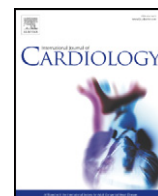




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## Novel self-expandable, stent-based transcatheter pulmonic valve: A preclinical animal study

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### ABSTRACT

**Background:** Because transcatheter implantation of pulmonary valve is indicated for limited-size dysfunctional right ventricular outflow tract only as a balloon-expandable stent, we investigated the feasibility of a large-diameter self-expandable valved stent and the durability of the valve after >6 months.

**Methods:** We made a nitinol-wire-based, self-expandable valved stent with leaflets made from porcine pericardium. The porcine pericardium was treated with  $\alpha$ -galactosidase, glutaraldehyde, and glycine after decellularization. After cutting the inguinal or cervical area, we implanted a valved stent in 12 sheep through the femoral or jugular vein by using an 18-Fr delivery catheter, controlling the catheter handles and hook block under fluoroscopic and echocardiographic guidance.

**Results:** The mean body weight of sheep was 43.9 kg. We successfully implanted valved stents (diameter: 24 mm in 7 sheep, 26 mm in 5 sheep) in good position in 8 sheep, in the main pulmonary artery (PA) in 2 sheep, and in the right ventricular outlet tract (RVOT) in 2 sheep. We sacrificed 8 sheep (6 sheep in good position, 1 sheep in the main PA, and 1 sheep in the RVOT) after >6 months. Five of the 6 sheep implanted in good position showed well-preserved valve morphology at the time of sacrifice. Histologic findings after routine sacrifice showed well-maintained collagen wave structure and no visible calcification in all explanted valve leaflets.

**Conclusions:** Transcatheter implantation of a nitinol-wire-based, self-expandable valved stent in the pulmonic valve was feasible, and stents implanted in good position showed well-preserved valve leaflets with functional competence in the mid-term results.

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### 1. Introduction

Diverse congenital heart diseases involving the pulmonary artery (PA), such as tetralogy of Fallot with or without pulmonary atresia, or transposition of the great arteries with pulmonary stenosis, require implantation of an artificial conduit between the right ventricle (RV) and the PA. Because these conduits finally degenerate and result in pulmonary regurgitation (PR) and/or stenosis and progressive RV dilation and eventual failure, patients need repetitive surgery for conduit revision. Since the first successful percutaneous pulmonary valve implantation (PPVI) in 2000 by Bonhoeffer et al. for a 12-year-old boy [1], the Melody valve has received European and Canadian approval in 2006 and approval from the US Food and Drug Administration in

2010. However, the US Food and Drug Administration still limits the indication for PPVI using Melody valve to patients with a limited-size RV-to-PA conduit with more than moderate PR, and/or stenosis of the right ventricular outflow tract (RVOT) (mean gradient,  $\geq 35$  mm Hg) [2,3].

The use of PPVI for diverse dysfunctional RVOT lesions including conduit malfunction with a large diameter greater than 22 mm is still under investigation. Besides the clinically available balloon-expandable percutaneous pulmonary valves such as the Melody valve [4,5] and the Edwards SAPIEN valve [6], several types of self-expandable pulmonic valves have been investigated in preclinical studies for future human use [7–9]. However, any self-expandable stent has not been applied for clinical use until now.

We developed a large-diameter (up to 26 mm) self-expandable stent with a relatively low profile from a nitinol wire backbone with valve leaflets made from porcine pericardial tissue. Then, we performed a preclinical study to investigate the feasibility of self-expandable valved stents through the transcatheter approach and the durability of the tissue valve after >6 months.

