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MAIN TEXT ARTICLE

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A preclinical trial of perventricular pulmonary valve implantation: Pericardial versus aortic porcine valves mounted on self-expandable stent

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Abstract

Perventricular pulmonary valve implantation (PPVI) of a xenograft valve can be a less invasive technique that avoids cardiopulmonary bypass in patients who require pulmonary valve replacement. We compared the hemodynamics, durability, and histologic changes between two different xenogenic valves (pericardial vs. aortic valve porcine xenografts) implanted into the pulmonary valve position using a PPVI technique and evaluated the safety and efficacy of PPVI as a preclinical study. In 18 sheep, pericardial (group porcine pericardial [PP], n = 9) or aortic valve (group porcine aortic valve [PAV], n = 9) xenogenic porcine valves manufactured as a stented valve were implanted using a PPVI technique. The porcine tissues were decellularized, alpha-galactosidase treated, fixed with glutaraldehyde after space-filler treatment, and detoxified to improve durability. Hemodynamic and immunohistochemical studies were performed after the implantation; radiologic and histologic studies were performed after a terminal procedure. All stented valves were positioned properly after the implantation, and echocardiography and cardiac catheterization demonstrated good hemodynamic state and function of the valves. All the anti-α-Gal IgM and IgG titers were below 0.3 optical density. Computed tomography of extracted valves demonstrated no significant differences in the degree of calcification between the two groups ($P = .927$). Microscopic findings revealed a minimal amount of calcification and no significant infiltration of macrophage or T-cell in both groups, regardless of the implantation duration. The PPVI is a feasible technique. Both stented valves made of PP and PAV showed no significant differences in hemodynamic profile, midterm durability, and degree of degenerative dystrophic calcification.

KEYWORDS

animal study, congenital heart disease, pulmonary valve replacement, tissue engineering

1 | INTRODUCTION

Many congenital heart disease patients who undergo surgical reconstruction for right ventricular outflow tract (RVOT) may gradually develop the malfunction of the pulmonary valve (PV) or stenosis of the RVOT and eventually require repetitive surgical interventions. To date, surgical approach under cardiopulmonary bypass has been regarded as a

standard treatment for a dysfunctional PV; however, less invasive strategies are being increasingly proposed since the first report by Bonhoeffer and colleagues on percutaneous PV implantation.[1] Percutaneous PV implantation has advantages over surgical interventions by avoiding cardiopulmonary bypass. However, percutaneous PV implantation has limitations in patients with poor peripheral vascular access, anatomical variations, and previous prosthetic valve implantation in the tricuspid or pulmonary position[2] Perventricular PV implantation (PPVI) also has the advantage of avoiding cardiopulmonary bypass. In addition, PPVI has broader indications than a percutaneous approach and can be performed even in small patients with vascular access difficulties and in patients with a relatively large pulmonary annulus.

Of the types of prosthetic valves used for valve replacement, bioprosthetic valves have been reported to have limitations in long-term durability compared with mechanical valves.[3] 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.[4] Bioprosthetic valves commonly have been fixed with glutaraldehyde (GA) to increase tissue stability and reduce antigenicity; however, this fixation process may cause the tissue to be more prone to calcification.[5] We previously reported that novel tissue treatment techniques, including GA fixation with simultaneous use of multiple anticalcification methods, decellularization, immunological modification with α-galactosidase, organic solvent treatment, and detoxification, were effective in preventing the calcification of bioprostheses.[6-8] In the present study, we treated porcine pericardial (PP) and aortic valve tissue using the aforementioned novel tissue treatment techniques, developed a stented valve with a Nitinol wire backbone, and performed a preclinical study in sheep. The aims of the study were: (a) to evaluate the safety and efficacy of PPVI as a preclinical animal study, and (b) to compare hemodynamic profiles, durability, and degenerative histologic changes between the porcine pericardial and aortic valve xenografts which were implanted using the PPVI technique.

2 | MATERIALS AND METHODS

2.1 | Tissue preparation and fixation

Fresh porcine pericardial tissue and aortic valves were obtained from the local slaughterhouse. The porcine pericardial tissue and porcine aortic valves were washed and decellularized using normal saline, peracetic acid, ethanol, sodium dodecyl sulfate, Triton X-100, and sodium lauroyl sarcosinate. After decellularization, the tissues were treated with α-galactosidase, followed by space-filler treatment using polyethylene glycol. The tissues were fixed with GA, treated with organic solvent (ethanol and octanol), and detoxified using glycine solution. All the processes were performed according to our tissue preparation protocol (Supporting Information Appendix).

2.2 | Preparation of the self-expandable valved stent and delivery system

An initial outer stent was knitted using a single-strand Nitinol wire with 0.2032 mm (0.008 inch) thickness (Taewoong Medical Co., Gyeonggi-do, Republic of Korea). The initial valve diameter ranged from 18 to 26 mm. 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 (PAV) tissues were tightly hand-sewn to the stent wall with 5-0 braided polyester to allow good coaptation.

