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**Development of an *in vivo* tissue-engineered autologous heart valve (the Biovalve): Preparation of prototype model**

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## **ABSTRACT**

*Objective:* This study aimed to develop an autologous heart valve without using any traditional *in vitro* tissue-engineering methods, which necessitate complicated cell management protocols under exceptionally clean laboratory facilities.

*Methods:* An autologous heart valve construct comprising of trileaflets was prepared by using a specially designed mold. The mold was prepared by covering a silicone rod with a crown-shaped tubular polyurethane scaffold containing 3 horns. The mold was implanted in the dorsal subcutaneous space in Japan White rabbits for 4 weeks. After harvesting, the implanted trileaflet valve-shaped structure with an internal diameter of either 5 or 20 mm was obtained by trimming the membranous tissue formed between the horns located around the silicone rod. The valve substitute was examined both macroscopically and histologically. The tensile strength of the leaflets was measured to rupture. The degree of regurgitation in valve function was evaluated using a flow circuit by calculating the ratio of the regurgitation volume to the forward flow volume.

*Results:* After implantation, the mold was completely covered with connective tissues consisting mostly of collagen and fibroblasts. Harvesting of the mold was straightforward since there was little adhesion between the formed tissue and the native skin tissue. The trileaflet heart valve construct was obtained following withdrawal of the inserted rods and trimming of the membranous tissues formed between the horns of the scaffold. It was firmly attached to the scaffold, the interstices and surface of which revealed connective tissues comprising of similar components to that of the leaflet tissue. Although mechanical properties of the leaflet tissue were rather less than those of the native porcine aortic valve leaflets, satisfactory valvular functions were demonstrated under pulsatile conditions using a flow circuit. No regurgitation was observed under retrograde hydrostatic pressures of up to 60 mm Hg, the physiological pressure acting on aortic valves during retrograde aortic flow.

*Conclusion:* An autologous, *in vivo* tissue-engineered, trileaflet, valve-shaped construct, named Biovalve, was developed using our enterprising *in body* tissue architecture technology. The Biovalves have the potential to be ideal prosthetic heart valves with excellent biocompatibility and possible to the growth of recipients' organ.

## INTRODUCTION

Prosthetic valve-replacement operations are currently regarded as the standard treatment for end-stage heart valve diseases; however, several clinical problems resulting from the use of these artificial valves exist. Lifetime anticoagulation therapy is necessary in the case of mechanical valves (1-4) while the structure of the xenogeneic valvular leaflets gradually deteriorates in the case of bioprosthetic valves.(5-7) Both types of prosthetic valves are unsuitable for pediatric patients due to a lack of their ability to grow.(4) In recent developmental studies, to acquire the characteristics of enhanced maturation such as anticoagulation, self-repair, tissue regeneration, and adaptability to growth, autologous valve prostheses have been developed by using *in vitro* tissue-engineering technology.(8-11) Some investigators have succeeded in the implantation of the *in vitro* tissue-engineered heart valves in animals and humans by using scaffolds based on either decellularized natural tissues or biodegradable synthetic polymers.(11-21) However, it is difficult to fabricate reproducible valves with a certain level of maturity.

For the development of autologous prosthetic tissues, we have embarked on a novel and practical concept in regenerative medicine, namely, the *in body* tissue architecture technology, which is based on the tissue-encapsulation phenomenon of foreign materials in living bodies.(22-24) The use of a recipient's body to construct organ tissues had been pioneered by Sparks and colleagues, who constructed arterial bypass grafts in recipients' subcutaneous spaces. In 1968, they clinically applied the silicone mandril method to arterial bypass.(25,26) Similarly, Campbell successfully constructed arterial grafts in the peritoneal cavity of animals using silastic tubes.(27,28) For *in vivo* cell seeding in bovine pericardium, Flameng and colleagues also utilized foreign body reaction through intraperitoneal implantation.(29,30)

This technique has the following advantages: the prostheses induce no immunological rejection; they exhibit nontoxic biocompatibility; and they might adapt to the recipient's growth. In addition, the tissue prostheses can be fabricated in a wide range of shapes and sizes to suit each individual recipient. Most importantly, neither complex cell management *in vitro* nor exceptionally clean laboratory facilities are required, both of which

are expensive and time consuming.