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<sup>1</sup> This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

**Table 1**  
Inventory of the valved stents in the pulmonic position.

Type	D type	M type
Wire thickness	Can be folded in longitudinal axis 0.008 in. (0.2 mm)	No folding in longitudinal axis 0.010 in. (0.25 mm)
Delivery system	18 Fr	18 Fr
Valve wall	Full covered	Partially covered
Diameter × total length	20 mm × 24 mm	20 mm × 30 mm
	22 mm × 25 mm	22 mm × 33 mm
	24 mm × 28 mm	24 mm × 36 mm
	26 mm × 33 mm	26 mm × 38 mm
Radial force	0.17–0.20 kgf	0.40–0.5 kgf

## 2. Methods

### 2.1. Preparation of the valved stent

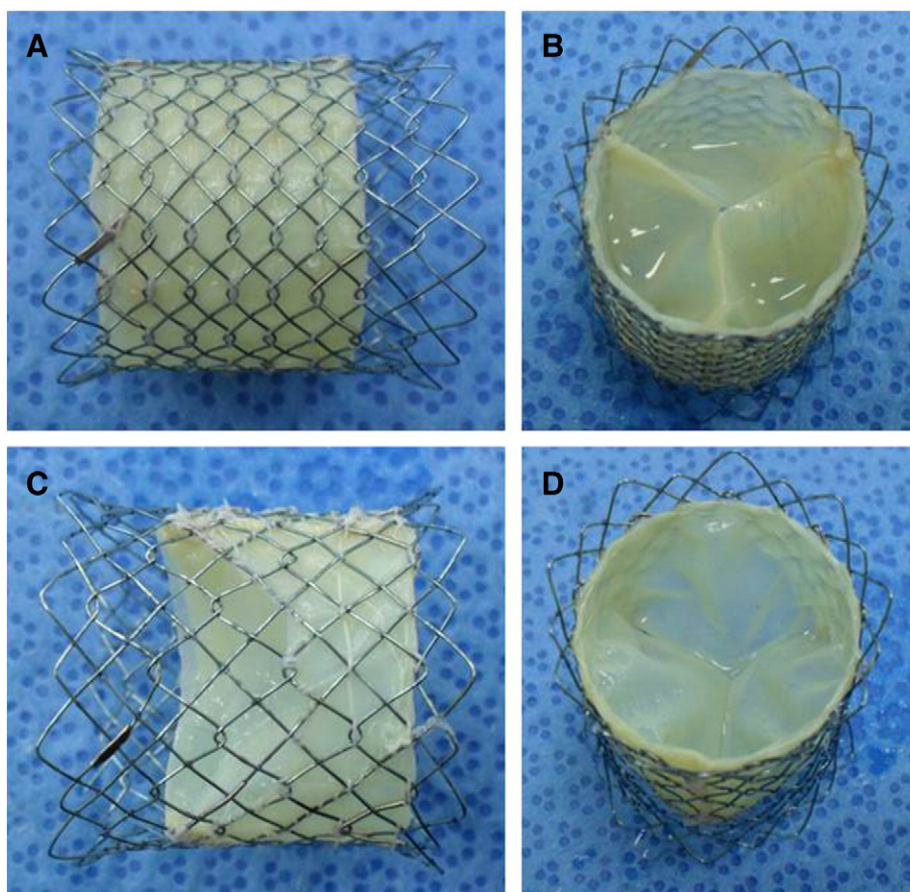
An initial outer stent was knitted using a single-strand nitinol wire with 0.008-in. thickness (Taewoong Medical Co., Gyeonggi-do, Republic of Korea). The initial valve diameter (D type in Table 1 and Fig. 1A,B, sheep 1–9) ranged from 20 mm to 26 mm, with the overall ratio of stent height to the valve diameter of approximately 1.1–1.2. Both ends of the stent were flared to 4 mm wider than the valve diameter for stable positioning (Fig. 1A,C). During the preclinical study, the stent was modified as an M type (Table 1 and Fig. 1C,D, sheep 10–12) with a 0.010-in.-thick double-strand wire, which increased the radial force to >2 times that of the initial stent. Furthermore, the wall of the M-type valved stent was covered partially to decrease the overall stent diameter and for ease in stent crimping.