We developed a self-expandable trans-catheter delivery system. The proximal area of the delivery catheter had a valved stent loading zone with a 17.5 mm conical tapered tip. The diameter of the outer sheath in the stent loading zone was 18 Fr, and the diameter of the catheter shaft was 14 Fr. 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 by crimping it into the delivery catheter just before the catheter exchange. A portion of the valved stent was immersed in a saline solution until introduction (Figure 1).

2.3 | Preparation of animals

Eighteen healthy sheep (Ovis aries, Dae Gwan Ryung, Kangwon-do, Republic of Korea) of median age 18.0 [16.0, 19.5] months and median body weight 38.5 [36.6, 40.9] 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 U.S. National Research Council Committee. This study was approved by the Ethical Committee of Seoul National University Hospital (Protocol approval No. 13-2011-003-3).

2.4 | Implantation of the stented valve and follow-up

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

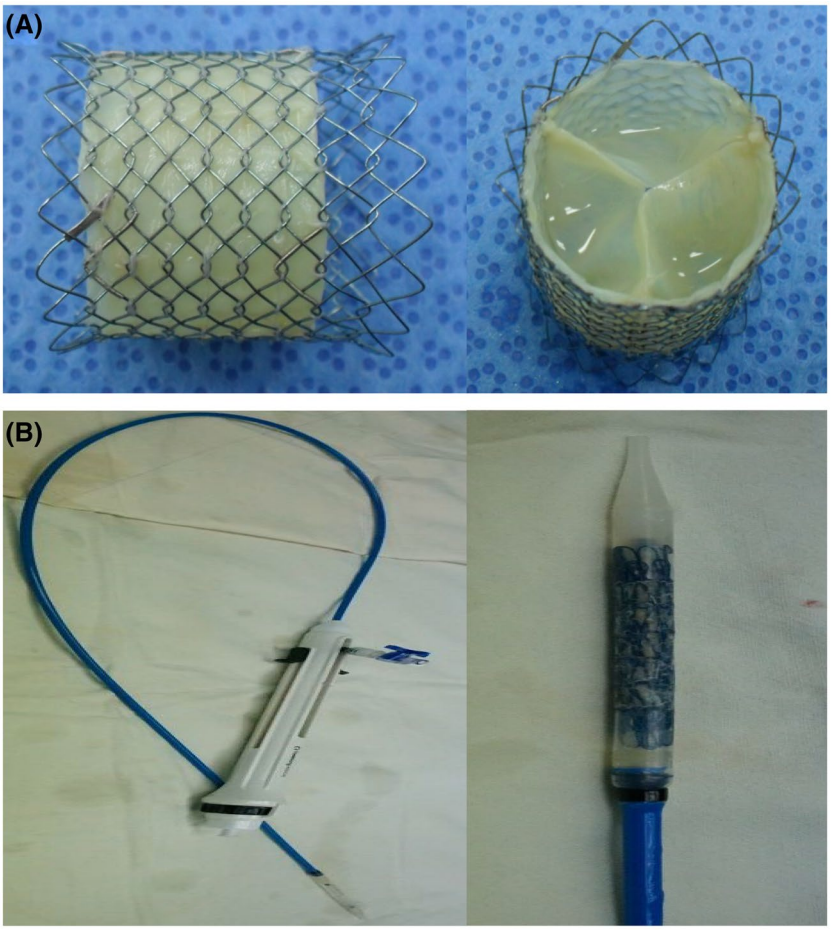

FIGURE 1 Valved stent (A) and the delivery catheter (B). The proximal part of the delivery catheter has a loading area for a valved stent with a 17.5 mm conical tapered tip

intramuscular injection of atropine (0.05 mg/kg), and intraperitoneal injection of xylazine (0.1 mg/kg) and ketamine (16 mg/kg). Animals were intubated, mechanically ventilated using isoflurane, and 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 [RV], pulmonary arterial [PA], and aortic pressure) and angiography were performed. A left thoracotomy was performed, and the thoracic cavity was entered through the fifth intercostal space. After opening the pericardium, purse-string sutures were placed near the RV apex, and PPVI was performed using the delivery catheter. Single-plane C-arm fluoroscopy and transthoracic echocardiography were used to guide valve deployment into a good position within the native PV by meticulous control of the catheter handle. After the stented valve was implanted, the RV and PA pressures were measured again by catheter 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. For perioperative antibiotic prophylaxis, intravenous cefazolin (20 mg/kg) was administered preoperatively, and intravenous penicillin G (3 mg/50 kg) was used postoperatively. Intravenous meloxicam (0.04 mL/kg) was used as postoperative analgesic. Animals survived for up to a maximum of 18 months after implantation. Terminal procedures were scheduled after 10 months postoperatively (>300 days) and efforts were made to keep the follow-up duration of the two groups similar. Angiography and echocardiography were performed prior to the scheduled terminal procedure, and a bolus injection with KCl (2 mol/L) was administered in a terminal procedure.

