implantation) and group PAV (animal #12, 376 days post-implantation) are presented in Figure 3. When compared with the H&E staining results of fresh porcine pericardial and aortic valve tissues, effective decellularization with minimal destruction of collagen fibers was clearly seen in tissues treated by multiple anti-calcification methods.

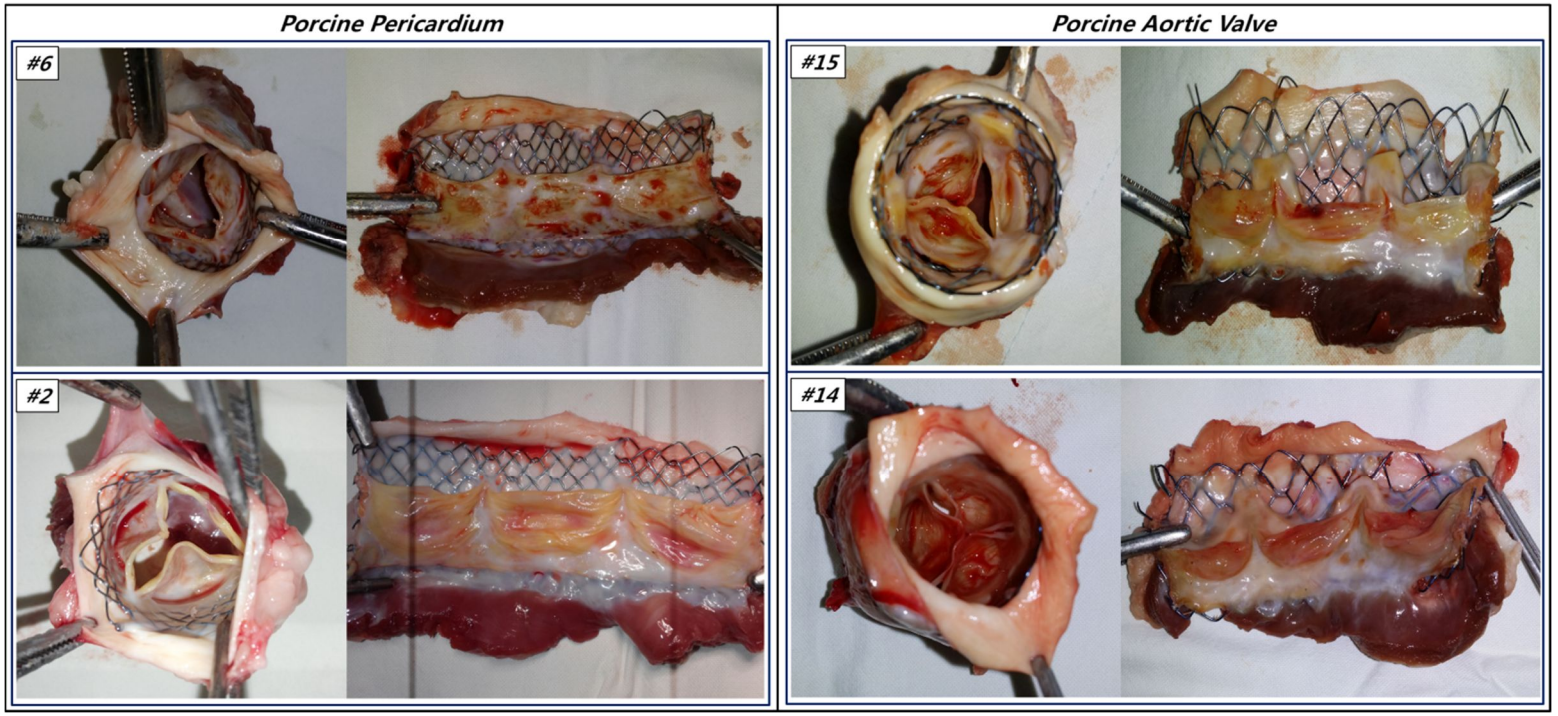

Figure 2. Gross examination of typical cases of extracted stented valves from porcine pericardial xenograft group (animal #6, #2) and porcine aortic valve xenograft group (animal #15, #14). No gross calcifications or RVOT obstructions are seen.