Porcine pericardium was used for making the valve in the stent and was treated for maximal tissue preservation, following the previously published methods from our xenotransplantation research center [10–13]. Decellularization with 0.25% sodium dodecyl

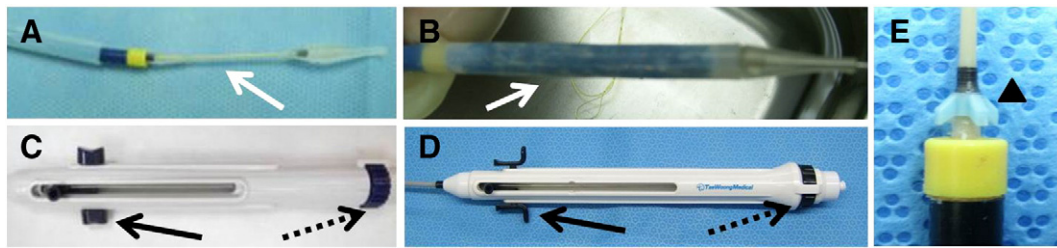
sulfate and 0.5% Triton X-100, treatment with 0.1 units/mL  $\alpha$ -galactosidase ( $\alpha$ -gal) (to reduce immunogenicity), space filler treatment with 25% polyethylene glycol, 0.5% glutaraldehyde (GA) fixation with solvent (75% ethanol + 5% octanol), and finally detoxification with 0.1 M glycine were performed. The 3 leaflets from the treated porcine pericardium were tightly hand sewn to the stent wall with 5–0 braided polyester to allow their good coaptation (Taewoong Medical Co.) (Fig. 1B,D).

### 2.2. Preparation of the delivery system

We developed an initial transcatheter delivery system with a self-expandable nature (Taewoong Medical Co.), as in Fig. 2. The proximal part of the delivery catheter has a valved stent loading area with a 17.5-mm conical tapered tip for smooth vessel introduction (Fig. 2A,B). 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 roll counterclockwise, the outer sheath could be pulled back to the proximal part of the stent area and the self-expandable valved stent could be completely deployed by pulling the lever (Fig. 2C,D).



**Fig. 1.** Morphology of the initial valved stent (D type; A, B) and the modified valved stent (M type; C, D). The valve diameter ranged from 20 mm to 26 mm, and both ends of the stent were flared to 4 mm wider than the valve diameter. The wall of the M-type stent was covered partially to decrease the overall valved stent diameter. The valves in the stent showed good coaptation grossly (B, D).



**Fig. 2.** Valved stent delivery catheter. The proximal part of the delivery catheter has a valved stent loading area with a 17.5-mm conical tapered tip (white arrows in A, B). By turning the roll counterclockwise (dotted arrows in C, D) and pulling the lever (thick arrows in C, D), the valved stent can be deployed completely with a self-expandable nature. A hook block (arrowhead in E) was helpful for controlled deployment at the targeted area.

During this preclinical study, we made a hook block at the proximal part of the stent loading area for good positioning of the valved stent (Fig. 2E). By simply hooking the proximal end of the nitinol wires at the hook block, controlled deployment and subsequent good positioning of the valved stent at the target area could be achieved (sheep 10–12 in Table 2).

The valved stent was loaded by hand crimping into the delivery catheter just before catheter exchange, and a portion of the valved stent was immersed in saline solution until vessel introduction.

### 2.3. Preparation of animals

Twelve sheep aged approximately 24 months (Dae Gwan Ryung, Kangwon-do, Republic of Korea) were prepared for this preclinical study. The mean body weight of the animals was 43.9 kg. All sheep 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 [14]. 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 valved stent

Under general anesthesia and mechanical ventilation, the inguinal area or the cervical area was cut to expose the femoral or jugular vein and the femoral or carotid artery. After insertion of a 6-Fr sheath in the vein and a 5-Fr sheath in the artery, routine hemodynamic study (measurement of the right atrium, RV, PA, and aortic pressure) and angiography at just below the pulmonary valve of the RVOT were performed. Then, we inserted an 18-Fr-long delivery catheter, with the valved stent mounted, through a 0.035-in. Terumo Glide wire (Terumo Medical Corp., Somerset, NJ, USA) or a 0.035-in. Amplatzer stiff exchange wire (Boston Scientific Corp., Miami, FL, USA) in the distal PA. Single-plane C-arm fluoroscopy (Fig. 3A) and transthoracic echocardiography were used to guide the deployment into a good position within the native pulmonary valve, by controlling the catheter handle meticulously. In the late period of this preclinical study, a hook block was useful for stable positioning at the target area (sheep 10–12 in Table 2). After the valved stent implantation, the RV and PA pressures were again measured to check the pressure gradient across the implanted valved stent.