The purpose of this study was to develop an autologous heart valve substitute by a more convenient and undemanding, compared to the traditional *in vitro* tissue engineering technique. We hereby describe, firstly, a fabrication method for the valvular structure named Biovalve, by using a specially designed mold. Subsequently, the results of examining the mechanical and histological properties of the Biovalve are described. In addition, we discuss the potential and limitations of the Biovalve.

## **METHODS**

### **Preparation and Characterization of the Biovalve.**

The molds were specially designed by covering a silicone rod with a crown-shaped tubular segmented polyurethane scaffold consisting of 3 isosceles triangular horns having a microporous wall structure (internal diameter of the scaffold, 5 or 20 mm; wall thickness, 1 or 3 mm; micropore diameter, 50-100  $\mu\text{m}$ ) fabricated by Bridgestone Corp. (Tokyo, Japan). A tapered silicone rod (diameter, 4 mm (top) and 7 mm (bottom); height, 15 mm) manufactured by Taiyo Kogyo Corp. (Tokyo, Japan) was used for the 5 mm scaffold (Fig. 1A). In the case of the 20 mm scaffold, a cylindrical silicone rod (diameter, 20 mm; height, 45 mm) obtained from Taiyo Kogyo Corp. was used. The mold was placed in the dorsal subcutaneous space of Japan White rabbits (n = 4; average weight, 2 kg). All of the experimental animals received humane care according to the Principles of Laboratory Animal Care formulated by the National Institutes of Health (National Institutes of Health publication no. 56-23, revised 1985). The research protocol was approved by the ethics committee. Anesthesia was induced in the rabbits by intramuscular injection of a mixture of ketamine (62.5 mg/kg) and xylazine (8.3 mg/kg) and maintained by a bolus intramuscular injection of a quarter of the initial doses. After 4 weeks of placement, the implants were harvested. The Biovalves were obtained from the implants after trimming of the peripheral fragile irregular redundant tissues; 3 notches were created on the rigid membranous tissues formed between the 3 horns of the scaffolds, and the molds were removed.

For histological examination, the Biovalves were fixed with 10% buffered formalin solution,

embedded in paraffin, cut into thin cross-sections (thickness, 3~5  $\mu\text{m}$ ), mounted, and stained with hematoxylin-eosin and sirius red. The Biovalve was fixed with 10% buffered formalin solution, embedded in paraffin, cut into the cross sections (thickness: 3~5  $\mu\text{m}$ ), and mounted for histological (hematoxylin-eosin (HE) and Elastica–van Gieson staining (EVG)) studies. Immunohistochemistry was performed using monoclonal antibodies against vimentin (DAKO Japan, Kyoto, Japan),  $\alpha$ -SMA (DAKO Japan, Kyoto, Japan), and desmin (DAKO Japan, Kyoto, Japan). The sections were treated with 0.3%  $\text{H}_2\text{O}_2$  for 20 min to inhibit endogenous peroxidase activity and then incubated overnight with the primary antibody (4°C). After the treatment with biotinylated secondary antibodies, the sections were incubated with peroxidase-conjugated streptavidin for 30 min. Finally, visualization was carried out using 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB) in 0.05 mol/L Tris-HCl buffer containing 0.005%  $\text{H}_2\text{O}_2$  for 5 min.

### **Physical Properties of the Biovalves.**

Measurements of the thickness and tensile strength of the Biovalve leaflets were performed using a tensile strength machine (RE-3305; Yamaden Co., Ltd., Tokyo, Japan). The specimens (size, 5 mm  $\times$  10 mm) were stress-loaded to rupture at a rate of 0.5 mm per second. The tensile strength denoted the amount of force required for rupture to occur. The apparent elastic modulus was determined as the slope of the initial linear section of the stress-strain curve.

Regurgitation of the Biovalves was measured for a period of 1 minute under static pressure in a closed structure, 5 mm in size, using a specially designed flow-loading circuit that was capable of generating hydrostatic pressure by altering the revolutions of the roller pump (pressure, 12-200 mm Hg). The degree of regurgitation was estimated by the ratio of the regurgitation volume to the forward flow volume.

## **RESULTS**

After placement of the specially designed molds (Fig. 1A) into the subcutaneous pouches for 4 weeks, they became completely encapsulated with the newly developed

membranous connective tissues (Fig. 1B). The implants could be easily harvested by cutting the surrounding tissues because the newly developed connective tissues and the original subcutaneous tissues were connected by very fragile irregular redundant tissues. Connective tissues were also formed in between the 3 horns of the crown-shaped scaffold. The membranous tissues were thin and homogenous in nature. After withdrawing the inserted silicone rod, the tubular connective tissue structure embedded within the scaffold was obtained. Biovalves were obtained after cutting of the membranous tissue formed between the horns into the predesigned shape. By changing the size of the mold, two different kinds of the Biovalves, with an internal diameter of either 5 or 20 mm were obtained (Fig. 1C). The larger Biovalve may fit adult human aortic valves.