2.5 | Enzyme-linked immunosorbent assay (ELISA)

Blood samples of 4 mL per animal were collected before implantation, immediately after implantation, 2 weeks after implantation, and after the terminal procedure. Anti-α-Gal serum IgM and IgG antibody titers were measured by ELISA (Supporting Information Appendix).

2.6 | Immunohistological study

The heart was explanted after the terminal procedure, and the gross morphology was inspected. Representative

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)

Animals	Age (months)	Body weight (kg)	Stent diameter (mm)	Type of tissue	F/U duration (d)	Preop. peak PG (RV-PA) (mm Hg)	Immediate postop. peak PG (RV-PA) (mm Hg)	Result
# 4	16	37.5	24	PP	0	5	7	Died
# 3	18	40.5	26		43	0	0	Died
# 18	24	47.5	26		87	2	2	Died
# 17	22	45.5	24		136	2	2	Died
# 16	18	41.0	26		352	4	4	Sacrificed
# 6	16	37.5	24		360	4	5	Sacrificed
# 5	18	38.0	26		402	8	9	Sacrificed
# 2	17	36.0	26		437	4	4	Sacrificed
# 1	18	39.0	26		478	5	5	Sacrificed
# 7	16	36.5	26	PAV	7	3	6	Died
# 13	16	37.0	22		48	3	3	Died
# 9	10	17.5	18		98	1	1	Died
# 11	20	39.5	24		139	2	2	Died
# 8	18	39.5	26		173	2	5	Died
# 15	22	42.5	26		258	7	5	Died
# 12	14	33.0	24		376	1	1	Sacrificed
# 14	26	50.0	22		530	5	8	Sacrificed
# 10	10	19.5	18		546	1	1	Sacrificed

Note: 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. Peak pressure gradient between RV and PA was obtained by catheter angiography.

sections were obtained and histology of the bioprosthesis tissue samples was examined by hematoxylin-eosin (H&E) stain, Masson's trichrome stain, and von Kossa stain. Immunostaining was performed against CD11b and CD25 in order to detect macrophages and T-cells of the animals, respectively (Supporting Information Appendix).

2.7 | Qualitative and quantitative calcification analysis

The degree of calcification of the extracted stented valves was measured by both qualitative (high-definition 64-slice computed tomography) and quantitative (using hydrolysate of the tissue samples) analyses (Supporting Information Appendix).

2.8 | Statistical analysis

Statistical analysis was performed with the SPSS software package (version 22.0; SPSS, Inc., Chicago, IL, USA). Data were expressed as median [interquartile range]. A P value of <.05 was considered statistically significant. Comparisons between groups were performed using the Mann-Whitney U-test. The autoregressive linear mixed-effects model was applied in order to analyze the changes in serum antibody titers and to assess the difference over time between the groups.

3 | RESULTS

3.1 | Overall outcomes

In group PP, five of nine animals survived >300 days (median 352 days [87, 402]). Three animals died of infection and one 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, three animals survived >300 days (median 173 days [98, 376]). Five animals died of infection and one 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.

Echocardiography and cardiac catheterization 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. Echocardiographic studies which were performed immediately after implantation, during the follow-up period based on the animal's condition, and before the terminal procedure, did not demonstrate any significant pulmonary regurgitation or stenosis in both groups. Cardiac catheterization demonstrated that preoperative median pressure gradients between the RV and PA were 4.0 mm Hg [2.0, 6.0] and 2.0 mm Hg [1.0, 3.0] in groups PP and PAV, respectively (P = .297). Immediately after implantation, median pressure gradients between the RV and PA were 4.0 mm Hg ([3.0, 5.0]) and 5.0 mm Hg [2.0, 8.0] in groups PP and PAV, respectively (P = .863). The median pressure gradients measured before the terminal procedure were 5.0 mm Hg [4.0, 6.0] and 6.0 mm Hg [4.0, 8.0] in groups PP and PAV, respectively (P > .999).

3.2 | Enzyme-linked immunosorbent assay

Anti-α-Gal serum IgM and IgG antibody titers were <0.3 optical density (dilution ratio of 1:10) in all animals, regardless of the time point. There were no differences in anti-α-Gal serum IgM titers between the two groups. Anti-α-Gal serum IgG titers also showed no significant differences between the two groups at pre-implantation, immediately after the implantation, and before the terminal procedure. However, anti-α-Gal IgG titers were higher in group PP than in group PAV at 2 weeks after implantation (P = .006) (Table 2). The changes in anti-α-Gal IgM and anti-α-Gal IgG antibody titers throughout the observed time points showed no significant difference between the two groups (P = .341 and P = .956, respectively) (Figure 2).

3.3 | Immuno-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 (Figure 3). In both groups, H&E staining showed well-decellularized features with a minimal amount of inflammatory cellular infiltrates in the matrix, and Masson's trichrome and von Kossa staining revealed intact collagen fibers without significant calcific deposits (Figure 4). 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. Immunostaining using fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-CD11b and FITC-conjugated monoclonal anti-CD25 antibodies revealed few inflammatory cells in the tissue

TABLE 2 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

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*	.438	.837	.230	.620
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*	.190	.252	.006	.073

**P* values, comparing the antibody titers of porcine pericardial and porcine aortic valve xenograft groups at a specific time point.