### 2.5. Follow-up and sacrifice schedule

The sheep were extubated after careful evaluation by an animal anesthesiologist and given post-procedural care. One month after the procedure, routine transthoracic echocardiography was performed with a 6-MHz probe to evaluate the function of the implanted

pulmonic valve (the degree of stenosis and regurgitation). After >6 months, transthoracic echocardiography and cardiac catheterization were performed to evaluate the degree of PR and measure the pressure gradient between the RV and the main PA before animal sacrifice. Then, the sheep were sacrificed by bolus injection with 2 M KCl.

After sacrifice, the sheep heart was explanted and the gross morphology of the heart and pulmonary trunk was inspected, and then the implanted valved stent was harvested as a whole pulmonary trunk from the RVOT. After rinsing the implanted valved stent, the valve status and endothelialization pattern of the stent wall were investigated grossly and microscopically.

## 3. Results

The overall outcomes of the implantation of transcatheter pulmonic valved stents are presented in Table 2. We successfully implanted a valved stent in 12 sheep, through the femoral vein in 8 sheep and the jugular vein in 4 sheep; 8 sheep were implanted in a good position (Fig. 3B,C), 2 sheep in the distal main PA, and 2 sheep in the RVOT. Just after implantation, transthoracic Doppler echocardiography showed good valve function without pulmonary stenosis or regurgitation. From the autopsy findings of the third sheep in Table 2, which died of an unknown cause (presumably of an infectious origin), we found the internal side of the stent wall in the RVOT and the outer side of the stent in the main PA to be well endothelialized at 3 months after valved stent implantation (Fig. 4A).

After >6 months of follow-up, we sacrificed 8 sheep (6 sheep implanted in a good position, 1 sheep in the main PA, and 1 sheep in the RVOT). Transthoracic echocardiography before sacrifice showed no PR in 5 sheep, trivial PR in 2 sheep, and mild PR in 1 sheep. Cardiac catheterization performed just before sacrifice in 8 sheep showed that the systolic pressure gradient between the RV and the main PA was  $6 \pm 3.8$  mm Hg (range, 1–11 mm Hg) and the mean RV systolic pressure was  $22.9 \pm 3.2$  mm Hg (range, 18–26 mm Hg).

From the autopsy results after routine sacrifice, 5 of 6 sheep with a good position of the valved stent showed well-preserved valve leaflets

**Table 2**

Overall outcomes of implantation of transcatheter pulmonic valved stents.