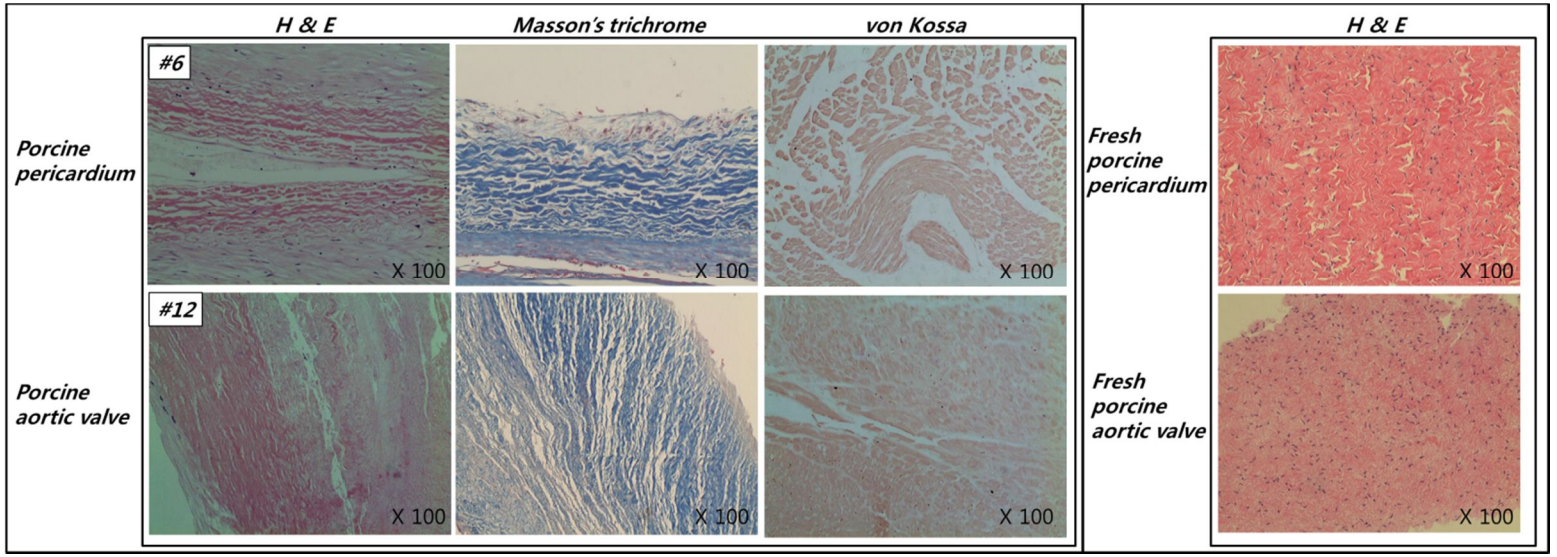

Figure 3. Light microscopy findings of the extracted stented valves (H&E, Masson's trichrome and von Kossa staining) from porcine pericardial and aortic valve xenograft groups. Effective decellularization with minimal destruction of collagen fibers and without significant calcific deposits was clearly seen. The H&E staining results of fresh porcine pericardial and aortic valve tissue were presented for comparison.

Immunofluorescence

Immunostaining using FITC-conjugated monoclonal anti-CD11b and FITC-conjugated monoclonal anti-CD25 antibodies revealed few inflammatory cells in the tissue samples. Typical findings from extracted valves from group PP (animal #18, 87 days post-implantation; animal #17, 136 days post-implantation; animal #6, 360 days post-implantation) and group PAV (animal #13, 48 days post-implantation; animal #11, 139 days post-implantation; animal #14, 530 days post-implantation) are presented in Figure 4-1 (immunostaining with FITC-conjugated monoclonal anti-CD11b antibody) and Figure 4-2 (immunostaining with FITC-conjugated monoclonal anti-CD25 antibody). Anti-CD11b and anti-CD25 antibodies were used as markers to detect macrophages and T-cells of the animals, respectively. No significant infiltration of macrophage or T-cell was observed in both groups PP and PAV, regardless of the implantation duration. The cells that were visualized under DAPI II counterstain seem to be nonspecific, non-inflammatory cells.

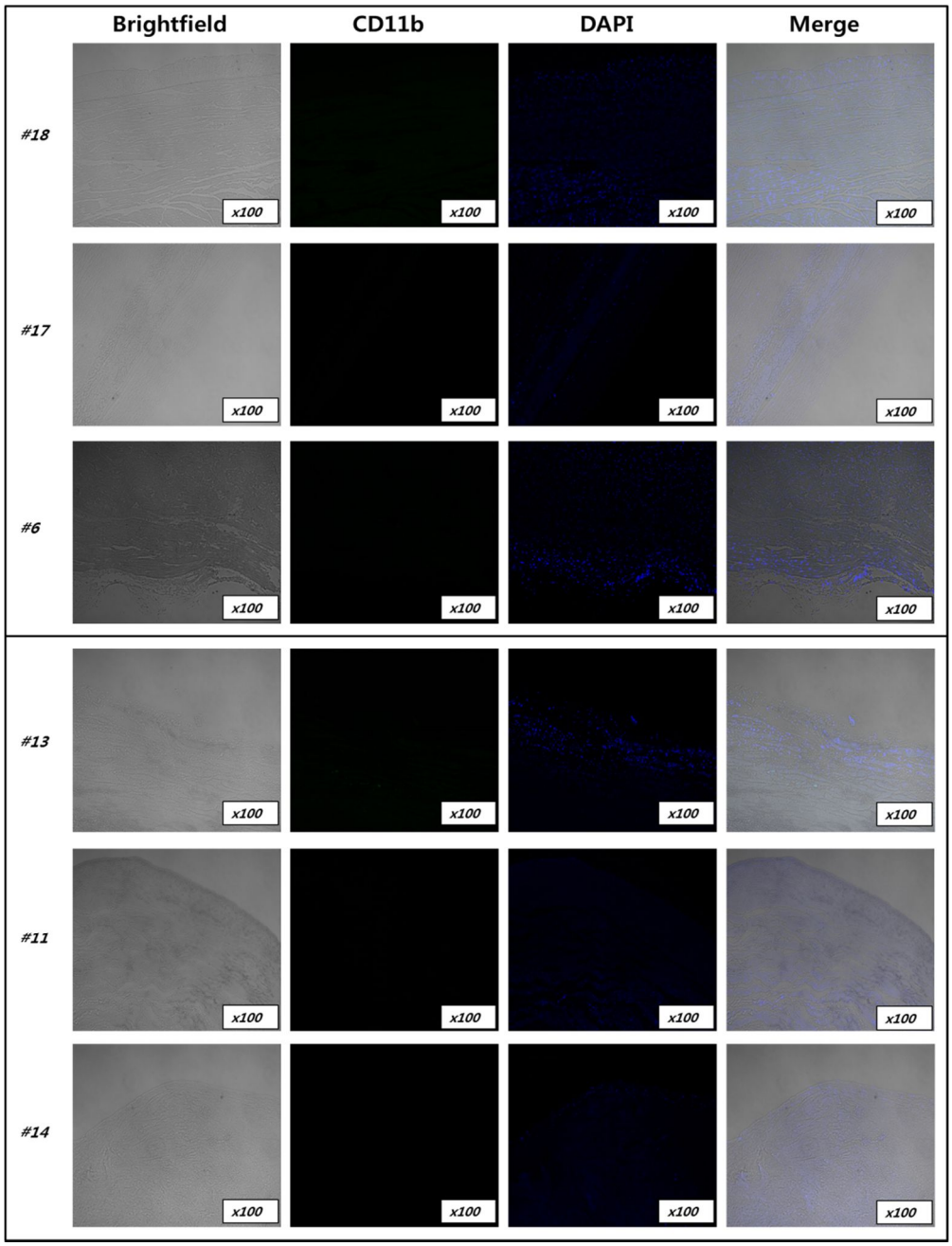

Figure 4-1. Immunostaining with FITC-conjugated monoclonal anti-CD11b (Green). The nuclei were counterstained with DAPI II. Typical findings from the animal #18, #17, #6, #13, #11, and #14 are presented.