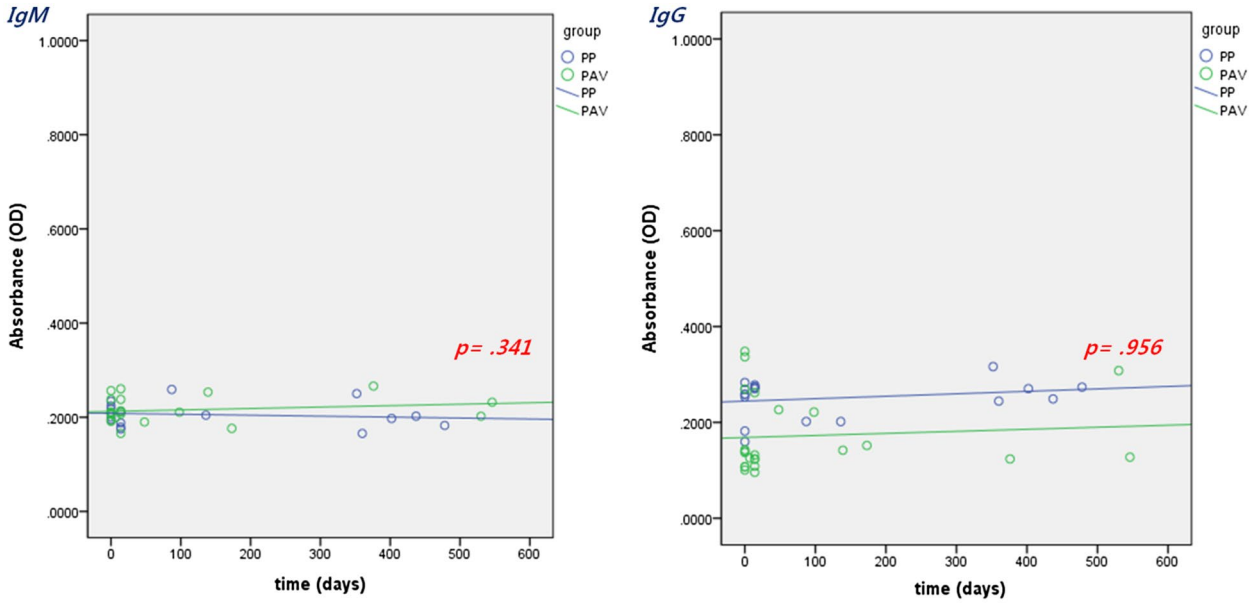

FIGURE 2 The scatterplot and fitted lines of anti-α-Gal serum IgM and IgG antibody titers (PP, antibody titers of porcine pericardial xenograft group; PAV, antibody titers of porcine aortic valve xenograft group). According to the linear mixed effect model analysis, the changes in anti-α-Gal IgM and IgG antibody titers over time showed no significant difference between the two groups ($P = .341$ and $P = .956$, respectively)

samples (Figure 5). Anti-CD11b and anti-CD25 antibodies were used as markers to detect macrophages and T-cells of the animals, respectively. No significant macrophage or T-cell infiltration was observed in either group, regardless of the implantation duration.

3.4 | Qualitative and quantitative calcification analysis

Computed tomography (CT) of the extracted valves revealed a low degree of calcification, regardless of implantation