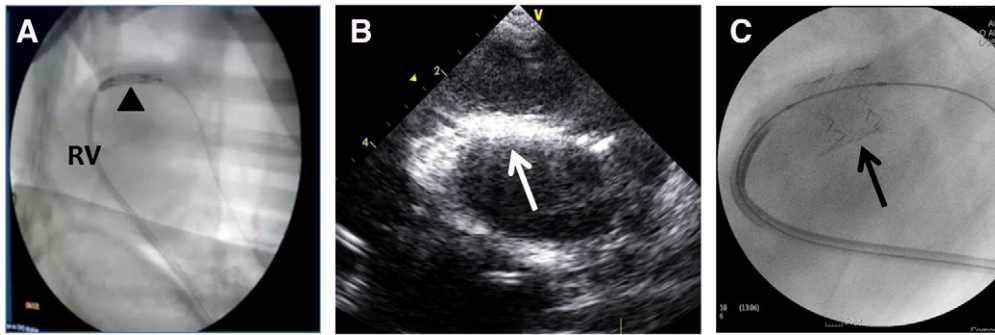
Sheep	Body weight (kg)	Valve diameter (mm)	Stent type	Hook block	Route	Valve position	F/U duration (months)	PR <sup>a</sup>	Peak PG <sup>b</sup> (RV-PA) (mm Hg)	Result	Valve morphology <sup>c</sup>	Calcium deposition (μg/mg)
1	34.5	24	D	–	FV	Good	6	Mild	11	Sacrificed	Tissue loss	4.4
2	45	24	D	–	FV	PA distal	6	No	3	Sacrificed	Attached to wall	4.0
3	51	26	D	–	FV	Good	2.5	No	NA	Died	Thick leaflet	6.8
4	45	26	D	–	FV	RVOT	6	No	8	Sacrificed	Attached to wall	4.4
5	47	26	D	–	FV	Good	6	No	11	Sacrificed	Good	5.38
6	41	24	D	–	FV	Good	6	Trivial	1	Sacrificed	Good	4.4
7	39	24	D	–	JV	Good	6	No	7	Sacrificed	Good	3.2
8	48	24	D	–	FV	RVOT	4	No	NA	Died	Attached to wall	3.2
9	39	24	D	–	FV	PA distal	2	No	NA	Died	Attached to wall	3.2
10	45	26	M	+	JV	Good	4	Moderate	NA	Died	Tissue loss	4.4
11	40	24	M	+	JV	Good	10	Trivial	1	Sacrificed	Good	3.92
12	53	26	M	+	JV	Good	7	No	6	Sacrificed	Good	4.07
Mean	43.9	24.8							6			

FV, femoral vein; JV, jugular vein; PR, pulmonary regurgitation; RV, right ventricle; PA, pulmonary artery; RVOT, right ventricular outflow tract; NA, not applicable.

<sup>a</sup> From the final transthoracic echocardiography.

<sup>b</sup> From cardiac catheterization at the time of sacrifice.

<sup>c</sup> From autopsy findings.



**Fig. 3.** Valved stent shape during the procedure. Single-plane C-arm fluoroscopy shows good positioning of the valved stent before implantation (arrowhead in A). After successful implantation of the valved stent, we confirmed the position of the implanted valved stent with transthoracic echocardiography (white arrow in B) and C-arm fluoroscopy (black arrow in C). RV: right ventricle.

with functional competence at the time of sacrifice; however, 1 sheep showed some valve tissue loss. On the other hand, 2 sheep implanted in the RVOT or the main PA showed loss of valve function with attachment of valve leaflets to the stent wall. The representative well-preserved gross valve morphology at a good position 6 months after implantation is shown in Fig. 4B,C. On histologic examination, we found no evidence of dense intimal proliferation and visible calcification in all explanted valve leaflets. We also found a well-maintained collagen wave structure on hematoxylin and eosin and Masson's trichrome staining (Fig. 5B,C). In sheep 11, which had been implanted with valve for >10 months, the calcium deposition level was low (3.92  $\mu\text{g}/\text{mg}$ ) and similar to the 6-month level (Table 2 and Fig. 5C).

Four sheep (2 sheep implanted in a good position, 1 sheep in the main PA, and 1 sheep in the RVOT) died during follow-up between 2.5 and 4 months. The cause of death was suspected to be pneumonia in sheep 8 and 9, and suspicious infection in sheep 3 and 10 (Table 2). From the autopsy findings after death, the valve seemed thick in 2 sheep and attached to the stent wall in 1 sheep, with some tissue loss in 1 sheep.

#### 4. Discussion

In this preclinical study, we introduced a newly made large-diameter self-expandable valved stent with relatively low profile from a nitinol wire and valve leaflets made from porcine pericardial tissue. The transcatheter implantation of the valved stent was feasible without technical difficulty, and the implanted tissue valve showed generally good function with well-preserved collagen wave structure and no significant calcium deposition, in the case of the valve implanted in a good position, for >6 months. Besides the technical feasibility, we also showed the durability of the tissue valve in this study.