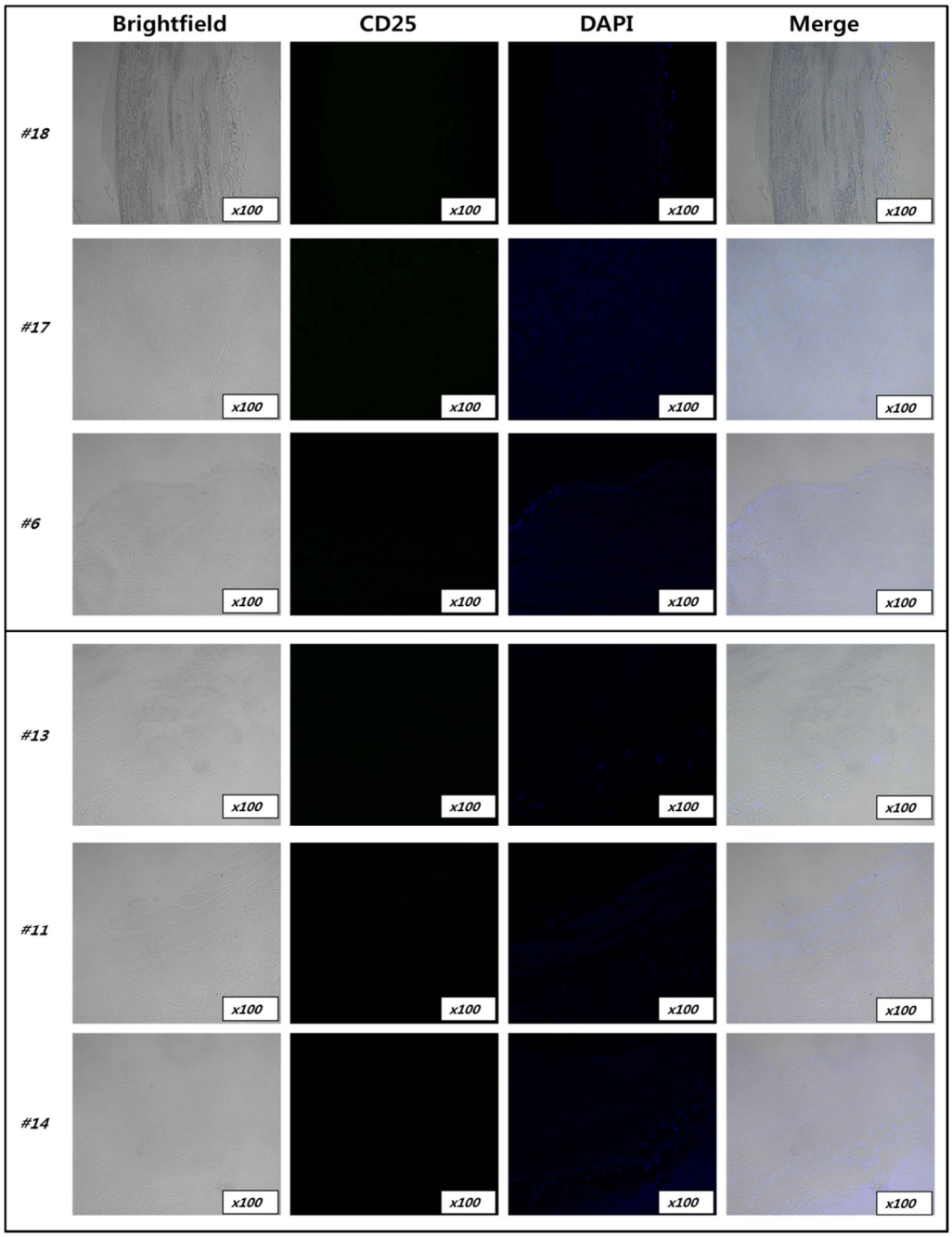

Figure 4-2. Immunostaining with FITC-conjugated monoclonal anti-CD25 (Green). The nuclei were counterstained with DAPI II. Typical findings from the animal #18, #17, #6, #13, #11, and #14 are presented.

Qualitative and quantitative calcification analysis

Computed tomography (CT) of the extracted valves revealed a low degree of calcification, regardless of implantation duration (Figure 5). Area of calcification (mm^2) and volume of calcification (mm^3) of harvested stented valves (stented valves from animals #4, #18, #17, #16, #7, #13, #11, #15, #12, #14, and #10) were calculated using CT images, and showed no significant differences between the groups ($p = 0.927$) (Table 4).

When the quantity of calcifications were analyzed using hydrolysate, median calcium contents of harvested stented valves were 2.13μg/mg [1.18μg/mg, 2.6μg/mg] and 0.95μg/mg [0.67μg/mg, 1.13μg/mg] in groups PP and PAV, respectively, demonstrating significant differences between the 2 groups ($p = 0.019$) (Figure 6).