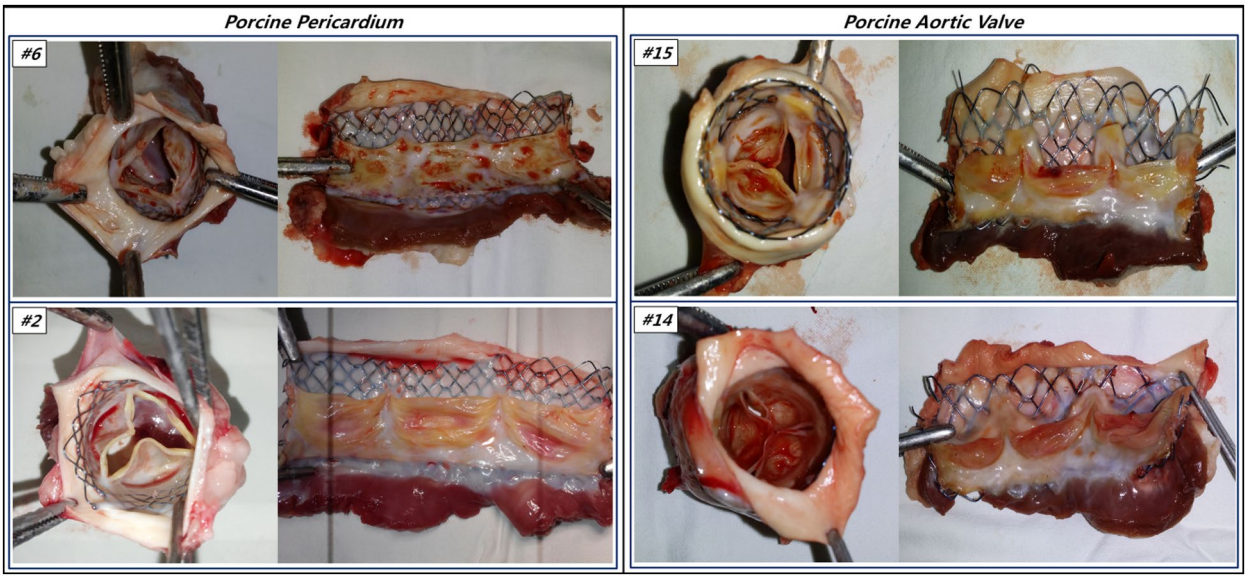

FIGURE 3 Gross examination of typical cases of extracted stented valves from porcine pericardial xenograft group (animal #6, #2) and porcine aortic valve xenograft group (animal #15, #14)

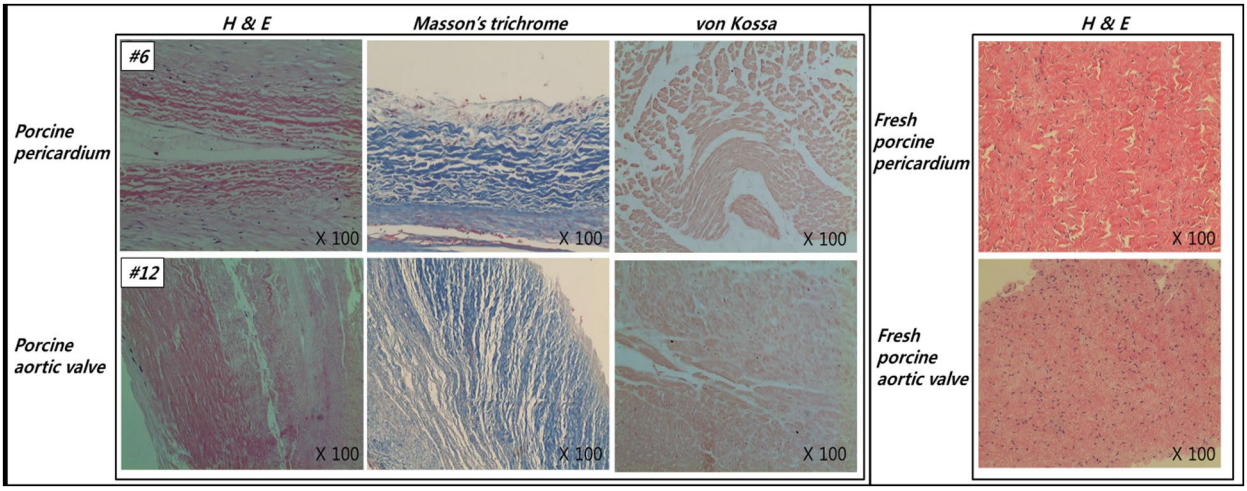

FIGURE 4 Light microscopy findings of the extracted stented valves (H&E, Masson's trichrome, and von Kossa staining) from porcine pericardial and aortic valve xenograft groups. The H&E staining results of fresh porcine pericardial and aortic valve tissue were presented for comparison

duration (Figure 6). Area of calcification (mm^2) and volume of calcification (mm^3) of harvested stented valves were calculated using CT images and showed no significant differences between the groups ($P = .927$). When the number of calcifications was analyzed using hydrolysate, median calcium contents of harvested stented valves were 2.13 μg/mg [1.18, 2.6] and 0.95 μg/mg [0.67, 1.13] in groups PP and PAV, respectively. Although the statistical analysis revealed significant differences between the two groups ($P = .019$), the absolute values of calcifications were low in both groups.

4 | DISCUSSION

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 showed minimal structural deterioration and low levels of calcification regardless of implantation duration.

Since the first clinical percutaneous PV implantation,[1] a large number of clinical implantations have been