In longitudinal sections of the Biovalve, it was observed that the luminal surface at the border between the leaflet structure and the scaffold was extremely smooth without any seam (Fig. 2A). Coaptation and opening of the leaflets were well-balanced (Fig. 2B and C). Histologic analysis confirmed that in circumferential sections, the leaflets and polyurethane crowns were tightly connected to each other and unified with the same newly-formed connective tissues (Fig. 3A and C). The thickness of the leaflets was about 200  $\mu\text{m}$ . Interestingly, rich angiogenesis was induced near the scaffold (Fig. 3B).

EVG staining revealed the absence of elastic fibers in the membranous leaflet tissue: however, rich collagen fibers were present (Figure 4A). Immunohistochemistry revealed that fibroblasts and myofibroblasts were the most predominant cell types in the leaflet tissues. Smooth muscle cells were not observed (Figure 4B, C and D).

Mechanical properties of the prepared leaflet tissues were evaluated by estimating their tensile strength. The load-deformation relationships obtained by causing expansion of the leaflets were compared amongst the two different sizes of the Biovalves and porcine aortic valves of 20 mm in diameter (radial and circumferential directions) (Fig. 5). The relation curve of leaflets from the 20 mm Biovalves was equivalent to that from the porcine aortic valves in the radial direction. Table 1 summarizes the mechanical properties of the leaflets. The thickness of the 20-mm Biovalve leaflets was 3 times that of the 5-mm Biovalves; this difference was reflected in their respective mechanical properties. The 20-mm Biovalve

leaflets endured more than 150% deformation. Both tensile strength and elastic modulus values of the 20-mm Biovalves were equivalent to those of the porcine leaflets in the radial direction; however, these values were about half of those of the porcine leaflets in the circumferential direction. Nevertheless, these values were twice as much as those of the 5-mm Biovalves.

The 5-mm Biovalves exhibited excellent performances in terms of tight closure during the diastolic phase and quick opening during the systolic phase. No major regurgitation was observed under retrograde static pressures of up to 60 mm Hg (retro/ante ratio = 0) during pulsatile flow conditions (Figure 6). Even at a pressure of more than 100 mm Hg, the ratio of the regurgitation volume to the forward flow volume of the circuit was approximately 0.1.

## **DISCUSSION**

Our goal in creating ideal heart valve prostheses is to ensure their biocompatibility, satisfactory valvular function, and life-long adaptability to the recipients' bodies. To acquire biocompatibility, autologous cell-incorporated heart valves have been developed with the help of the recent advances in *in vitro* tissue-engineering techniques. Some investigators have successfully implanted valve substitutes during animal experiments and in the clinical setting (20,31-33); autologous bone marrow cells and vascular-composing cells, both of which were carefully harvested from the recipient's bodies, were used as cell sources in these instances. However, these procedures require complicated cell-management protocols, including cell harvesting, seeding of the cells to appropriate scaffolds, and culturing of them for the development of neotissues in bioreactors under strictly sterile conditions; all of these procedures require enormous amounts of time and money. When a homograft or xenograft instead of polymeric materials is used as the scaffold, complete decellularization is indispensable to exclude all immunological sources in order to avoid subsequent calcification.(11) These complex procedures could limit their safety, dependability, and popularity in the future. Tissue-engineered valves are now used only in the cases of pulmonary position under low pressures of up to 40 to 50 mm Hg because, after culture and

development in a bioreactor, they are not matured enough to sustain the high pressure of the arterial circulation systems.(16-21)

In comparison, the *in body* tissue architecture technology, which we have described above, used a living body as a "reactor" to fabricate valvular tissues. While fabricating the autologous implants by means of this technique, we utilized and controlled the extracellular matrix components produced by the body's own fibroblasts or myofibroblasts that exist abundantly in subcutaneous tissues.(24,32-36) Therefore, the preparation method of the Biovalves was straightforward, safe, and reproducible. Only two processes were necessary, namely, embedding of a synthetic scaffold into the recipients' subcutaneous spaces and harvesting and trimming of the regenerated tissues. Since the Biovalves were produced autonomically in the recipients' bodies, neither complicated cell-management methods nor development of neotissues *in vitro* was essential. Additionally, the risk of bacterial contamination and transmission of infection to the recipients are considered less in this technique.