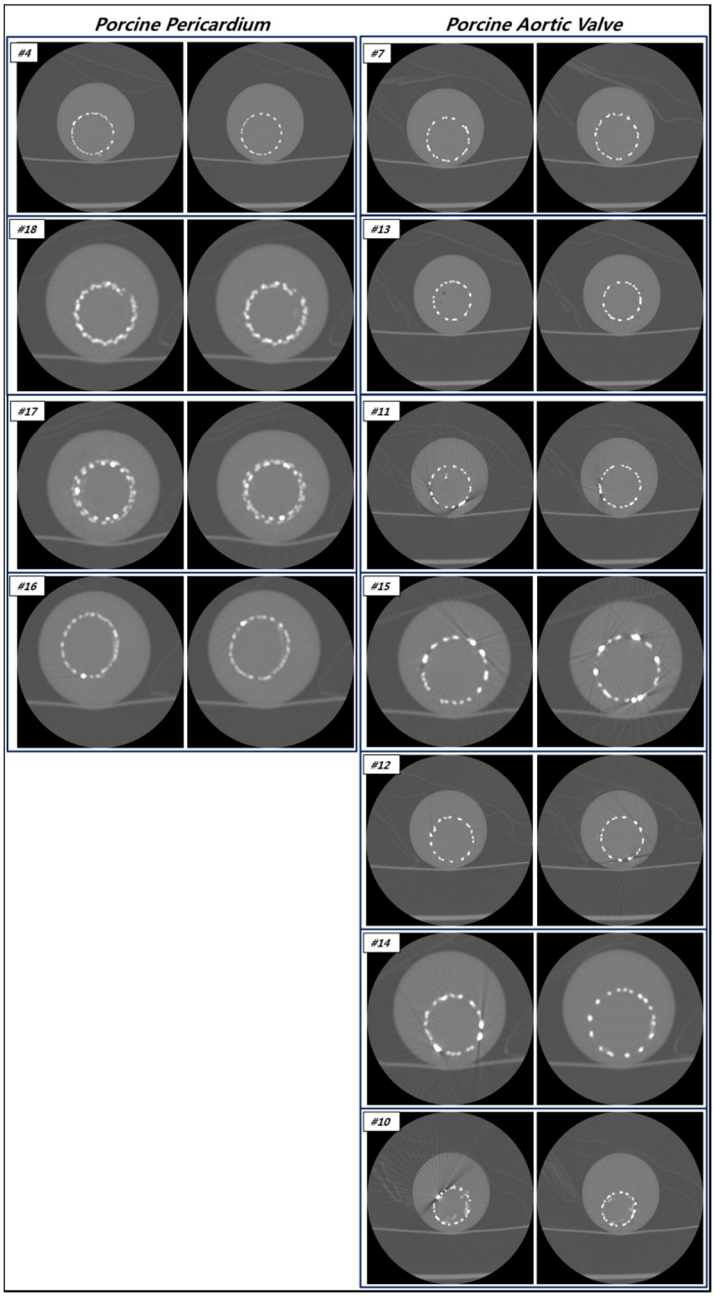

Figure 5 Computed tomography findings of the extracted stented valves from porcine pericardial group (animals #4, #18, #17, and #16) and porcine aortic valve group (animals #7, #13, #11, #15, #12, #14, and #10).

Table 4. Area of calcification (mm^2) and volume of calcification (mm^3) of extracted stented valves calculated using computed tomography images. The results showed no significant differences between the porcine pericardial and aortic valve xenograft groups ($p = 0.927$).

Type of tissue	**Sheep**	**Area of calcification (mm^2)**	**Volume of calcification (mm^3)**
Porcine pericardium	#4	0	0
	#18	13.65	17.07
	#17	2.09	2.61
	#16	0	0
Porcine aortic valve	#7	0.95	1.79
	#13	0	0
	#11	9.16	16.44
	#15	0	0
	#12	0	0
	#14	0	0
	#10	183.13	343.75
p-value		*0.927*	*0.927*

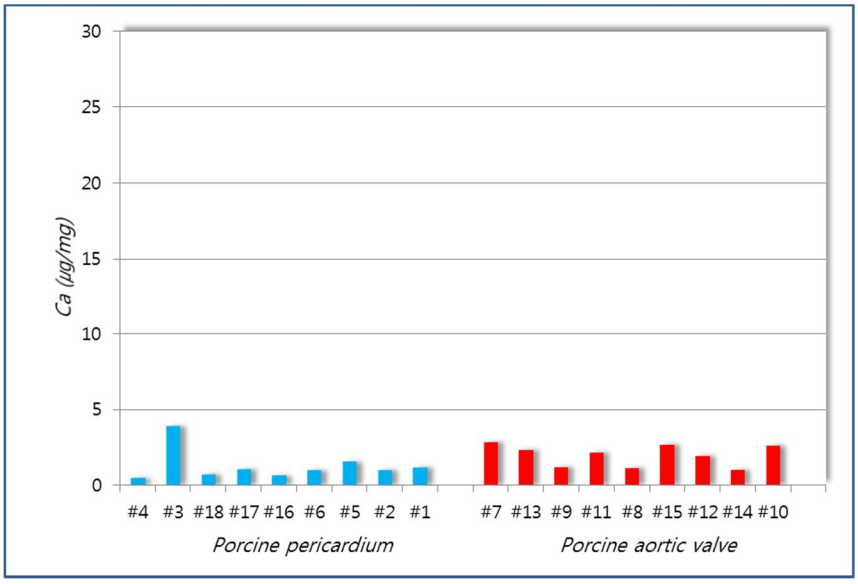

Figure 6. Quantity of calcifications analyzed using hydrolysate. Median calcium contents of harvested stented valves were 2.13μg/mg [1.18μg/mg, 2.6μg/mg] and 0.95μg/mg [0.67μg/mg, 1.13μg/mg] in porcine pericardial and aortic valve xenograft groups, respectively (p = 0.019).

4. Discussions

The present study revealed two main findings. First, all the stented porcine pericardial and aortic valve xenografts were placed in an appropriate position and demonstrated a low-pressure gradient between the RV and PA. Second, implanted stented valves showed preserved valve function and extracted valves revealed minimal structural deterioration and low levels of calcification regardless of implantation duration.