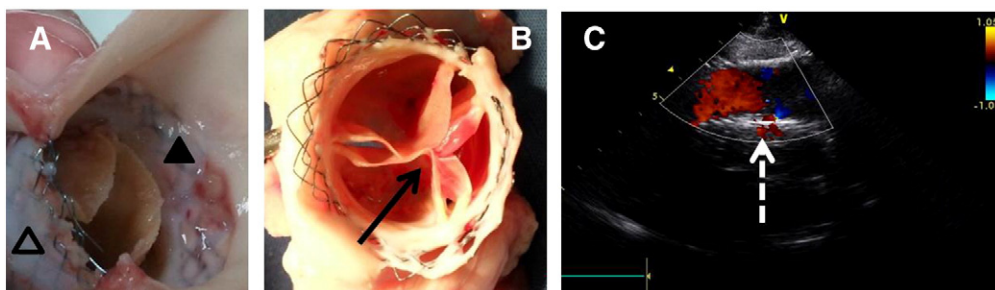
Although the Melody valve is currently available for human use, its diameter is limited to up to 22 mm, which is not useful for larger

dysfunctional RVOT lesions [7]. Furthermore, because of the balloon-expandable, covered stent in itself, the delivery system is inevitably large (22 Fr). These limitations have demanded new types of transcatheter valved stents, and several studies have been completed mainly as an animal study [8,9,15,16]. However, no other valves besides the Melody and Edward-SAPIEN valves have been applied for clinical use until now, which means more investigation is needed to overcome diverse dysfunctional RVOT lesions including native valve malfunction.

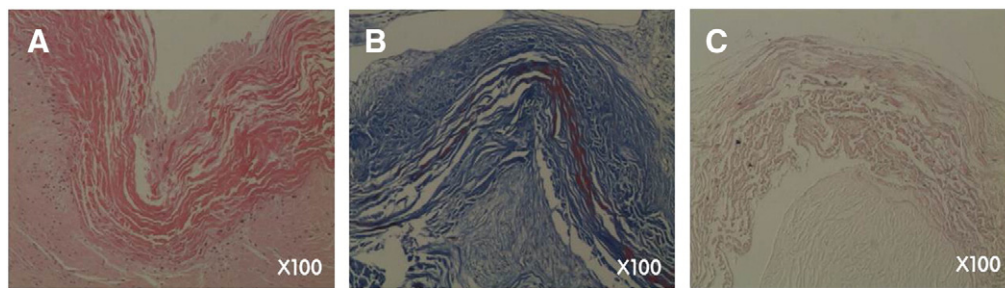
Self-expandable valved stents made of nitinol wire in our study have merit in this sense. First, stents made of nitinol wire have memory-shape property and a chronic outward force that increases 2-fold when the temperature is increased from 20 °C to 37 °C [17]. Because the transformation temperature of nitinol is set to 30 °C, a valved stent becomes stiffer when implanted in the human body [17]. Furthermore, the radial force of the valved stent was also doubled by increasing the wire thickness in this study (Table 1). Moreover, because nitinol loses its stiffness when cooled, the valved stent can be loaded well into a delivery system at or below room temperature. The second important property of the nitinol wire is its resistance to corrosion and low risk of rejection by the human body [17]. We could make large diameter valved stent up to 28 mm, which can be crimped in only 18 Fr delivery systems, from these properties of nitinol wire.

We also anticipate that our device could be implanted into the native RVOT lesions with large diameter greater than 22 mm (i.e. free pulmonary regurgitation after repair of tetralogy of Fallot) in the future human study, because larger diameter valve up to 28 mm and both flared ends of the stent in our study will be technically helpful for successful implantation.

The disadvantage of self-expandable stents is the difficulty of stable positioning at the targeted area compared with balloon-expandable stents. To improve stable positioning at the native PA valve area, we flared both ends of the valved stent to be 4 mm wider than each valve



**Fig. 4.** Valved stent at the time of sacrifice. Well-endothelialized internal side of the stent wall in the RVOT (closed arrowhead) and outer side of the stent in the main PA (open arrowhead) were seen (A). The implanted valve leaflets in good position were well preserved with good gross morphology (B), without pulmonary stenosis or regurgitation on transthoracic echocardiogram (C).