In this study, a microporous polyurethane tube cut into a simple crown shape was prepared as a scaffold. Polyurethanes, which are clinically used in blood pumps and arterial grafts, exhibited no toxicity and little biodegradation *in vivo*.(37,38) With regard to the use of the polyurethane, we have demonstrated its good biocompatibility in a discrete series of experiments using dogs and rabbits. In these experiments, no anomalous accumulation of inflammatory cells around the scaffolds was observed during the fourth week; this observation was also demonstrated in this study (Fig. 3). In addition, rapid and homogenous tissue infiltration was observed in the deep layer of the scaffolds. In the next stage of our research, we are planning the use of biodegradable scaffold materials so that attainment of proper adaptability for the growth of the tissue is possible. To form membranous tissues as leaflet parts between the horns of the scaffolds, silicone rods were inserted into the scaffolds in this study. Since the encapsulating tissues did not adhere to the silicone molds, the Biovalve leaflets could be easily obtained by extracting the rods and trimming away the redundant tissues.

In the histological examinations, the extracellular matrix of the Biovalve leaflets

was observed to be mainly composed of collagen fibers (Fig. 3, 4A). We could not reproduce exactly the same components of the native aortic valves, such as elastin, which is one of the major extracellular matrix proteins. However, it is presumed that maturation into highly differentiated tissues including those demonstrating elastic fiber formation could be induced under the mechanical stresses of pulsatile blood pressure and the opening and closing movements after implantation in the hemodynamic conditions *in vivo*. Indeed, in our implantation study on small-caliber tubular prostheses known as Biotubes, which were developed by the application of the *in body* tissue architecture technology, hierarchical vascular structure formation including the regeneration of elastic fibers was observed at 3 months after the implantation.(36)

Another structural difference between the Biovalves and the native valves was that their blood-contacting surface was thrombogenic in nature. However, upon implantation *in vivo*, natural antithrombogenicity could be obtained rapidly as discussed below. In animal experiments using Biotubes coated with nonthrombogenic agents such as argatroban, graft patency was excellent and complete endothelialization was induced within 2 weeks.(30) Therefore, implanted Bivalves are also expected to be completely covered with endothelial cells forthwith. We could control the strength and thickness of the regenerated membranous tissues to a certain degree by changing the size of the molds (Table 1). When we used a larger mold, the thickness of the membranous tissues increased and the tissues became stronger.

In regurgitation tests, the Biovalves showed high resistance to retrograde pressure (Fig 6). Even the 5-mm Biovalves endured 100 to 200 mm Hg hydrostatic pressures. Since the 20-mm Biovalves demonstrated better mechanical properties in strength or extensibility than the 5-mm Biovalves, we suggested that they also could sustain the high pressure extant in the arterial system.

We have created functional prototype models of autologous heart valvular tissues in subcutaneous spaces. The Biovalves have already achieved satisfactory levels in terms of mechanical properties and valvular functions.

One of our impending challenges in the development of the Biovalves is to improve

the design of the mold and scaffold to achieve more functional and biomimetic structures with the sinus of Valsalva. Moreover, we need to demonstrate the possibility of regeneration and tissue restoration, influence of physical stresses on valvular tissue formation, and adaptability to size discrepancy *in vivo*, especially in the arterial system. We intend to address these issues through animal implantation experiments. Subsequently, long-term function and tissue reorganization will be investigated in follow-up studies, in addition to the evaluation of surgical safety and the primary function of the Biovalves.

In conclusion, two sizes of *in vivo* tissue-engineered, autologous, trileaflet, valve-shaped structures known as Biovalves were successfully produced by the *in body* tissue-architecture technology. This new type bio-added valve prosthesis was, on the whole, satisfactory in terms of valvular functions and mechanical properties. Therefore, the Biovalves have the potential to be ideal prosthetic heart valves with high biocompatibility and possible to the growth of the recipients recipients' organ.