Since the first percutaneous pulmonic valve implantation in human [1], a large number of clinical implantations have been performed with satisfactory results [11, 12]. However, procedure-related complications, such as valve migration, coronary compression, or stent fractures, also have been reported [3, 13, 14]. Previous studies have suggested the ways to overcome those complications, including pre-procedural banding of the main pulmonary artery [15], performing selective coronary angiography [16], and pre-stenting of the RVOT [17]. Compared with percutaneous pulmonic valve implantation, the perventricular pulmonic valve implantation technique has advantages in that it can be applied to patients with poor peripheral access or difficult anatomy, and allows a shorter distance to the target site for larger stented valves. However, the aforementioned complications experienced by percutaneous pulmonic valve implantation were

still the most significant complications with the perventricular method. Previous studies suggested using an oversized stented valve compared to the pulmonary annulus size to prevent stent migration [18, 19]. In the present study, oversized valves were implanted and a significant valve migration was not seen in 18 animals. We expected that it would be easier to implant an oversized valve with perventricular technique than with percutaneous technique because larger stented valve can be used. Moreover, the self-expandable nature of the stented valves used in the present study might have further reduced the incidence of migration. Stents made of Nitinol wire have memory-shape property and a chronic outward force that increases 2-times when the temperature is increased from 20℃ to 37℃ [20]. Therefore, a stented valve mounted on Nitinol wire becomes stiffer when implanted and firmly implanted at appropriate position. Advantages of the perventricular pulmonic valve implantation technique are: (1) a larger-sized stented valve could be implanted in order to prevent stent migration, (2) the stented valve could be implanted precisely because of a shorter distance to the target site, and (3) the self-expandable nature of the stented valve makes it possible for the stented valve to be firmly implanted at appropriate position and may reduce the valve migration rate. In the present study, there was one procedure-related mortality due to coronary artery compression caused by the stented valve in an animal with anomalous coronary artery anatomy. We suggest performing coronary angiography during the procedure to avoid such fatal

complications.

In the present study, 8 of 18 animals (44.4%) survived >300 days, with 546 days the longest length of survival. Although more than half of the animals died before the scheduled terminal procedure of 300 days post-implantation, autopsy findings revealed that none of the deaths were related to cardiac problems, except in the single procedure-related mortality with anomalous coronary artery anatomy. No significant pulmonary regurgitation or stenosis was observed in any animal, and low-pressure gradients between the RV and PA were maintained throughout the study period. There were no significant differences in pressure gradients between the 2 groups. Both porcine pericardial and aortic valve xenografts showed preserved valve function and good hemodynamic performances. Echocardiographic and cardiac catheterization studies showed excellent hemodynamic properties and showed no differences between the two stented valves.

Bioprosthetic valves made of xenogenic tissues have advantages of lower thrombogenicity and good hemodynamic profiles. However, dystrophic degenerative calcification reducing long-term durability is a main limitation, and multiple mechanisms such as immunological, chemical, hemodynamic and mechanical factors have been demonstrated to be involved in calcification process [6]. Glutaraldehyde (GA) commonly has been used to fix bioprosthetic tissues for increasing tissue stability and reducing antigenicity; however, that GA fixation

process may cause the tissue to be more prone to calcification [7]. Free aldehyde groups of GA, tissue phospholipids, and residual non-viable connective tissue cells of the bioprosthetic tissue have been studied as causal factors involved in the calcification process [21, 22]. Detoxification with amino acids to block the free aldehyde groups [23, 24], removal of bioprosthetic tissue phospholipids with various alcoholic solutions [25, 26], and other cross-linking agents [22, 27] have been used to suppress factors involved in calcification process. Galα1,3-Galβ1-4GlcNAc-R (α-Gal) epitope has also been suggested as one of the most important antigens involved in immune response against xenogenic tissues [28, 29]. The α-Gal epitope exists as a cell surface molecule in most species, except humans and old world monkeys [30]. In this regard, implanting porcine xenograft tissues into sheep is a type of concordant xenotransplantation, because both donor and recipient have α-Gal epitopes and will not provoke an anti-α-Gal immune response. The present study showed that both anti-α-Gal IgM and IgG titers were low throughout the time period. Although anti-α-Gal IgG antibody titers were lower in group PAV than in group PP, they did not have a clinical implication because of the low absolute value of the antibody titers. In gross examination, the extracted valves did not show gross calcifications or RVOT obstructions, regardless of groups and implantation duration. Microscopic findings also revealed well-decellularized tissues, with intact collagen fibers and minimal calcific deposits in both groups. In the previous studies from our group, the novel

tissue valve preservation techniques, including simultaneous use of multiple anticalcification methods, decellularization, immunological modification with α-galactosidase, organic solvent treatment, and detoxification, were effective in preventing dystrophic calcification of bioprostheses [8-10]. Although our tissue preservation technique is a laborious and time-consuming protocol, it seems to be an efficient method in decellularization and tissue stabilization. The immunofluorescence results in the present study showed that the tissues revealed no significant infiltration of macrophages or T-cells in both groups, regardless of implantation duration. Our tissue preservation protocol revealed to be efficient in suppressing the immune response against xenogenic tissues. Calcium contents also were low in both groups. Although the calcium contents in group PP were low in calcium quantification using hydrolysate, the CT analysis showed no significant differences between the two groups. In the present study, we did not find any correlation in the degree of calcification between the two calcification measurements (using hydrolysate of sampled tissue versus CT analysis), which seemed to be caused by differences in diagnostic accuracy of the two method. The present study may conclude that the degree of calcification was low in both groups and that there were no differences in degree of calcification between the two xenografts, because the absolute values of calcium contents were low.

Xenogenic pericardial and aortic valve tissues have been used widely for cardiac bioprostheses. The histologic structures of the two tissues are different.

Aortic valve cusps are composed of three layers: fibrosa, spongiosa, and ventricularis layers. The fibrosa layer faces the aorta and is primarily composed of collagen fibers. The ventricularis layer faces the left ventricle and is composed of elastin and collagen. The central spongiosa layer is loosely arranged collagen layer and mainly composed of galycosaminoglycans and water [31, 32]. In contrast, pericardial tissue is a relatively homogenous sheet of laminated collagen without clear layers [31]. The types of component collagen fibers are also different: the aortic valve cusp contains type I and type III collagen fibers; however, the pericardium is predominantly composed of type I collagen [33]. Despite the differences in histologic structure of the pericardial and aortic valve tissues, calcification of the two tissues has been shown to be qualitatively, quantitatively, and mechanistically similar [34, 35]. Similarly, the calcification of the xenogenic tissues of the two groups was qualitatively and quantitatively similar in the present study.