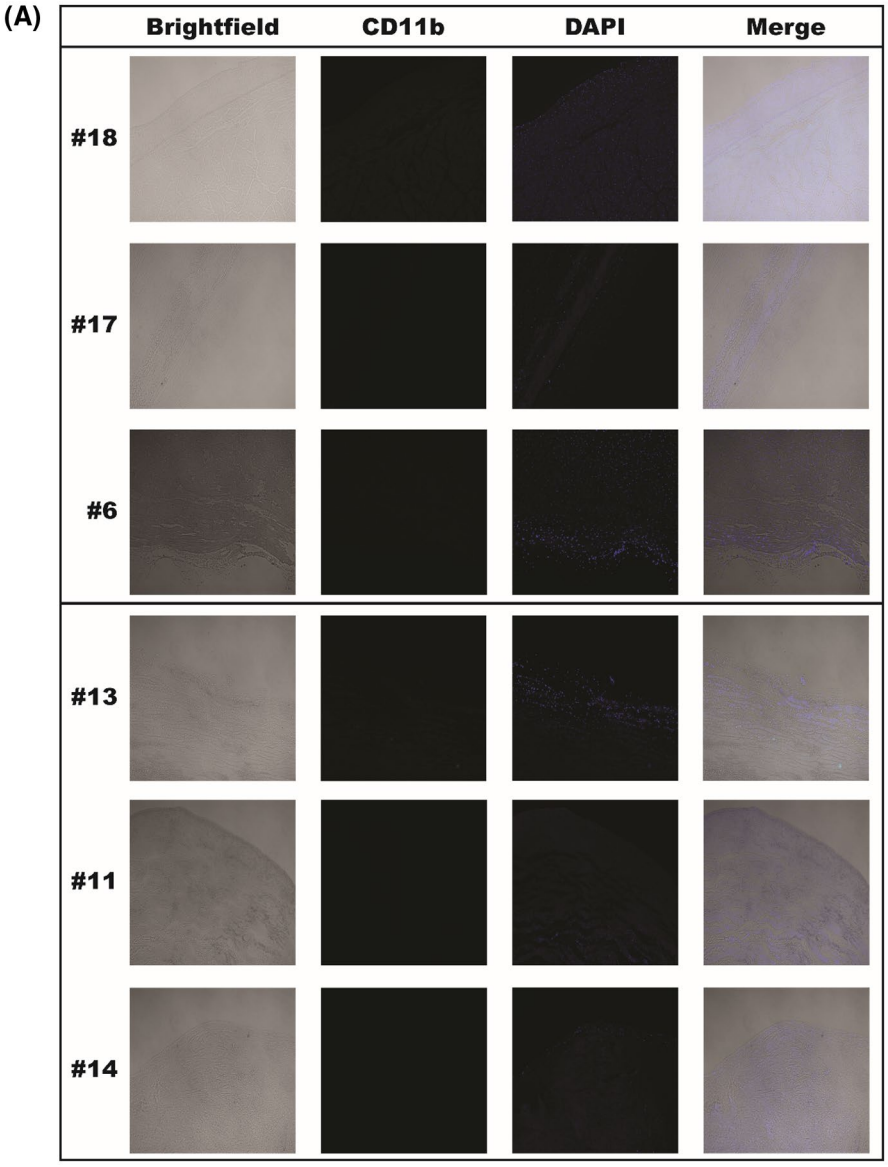

FIGURE 5 A, Immunostaining with FITC-conjugated monoclonal anti-CD11b (Green). The nuclei were counterstained with DAPI II. Typical findings from the animal #18, #17, #6, #13, #11, and #14 are presented. B, Immunostaining with FITC-conjugated monoclonal anti-CD25 (Green). The nuclei were counterstained with DAPI II. Typical findings from the animal #18, #17, #6, #13, #11, and #14 are presented

performed with satisfactory results.[9,10] However, procedure-related complications, such as valve migration, coronary compression, or stent fractures also have been reported.[11,12] Stent migration, one of the most important procedure-related complications, can be prevented by an oversized stented valve compared to the pulmonary annulus size.[13,14] Compared with percutaneous PV implantation, the PPVI technique has advantages in that it allows a relatively larger stented valve to be delivered and thereby cause a lower pressure gradient after implantation. In addition, it can be applied to patients with poor peripheral access or difficult anatomy. Stents made of Nitinol wire have a memory-shape property and a chronic outward force that increases 2-times when the temperature is increased from 20 to 37°C.[15] Therefore, a stented valve mounted on Nitinol wire becomes stiffer when implanted and becomes firmly implanted in an appropriate position. The feasibility and good short-term effectiveness of self-expandable stented valves have been previously demonstrated.[16] In the present study, significant valve migration was not seen in all 18 animals. The self-expandable nature of the oversized stented valves used in the present study may have reduced the incidence of migration.

In the present study, terminal procedures were scheduled after 10 months postoperatively (>300 days), and 8 of 18 animals (44.4%) survived >300 days with 546 days the longest