**Fig. 5.** Histologic findings of tissue valve leaflets 10 months after implantation in sheep 11. The valve leaflet showed no visible calcification and a well-maintained collagen wave structure on hematoxylin and eosin staining (A) and Masson's trichrome staining (B, blue). On von Kossa staining, there was no definite calcium deposition (C: 3.92  $\mu\text{g}/\text{mg}$ ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

diameter for anchoring at the distal main PA and RVOT. We also made a hook block (Fig. 2E) during this preclinical study for controlled deployment because this valved stent is not re-sheathable. With the help of a hook block, we could get some time to relocate the valved stent at the targeted area during deployment without springing forth from the delivery catheter. These modifications of the valved stent in this preclinical study would make deployment into the various RVOT lesions much easier in the future human clinical study.

Valve durability is another main concern about the replaced tissue valve at the pulmonic valve position. From a recent study about the long-term durability of bioprosthetic valves in the pulmonary position, about 80% of implanted bioprosthetic valves required reoperation or showed valve dysfunction [18], and it has been well known that adult-sized valved conduits from xenografts >18 mm have a mean interval from freedom of conduit exchange of 12.5 years [19]. In the case of PPVI, because only short-term results have been reported until now, a large-scale patient study is needed to prove that PPVI can ultimately reduce the number of cardiac operations during the lifetime of patients [4,5]. In our study, we observed the function and morphology of the implanted valve for >6 months in 8 sheep and found relatively well-preserved gross valve morphology and hemodynamic stability in most sheep implanted with the valved stent in a good position.

Bioprosthetic tissues are conventionally cross-linked with GA to impart tissue stability, reduce antigenicity, and maintain tissue sterility. However, GA-fixed bioprostheses are prone to calcification after implantation in humans because of tissue phospholipids, the free aldehyde groups of GA, and residual antigenicity [10–13]. Therefore, in this study, we made valve leaflets by using porcine pericardium to allow longer valve durability and less calcium deposition in the valve as the following methods: (1) decellularization was used to suppress the residual antigenicity of GA-fixed bioprosthesis [11]; (2)  $\alpha$ -gal was used to remove its epitopes effectively since removing  $\alpha$ -gal from the surface of the tissue valves can improve the valve durability [10,12]; (3) a space filler was used to fill the interstitial void spaces in GA-pretreated tissue with a macromolecular substance to prevent calcification [13]; (4) organic solvent was used to reduce the calcification potential of aldehyde-fixed tissues by the removal of phospholipids or conformational changes in collagen [11]; and (5) detoxification with glycine was used to remove the free aldehyde groups of GA [11].

Another interesting finding in this study is that the valved stent with malposition in the distal PA or RVOT usually showed loss of valve function, and the leaflets attached to the stent wall. We believe that this was because, owing to the well-functioning native pulmonary valve, the artificial valve could not move well and finally adhere to the stent wall through some kind of fibrin network. For the absence of PR in these sheep with malposed prosthesis, when we routinely sacrificed 6 months after implantation, the native valve showed good valve morphology. Because the native valves function well, we thought that there was no PR from the echocardiography in these sheep with malposed prosthesis.

#### 4.1. Study limitations

This study has some limitations. First, we did not complete routine anticoagulation after procedures such as heparin or antiplatelet agent prescription. We also did not give antibiotics to the sheep after the procedure. The results of this study might have been better if we had performed anticoagulation and given antibiotics after the procedures. For the clinical study, we will definitely do routine anticoagulation after the procedures and give antibiotics during the perioperative periods. Second, we used single-plane C-arm fluoroscopy and transthoracic echocardiography during deployment, which was not appropriate for valved stent implantation and resulted in malpositioning in 4 sheep in this study. In the clinical study, biplane C-arm fluoroscopy and transesophageal echocardiography should be used during insertion of the valved conduit for good target positioning.

#### 5. Conclusion

Transcatheter implantation of a nitinol-wire-based, self-expandable valved stent in the pulmonic valve position was feasible, and most valves showed well-preserved valve leaflets with functional competence in the case of a good valved stent position in the midterm results of this preclinical animal study.

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