In the present study, porcine pericardium was used instead of bovine pericardium, which commonly has been used as xenograft. Although both bovine and porcine pericardia have similar hemodynamic profiles and do not exhibit a significant difference in the degree of calcification [36, 37], there are histologic differences between pericardial tissues from the different species. Bovine pericardium has higher collagen content, thicker, and has coarser connective tissue fiber components than porcine pericardium [38, 39], whereas porcine

pericardium possesses more compact fiber components and relatively larger quantities of fat cells in regular layers [40]. In the present study, porcine pericardial xenograft was used because we expected that a xenograft with less rigid and thinner properties would be better for low-pressure system such as pulmonary valve position [41]. Although porcine pericardium is not yet being popularly used than bovine pericardium, it can also serve a role as material for bioprostheses.

Previous studies showed that pericardial valves had better durability and superior hemodynamics than porcine aortic valves [42, 43]; however, there were insufficient studies so far comparing hemodynamics and durability between the different types of xenografts at pulmonary position. In addition, the durability of bioprosthetic valves in the pulmonary position has been shown to be suboptimal [44]. Hemodynamic performance and durability of the bioprostheses are influenced by multiple factors, such as tissue type, tissue preservation methods, patients, and the position of the implanted valve. The results of the present study implied that both porcine pericardial and aortic valve tissues treated with our novel tissue treatment methods were excellent, and did not show any differences in hemodynamic properties and degenerative calcifications after long-term implantation into the sheep. Our novel tissue treatment method and implantation using a perventricular implantation technique could be used as an effective treatment option in patients requiring pulmonic valve replacement.

Limitations of the study

There are limitations to the present study that must be recognized. First, More than half of the animals died unexpectedly and data such as hemodynamic profile and serum anti-α-Gal antibody titers before death were not collected. Causes of death of the sheep were mostly gastrointestinal inflammation or anima l stress in cage. Large animal care and handling is a difficult area requiring many financial and labor resources. More meticulous care of the sheep and medical management should be performed in order to decrease such unexpected deaths. Second, calcium analysis demonstrated inconsistent results in the present study, which might result from small number of the animal samples, low absolute quantity of calcification, the tissue sampling sites, and instrumental errors. These limitations should be overcome and corrected in future studies.

Conclusions

Perventricular pulmonic valve implantation is a feasible technique that has a high success rate and produces a good hemodynamic profile. Our tissue treatment technique using simultaneous multiple anti-calcification methods, decellularization, immunological modification with α-galactosidase, organic solvent treatment, and detoxification proved to be an applicable method for treatment of bioprostheses. Both stented valves made of porcine pericardial and

aortic valve tissues showed no significant differences in hemodynamic profile, midterm durability and degree of degenerative dystrophic calcification.

5. References

1. Bonhoeffer P, Boudjemline Y, Saliba Z et al. *Percutaneous replacement of pulmonary valve in a right-ventricle to pulmonary-artery prosthetic conduit with valve dysfunction.* Lancet 2000;356:1403-5.

2. Lurz P, Coats L, Khambadkone S et al. *Percutaneous pulmonary valve implantation: impact of evolving technology and learning curve on clinical outcome.* Circulation 2008;117:1964-72.

3. Eicken A, Ewert P, Hager A et al. *Percutaneous pulmonary valve implantation: Two-centre experience with more than 100 patients.* Eur Heart J 2011;32:1260-5.

4. Luthra S, Westaby S, Ormerod O, Wilson N, Forfar C. *Transventricular pulmonary valve implantation in corrected truncus arteriosus.* Ann Thorac Surg 2012;93:660-1.

5. Vongpatanasin W, Hillis D, Lange R. *Prosthetic heart valves.* N Engl J Med 1996;335:407-16.

6. Gabbay S, Kadam P, Factor S, Cheung TK. *Do heart valve bioprosthesis degenerate for metabolic or mechanical reasons?* J Thorac Cardiovasc Surg 1988;95:208-15.

7. Manji RA, Zhu LF, Nijjar NK, Rayner DC, Korbutt GS, Churchill TA et

al. *Glutaraldehyde - fixed bioprosthetic heart valve conduits calcify and fail from xenograft rejection.* Circulation 2006;114:318-27.

8. Lim H-G, Kim GB, Jeong S, Kim YJ. *Development of a next-generation tissue valve using a glutaraldehyde-fixed porcine aortic valve treated with decellularization, α-galactosidase, space filler, organic solvent and detoxification.* Eur J Cardiothorac Surg 2014:ezu385.

9. Kim M-S, Jeong S, Lim H-G, Kim YJ. *Differences in xenoreactive immune response and patterns of calcification of porcine and bovine tissues in alpha-Gal knock-out and wild-type mouse implantation models.* Eur J Cardiothorac Surg 2015;48:392-9.

10. Kim M-S, Lim H-G, Kim YJ. *Calcification of decellularized and alpha-galactosidase-treated bovine pericardial tissue in an alpha-gal knock-out mouse implantation model: Comparison with primate pericardial tissue.* Eur J Cardiothorac Surg 2015:ezv189.

11. Khambadkone S, Coats L, Taylor A, Boudjemline Y, Derrick G, Tsang V et al. *Percutaneous pulmonary valve implantation in humans : Results in 59 consecutive patients.* Circulation 2005;112:1189-97.

12. Boone RH, Webb JG, Horlick E, Benson L, Cao QL, Nadeem N et al. *Transcatheter pulmonary valve implantation using the Edwards SAPIEN transcatheter heart valve.* Cathet Cardiovasc Interv 2010;75:286-94.

13. Cubeddu RJ, Hijazi ZM. *Bailout perventricular pulmonary valve*

implantation following failed percutaneous attempt using the Edwards SAPIEN transcatheter heart valve. Cathet Cardiovasc Interv 2011;77:276-80.

14. McElhinney DB, Hellenbrand WE, Zahn EM, Jones TK, Cheatham JP, Lock JE et al. *Short- and medium-term outcomes after transcatheter pulmonary valve placement in the expanded multicenter US melody valve trial.* Circulation 2010;122:507-16.