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## REFERENCES

1. Vongpatanasin W, Hillis D, Lange RA. Prosthetic heart valves. *N Engl J Med* 1996;335:407-416.
2. Bloomfield P, Wheatley DJ, Prescott RJ, Miller HC. Twelve-year comparison of a Bjork-Shiley mechanical heart valve with porcine bioprostheses. *N Engl J Med* 1991;324:573-579.
3. Hammermeister KE, Sethi GK, Henderson WG, Oprian C, Kim T, Rahimtoola S. A comparison of outcomes in men 11 years after heart-valve replacement with a mechanical valve or bioprosthesis. *N Engl J Med* 1993;328:1289-1296.
4. Cannegieter SC, Rosendaal FR, Briët E. Thromboembolic and bleeding complications in patients with mechanical heart valve prostheses. *Circulation* 1994;89:635-641.
5. Jamieson WRE, Munro AI, Miyagishima RT, Allen P, Burr LH, Tyers GFO. Carpentier-Edwards standard porcine bioprosthesis: Clinical performance to seventeen years. *Ann Thorac Surg* 1995;60:999-1007.
6. Grunkemeier GL, Jamieson WRE, Miller DC, Starr A. Actuarial versus actual risk of porcine structural valve deterioration. *J Thorac Cardiovasc Surg* 1994; 108:709-718.
7. Cosgrove DM, Lytle BW, Taylor PC, Camacho MT, Stewart RW, McCarthy PM, Miller DP, Piedmonte MR, Loop FD. The Carpentier-Edwards pericardial aortic valve. Ten-year results. *J Thorac Cardiovasc Surg* 1995;110:651-662.
8. Breuer CK, Mettler BA, Anthony T, Sales VL, Schoen FJ, Mayer JE Jr. Application of tissue-engineering principles toward the development of a semilunar heart valve substitute. *Tissue Eng* 2004;10:1725-1736.
9. Schoen FJ, Levy RJ. Tissue Heart Valves: Current challenges and future research perspectives. *J Biomed Mater Res* 1999;47:439-465.
10. Rabkin-Aikawa E, Mayer JE Jr, Schoen FJ. Heart valve regeneration. *Adv Biochem Engin/Biotechnol* 2005;94:141-179.
11. P. Simon, M.T. Kasimir, G. Seebacher, G. Weigel, R. Ullrich, U. Salzer-Muhar, E. Rieder, E. Wolner. Early failure of the tissue engineered porcine heart valve SYNERGRAFT™ in pediatric patients. *Eur J Cardio-thorac Surg* 2003;23:1002-1006.

12. Hilbert SL, Yanagida R, Souza J, Wolfenbarger L, Jones AL, Krueger P, Stearns G, Bert A, Hopkins RA. Prototype anionic detergent technique used to decellularize allograft valve conduits evaluated in the right ventricular outflow tract in sheep. *J Heart Valve Dis* 2004;13:831-840.
13. Steinhoff G, Stock U, Karim N, Mertsching H, Timke A, Meliss RR, Pethig K, Haverich A, Bader A. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits. *In vivo* restoration of valve tissue. *Circulation* 2000;102[supplIII]:III-50-III-55.
14. Shinoka T, Ma PX, Shum-Tim D, Breuer CK, Cusick RA, Zund G, Langer R, Vacanti JP, Mayer JE Jr. Tissue-engineered heart valves. Autologous valve leaflet replacement study in a lamb model. *Circulation* 1996;94[supplIII]:II-164-II-168.
15. Hoerstrup SP, Sodian R, Daebritz S, Wang J, Bacha EA, Martin DP, Moran AM, Guleserian KJ, Sperling JS, Kaushal S, Vacanti JP, Schoen FJ, Mayer JE Jr. Functional living trileaflet heart valves grown *in vitro*. *Circulation* 2000; 102[supplIII]:III-44-III-49.
16. Dohmen PM, Lembcke A, Hotz H, Kivelitz D, Konertz WF. Ross operation with a tissue-engineered heart valve. *Ann.Thorac. Surg* 2002;74:1438-1442.
17. Bader A, Steinhoff G, Strobl K, Schilling T, Brandes G, Mertsching H, Tsikas D, Froelich J, Haverich A. Engineering of human vascular aortic tissue based on a xenogeneic starter matrix. *Transplantation* 2000;70:7-14.
18. Shinoka T, Shum-Tim D, Ma PX, Tanel RE, Langer R, Vacanti JP, Mayer JE Jr. Tissue-engineered heart valve leaflets. Does cell origin affect outcome? *Circulation* 1997;96[supplIII]:II-102-II-107.
19. Matsumura G, Miyagawa-Tomita S, Shin'oka T, Ikada Y, Kurosawa H. First evidence that bone marrow cells contribute to the construction of tissue-engineered vascular autografts *in vivo*. *Circulation* 2003;108:1729-1734.
20. Stock UA, Nagashima M, Khalil PN, Nollert GD, Herden T, Sperling JS, Moran A, Lien J, Martin DP, Schoen FJ, Vacanti JP, Mayer JE Jr. Tissue-engineered valved conduits in