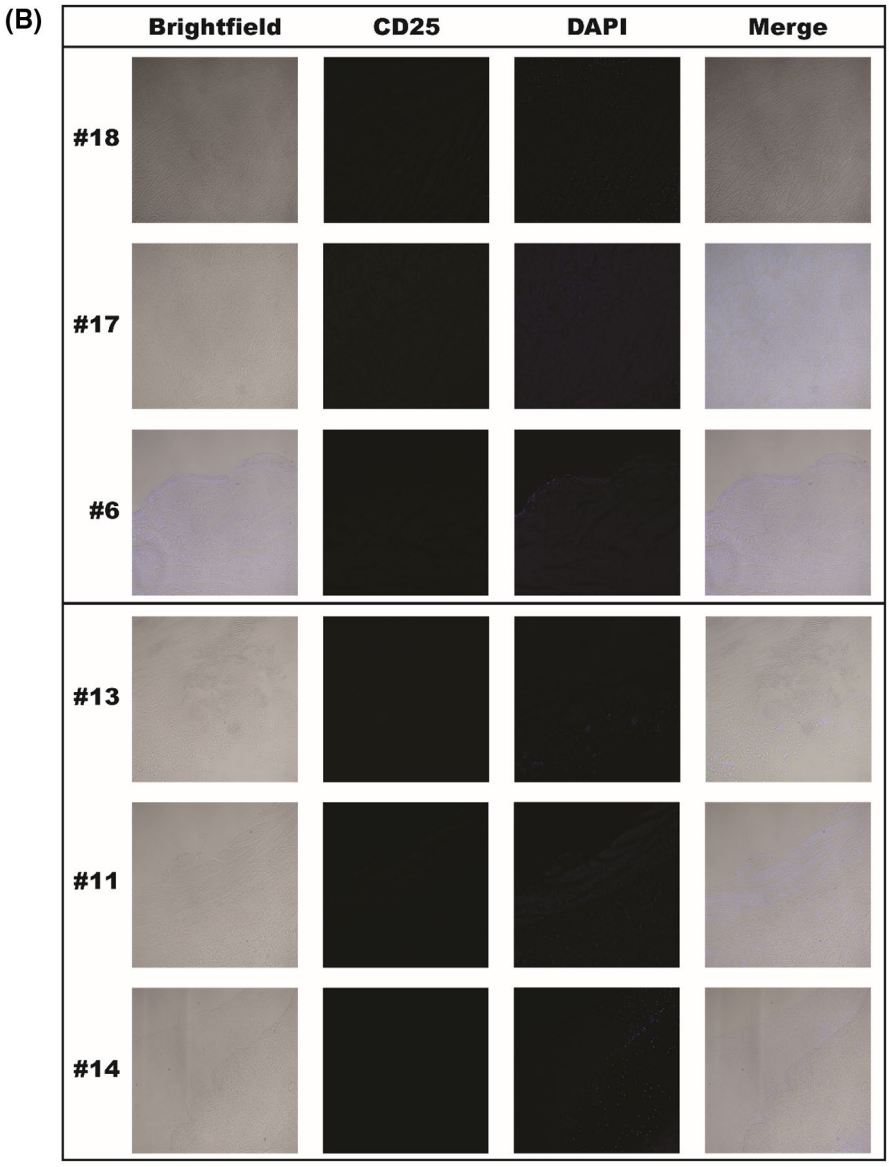

FIGURE 5 Continued

length of survival. Follow-up duration of the study was decided because the calcification of bioprostheses was reported to develop after implantation duration of 3 to 6 months in sheep.[17] 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 valve-related problems. Autopsy in eight sheep that died from infection before postoperative 10 months revealed enterocolitis and bezoars without any inflammatory findings or abnormalities in the heart. Inadequate long-term postoperative care of large experimental animals may have contributed to the condition. 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. 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 the advantages of lower thrombogenicity and good hemodynamic profiles. Xenogenic pericardial and aortic valve tissues have been used widely for cardiac bioprostheses. Dystrophic degenerative calcification reducing long-term durability is the main limitation of bioprosthetic valves, and multiple mechanisms such as immunological, chemical, hemodynamic, and mechanical factors have been demonstrated to be involved in the calcification process.[4] GA commonly has been used to fix bioprosthetic tissues for increasing tissue stability and reducing antigenicity; however, the GA fixation process may cause the tissue to be more prone to calcification.[5] Free