15. Travelli FC, Herrington CS, Ing FF. *A novel hybrid technique for transcatheter pulmonary valve implantation within a dilated native right ventricular outflow tract.* J Thorac Cardiovasc Surg 2014;148:e145-6.

16. Sridharan S, Coats L, Khambadkone S, Taylor AM, Bonhoeffer P. *Images in cardiovascular medicine. Transcatheter right ventricular outflow tract intervention: the risk to the coronary circulation.* Circulation 2006;113:e934-5.

17. Haas NA, Moysich A, Neudorf U, Mortezaeian H, Abdel-Wahab M, Schneider H et al. *Percutaneous implantation of the Edwards SAPIEN pulmonic valve: initial results in the first 22 patients.* Clin Res Cardiol 2013;102:119-28.

18. Meng G-W, Zhou J-Y, Tang Y, Ye Z-K, Zhang Y, Liu G-M et al. *Off-pump pulmonary valve implantation of a valved stent with an anchoring mechanism.* Ann Thorac Surg 2009;87:597-602.

19. Schreiber C, Hoerer J, Vogt M, Fratz S, Kunze M, Galm C et al. *A new treatment option for pulmonary valvar insufficiency: first experiences with implantation of a self-expanding stented valve without use of cardiopulmonary*

bypass. Eur J Cardio-Thorac Surg 2007;31:26-30.

20. Stoeckel D, Pelton A, Duerig T. *Self-expanding nitinol stents: material and design considerations.* Eur Radiol 2004;142:351-8.

21. Manji RA, Zhu LF, Nijjar NK, Rayner DC, Korbutt GS, Churchill TA et al. *Glutaraldehyde-fixed bioprosthetic heart valve conduits calcify and fail from xenograft rejection.* Circulation 2006;114:318-27.

22. Schoen FJ, Levy RJ. *Calcification of tissue heart valve substitutes: progress toward understanding and prevention.* Ann Thorac Surg 2005;79:1072-80.

23. Lee C, Kim SH, Choi S-H, 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.

24. Grimm M, Grabenwoger M, Eybl E, Moritz A, Bock P, Muller MM et al. *Improved biocompatibility of bioprosthetic heart valves by L-glutamic acid treatment.* J Card Surg 1992;7:58-64.

25. Vyavahare N, Hirsch D, Lerner E, Baskin JZ, Schoen FJ, Bianco R et al. *Prevention of bioprosthetic heart valve calcification by ethanol preincubation. Efficacy and mechanisms.* Circulation 1997;95:479-88.

26. Gratzer PF, Pereira CA, Lee JM. *Solvent environment modulates effects of glutaraldehyde crosslinking on tissue-derived biomaterials.* J Biomed Mater

Res 1996;31:533-43.

27. Chang Y, Hsu CK, Wei HJ, Chen SC, Liang HC, Lai PH et al. *Cell-free xenogenic vascular grafts fixed with glutaraldehyde or genipin: in vitro and in vivo studies.* J Biotechnol 2005;120:207-19.

28. Good AH, Cooper DKC, Malcolm AJ, Ippolito RM, Koren E, Neethling FA et al. *Identification of carbohydrate structures that bind human antiporcine antibodies: implications for discordant xenografting in humans.* Transplant Proc 1992;24:559-62.

29. Konakci KZ, Bohle B, Blumer R, Hoetzenecker W, Roth G, Moser B et al. *Alpha-Gal on bioprostheses: xenograft immune response in cardiac surgery.* Eur J Clin Invest 2005;35:17-23.

30. Badylak SF, Gilbert TW. *Immune response to biologic scaffold materials.* Semin Immunol 2008;20:109-16.

31. Schoen FJ, Levy RJ, Piehler HR. *Pathological considerations in replacement cardiac valves.* Cardiovasc Pathol 1992;1:29-52.

32. Liao J, Joyce EM, Sacks MS. *Effects of decellularization on the mechanical and structural properties of the porcine aortic valve leaflet.* Biomaterials 2008;29:1065-74.

33. Mannschott P, Herbage D, Weiss M, Buffevant C. *Collagen heterogeneity in pig heart valves.* Biochem Biophys Acta 1976;434:177-83.

34. Schoen FJ, Tsao JW, Levy RJ. *Calcification of bovine pericardium used*

in cardiac valve bioprostheses: implications for the mechanisms of bioprosthetic tissue mineralization. Am J Pathol 1986;123:134-45.

35. Schoen FJ, Levy RJ. *Calcification of tissue heart valve substitutes: Progress toward understanding and prevention.* Ann Thorac Surg 2005;79:1072-80.

36. Chambers JB, Rajani R, Parkin D, Rimington HM, Blauth CI, Venn GE et al. *Bovine pericardial versus porcine stented replacement aortic valves: early results of a randomized comparison of the Perimount and the Mosaic valves.* J Thorac Cardiovasc Surg. 2008;136:1142-8.

37. Sinha P, Zurakowski D, Kumar TK, He D, Rossi C, Jonas RA. *Effects of glutaraldehyde concentration, pretreatment time, and type of tissue (porcine versus bovine) on postimplantation calcification.* J Thorac Cardiovasc Surg. 2012;143:224-7.

38. Braga-Vilela AS, Pimentel ER, Marangoni S, Toyama MH, de Campos Vidal B. *Extracellular matrix of porcine pericardium: biochemistry and collagen architecture. J Membr Biol.* 2008;221:15-25.

39. Liao K, Seifter E, Hoffman D, Yellin EL, Frater RW. *Bovine pericardium versus porcine aortic valve: comparison of tissue biological properties as prosthetic valves.* Artif Organs. 1992;16:361-5.

40. Fentie IH, Allen DJ, Schenck MH, Didio LJ. *Comparative electron microscopic study of bovine, porcine, and human parietal pericardium, as*

materials for cardiac valve bioprostheses. J Submicrosc Cytol. 1986;18:53-65.

41. Ichikawa Y, Noishiki Y, Kosuge T, Yamamoto K, Kondo J, Matsumoto A. *Use of a bovine jugular vein graft with natural valve for right ventricular outflow tract reconstruction : A one-year animal study.* J Thorac Cardiovasc Surg 1997;114:224-33.

42. Gao G, Wu Y, Grunkemeier GL, Furnary AP, Starr A. *Durability of pericardial versus porcine aortic valves.* J Am Coll Cardiol 2004;44:384-8.

43. Eichinger WB, Botzenhardt F, Keithahn A, Guenzinger R, Bleiziffer S, Wagner I et al. *Exercise hemodynamics of bovine versus porcine bioprostheses : A prospective randomized comparison of the Mosaicand Perimount aortic valves.* J Thorac Cardiovasc Surg 2005;129:1056-63.

44. Lee C, Park CS, Lee C-H, Kwak JG, Kim S-J, Shim W-S et al. *Durability of bioprosthetic valves in the pulmonary position : Long-term follow-up of 181 implants in patients with congenital heart disease.* J Thorac Cardiovasc Surg 2011;142:351-8.

국문 초록

서론: 본 연구는, (1) 두 종류의 서로 다른 이종 조직 (돼지의 심낭과 대동맥 판막 조직) 판막을 경심실 폐동맥 판막 치환술로 양의 폐동맥 위치에 삽입하여 혈류역학적 차이와, 내구성과 퇴행성 변화의 차이를 비교하고, (2) 전임상 시험으로서 경심실 폐동맥 판막 치환술의 안정성 및 유효성을 검증하고자 함에 그 목적이 있다.

방법: 돼지의 심낭과 대동맥 판막 조직을 얻은 뒤, 기존의 연구를 바탕으로 하여 탈세포화, 이종항원의 제거, space-filler 처리 및 glutaraldehyde를 이용한 고정, 유기용매 처리 및 항독소화 처리 등의 조직 처리를 시행하여 조직 판막의 내구성을 높이고자 하였다. 상기 처리된 이종 조직에, 형상 기억 및 생체 내 형상 복원 소재인 Nitinol wire를 이용한 스텐트 판막을 제작하였고, 이 스텐트 판막을 이용하여, 18마리의 양을 대상으로 심장 초음파 검사와 혈관 조영술을 함께 시행하며 경심실 폐동맥 판막 치환술을 시행하였다 (PP 군, 돼지 심낭 조직 판막 이식군, n=9; PAV 군, 돼지 대동맥 판막 조직 판막 이식군, n=9). 치환술 이후 혈류역학적, 면역학적 검사를 시행하였고, 양을 희생한 이후, 방사선학적, 조직학적 검사 및 칼슘 정량 검사를 시행하였다.

결과: 모든 스텐트 판막은 적절한 위치에 삽입되었으며, 치환술 직후 우심실-폐동맥 간 압력 차이는 낮게 측정되었다 (압력차이의 중간값; PP 군 vs. PAV 군, 4.0 vs. 5.0 mmHg). 18마리의 양 중에서, 8마리의 양 (PP 군, n=5;

PAV 군, n=3)이 300일 이상 생존하였다. 생존 기간의 중간값은 PP 군과 PAV 군 각각에서 352일 [87일, 402일] 과 173일 [98일, 376일] 이었다. ; 최대 생존 기간, 546일). 계획 하에 희생된 8마리의 양 중 7마리의 양 (PP 군, n=4; PAV 군, n=3)에서 희생 전 (관찰 기간의 중간값 437일 [389일, 504일]) 심초음파 및 심도자 검사를 시행하였으며, 이 때 스텐트 판막의 혈류역학적 상태 및 기능은 양호하였다. 폐동맥판 역류 혹은 협착 소견은 없었으며, 우심실-폐동맥 간 압력 차이는 낮게 측정되었다 (압력차이의 중간값; PP군 vs. PAV 군, 5.0 vs. 6.0 mmHg). Enzyme-linked immunosorbent assay로 측정한 항-α-Gal IgM 및 IgG 항체의 역가는 0.3 이하로 낮게 측정되었으며, 칼슘 정량의 중간값은 PP 군과 PAV 군에서 각각 0.95μg/mg [0.67μg/mg, 1.13μg/mg] 와 2.13μg/mg [1.18μg/mg, 2.6μg/mg] 로 측정되었다. 적출한 판막을 육안적으로 관찰하였을 때 석회화 혹은 우심실 유출로 폐색 소견은 관찰되지 않았으며, 현미경학적으로도 양 군 모두에서 석회화 소견은 거의 관찰되지 않았다. Immunofluorescence 검사 결과, 양 군에서 대식세포나 T-세포의 침윤은 관찰되지 않았다. 적출한 판막 중 11개 (PP 군, n=4; PAV 군, n=7)에 대해 컴퓨터 단층 검사를 시행하였으며, 2 군간의 유의한 차이는 관찰되지 않았다 (p=0.927). 조직을 가수분해하여 칼슘을 정량화하였을 때, PP 군의 판막에서 PAV 군의 판막보다 높은 칼슘 정량을 보였다 (PP군, n=9, 2.13μg/mg [1.18μg/mg, 2.6μg/mg]; PAV 군, n=9, 0.95μg/mg [0.67μg/mg, 1.13μg/mg]; p=0.019).

결론: 경심실 폐동맥 스텐트 판막 치환술은 높은 성공률과 좋은 혈류역학적 결과를 보이는 시술 기법이다. 연구에 이용된, 탈세포화, 이종항원의 제거, 유기용매 처리 및 항독소화 처리 등 다양한 항석회화 처리를 근간으로 하는 조직 처리 방법은 이종 조직 처리에 있어서 적용 가능한 방법이다. 돼지의 심낭과 대동맥 조직을 이용하여 만든 스텐트 판막은 혈류역학 및 내구성, 석회화의 측면에서 유의한 차이를 보이지 않았다.

.

주요어: 이종이식, 생체 인공 삽입물, 심장 판막, 생명 공학, 생체 기능 재료, 석회화

학번: 2012-21673