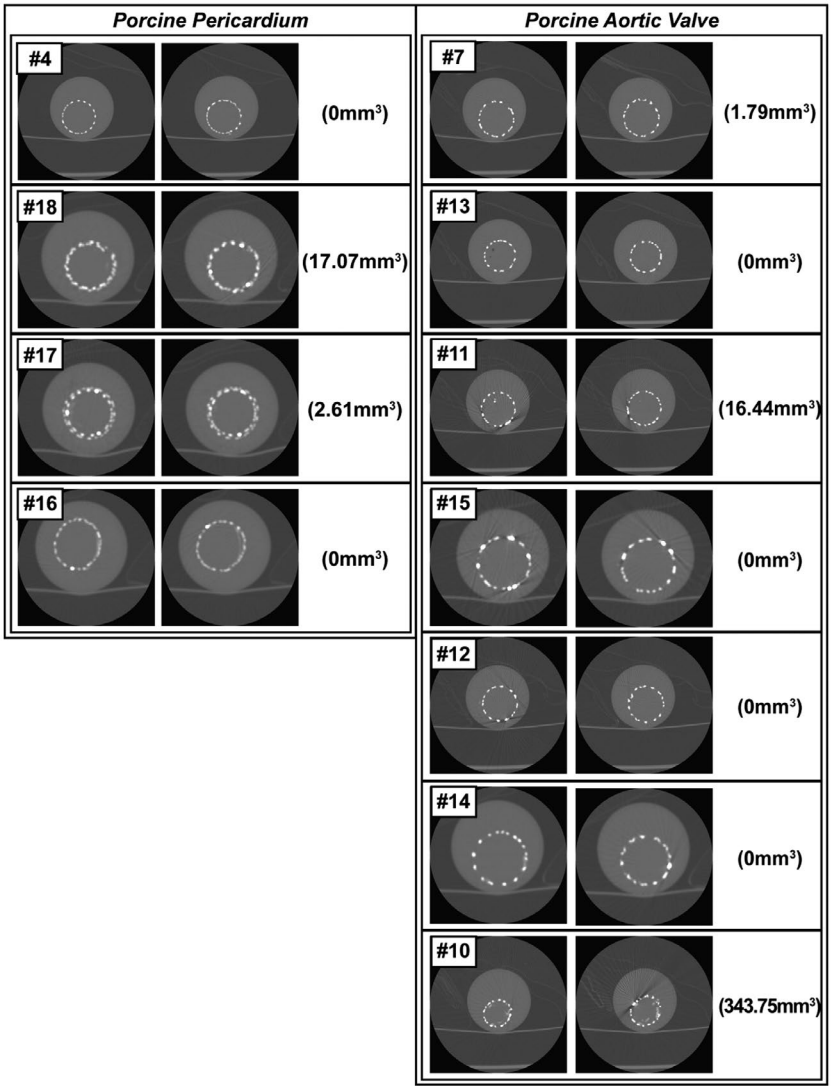

FIGURE 6 Computed tomography findings of the extracted stented valves from porcine pericardial group (animals #4, #18, #17, and #16) and porcine aortic valve group (animals #7, #13, #11, #15, #12, #14, and #10). The volume of calcification (mm^3) was calculated using computed tomography images, and shown in parentheses. There were no significant differences in the volume of calcifications between the porcine pericardial and aortic valve xenograft groups ($P = .927$)

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.[18,19] Detoxification with amino acids to block the free aldehyde groups,[20,21] removal of bioprosthetic tissue phospholipids with various alcohol solutions,[22,23] and other cross-linking agents[19,24] have been used to suppress factors involved in the 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.[25,26] The α-Gal epitope exists as a cell surface molecule in most species, except humans and old world monkeys.[27] 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, and the changes in the antibody titers over time showed no significant difference between the two groups. Although anti-α-Gal IgG antibody titers were higher in group PP than in group PAV at 2 weeks after implantation, 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 group 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 the dystrophic calcification of bioprostheses.[6-8] 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. Hence, our tissue preservation protocol was shown to be efficient in suppressing the immune response against xenogenic tissues.

The absolute value of calcium contents was low in both groups and CT analysis showed no significant differences between the two groups, although calcium quantification using hydrolysate revealed lower calcium contents in group PAV than in group PP. In the present study, we did not find any correlation in the degree of calcification between the two calcification measurements (hydrolysate of sampled tissue vs. CT analysis), which seemed to be caused by differences in the diagnostic accuracy of the two methods. The present study showed that the absolute values of calcium contents and degree of calcification were low in both groups and that there were no differences in the degree of calcification between the two xenografts.

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. Previous studies showed that pericardial valves had better durability and superior hemodynamics than porcine aortic valves[28,29]; however, the number of studies so far is insufficient for comparing hemodynamics and durability between the different types of xenografts at the pulmonary position. 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 in sheep. Our novel tissue treatment method and implantation using a perventricular implantation technique could be used as an effective treatment option in patients requiring PV replacement.

5 | LIMITATIONS OF THE STUDY

There are limitations to the present study that must be recognized. First, more than half of the animals died before the scheduled terminal procedures and data such as hemodynamic profile and serum anti-α-Gal antibody titers before death were not collected. Although the main cause of mortality seemed to be inadequate long-term postoperative care of large experimental animals, rather than immunological response against xenotransplantation or the procedure itself, the high mortality rate might cause bias. Second, we used adolescent sheep instead of juvenile sheep based on our previous calcification study using adolescent sheep.[30] Younger juvenile sheep would be a more appropriate model for evaluating calcification.[31] Third, computed tomography of extracted valves demonstrated no significant differences in calcification between groups PP and PAV. However, calcium analysis demonstrated inconsistent results in the present study, which may have resulted from the small number of samples, low absolute quantity of calcification, or instrumental errors. Fourth, a durability study of the implanted valves based on continuous heart rate measurement was not performed in the present study because it may not be feasible in a chronic animal study.

6 | CONCLUSIONS

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

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

AUTHOR CONTRIBUTIONS

Concept/design: M-S Kim, Lee, K-B Kim, H-G Kim, YJ Kim
Data collection/analysis/interpretation: M-S Kim
Drafting article: M-S Kim
Statistics: M-S Kim
Data analysis/interpretation: Lee, K-B Kim, H-G Kim, YJ Kim
Critical revision of the article: H-G Kim, YJ Kim
Approval of the article: YJ Kim

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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