- the pulmonary circulation. *J Thorac Cardiovasc Surg* 2000;119:732-740
21. Isomatsu Y, Shin'oka T, Matsumura G, Hibino N, Konuma T, Nagatsu M, Kurosawa H. Extracardiac total cavopulmonary connection using a tissue-engineered graft. *J Thorac Cardiovas Surg* 2003;126:1958-1962.
  22. Lee W, Park BY. The subcutaneous capsules for foreign body in fetal rabbits: preliminary report. *Yonsei Med J* 2001;42: 595-601
  23. Campbell JH, Efendy J, Campbell GR. Novel vascular graft grown within recipient's own peritoneal cavity. *Circ Res* 1999;85:1173-1178.
  24. Nakayama Y, Ishibashi-Ueda H, Takamizawa K. *In vivo* tissue-engineered small-caliber arterial graft prosthesis consisting of autologous tissue (Biotube). *Cell Transplantation* 2004;13:439-449.
  25. Sparks CH, Silicone mandril method of femoropopliteal artery bypass. Clinical experience and surgical technics. *Am J. Surg.* 1972;124:244-249.
  26. Hallin RW, Sweetman WR. The sparkes' mandril graft. A seven year follow-up of mandril grafts placed by Charles H. Sparks and his associates. *Am J Surg.*1976;132: 221-223.
  27. Campbell JH, Efendy J, Campbell GR. Novel vascular graft grown within recipient's own peritoneal cavity. *Circ Res.* 1999;85:1173-1178.
  28. Campbell JH, Efendy JL, Han C, Girjes AA, Campbell GR. Haemopoetic origin of myofibroblasts formed in the peritoneal cavity in response to a foreign body. *J Vasc Res.* 2000;37:364-371.
  29. Flameng W. A new approach to heart valve tissue engineering. *Int J. Cardiol.* 2004;95(1):S55-S56.
  30. De Visscher G, Verbeken E, Van Kerrebroeck C, Holvoet P, Flameng W. Recellularization of photo-oxidised pericardium using immature foreign body reaction in the construction of a tissue engineered heart vlave. *J Molecular and Cellular Cardiology* 2004;37:214.
  31. Hoerstrup SP, Kadner A, Melnitchouk S, Trojan A, Eid K, Tracy J, Sodian R, Visjager JF, Kolb SA, Grunenfelder J, Zund G, Turina MI. Tissue engineering of functional trileaflet

- heart valves from human marrow stromal cells. *Circulation* 2002;106[suppl]:I-143-I-150.
32. Sodian R, Hoerstrup SP, Sperling JS, Daebritz SH, Martin DP, Schoen FJ, Vacanti JP, Mayer JE Jr. Tissue engineering of heart valves: In vitro experiences. *Ann Thorac Surg* 2000;70:140-144.
  33. Sutherland FWH, Perry TE, Yu Y, Sherwood MC, Rabkin E, Masuda Y, Garcia GA, McLellan DL, Engelmayr GC Jr, Sacks MS, Schoen FJ, Mayer JE Jr. From stem cells to viable autologous semilunar heart valve. *Circulation* 2005;111:2783-2791.
  34. Sakai O, Zhou Y, Ametani A, Kanda K, Yaku H, Nakayama Y. Tissue organization acceleration technology *in vivo* in regenerative medicine: A wing-attached rod as a novel scaffold for “Biotube” grafts fabrication. *Artif Organs* 2005;29:737
  35. Sakai O, Kanda K, Yaku H, Sato T, Nakayama Y. Tissue organization acceleration technology *in vivo* in regenerative medicine: Promotion of angiogenesis by control-released nicotine. *Artif Organs* 2005;29:780
  36. Watanabe T, Huang H, Hayashida K, Okamoto Y, Nemoto Y, Kanda K, Yaku H, Nakayama Y. Development of small-caliber “Biotube” vascular grafts: Preliminary animal implantation study. *Artif Organs* 2005;29:733
  37. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, ed. *Biomaterials Science* New York: Academic Press 1996.
  38. Zdrahala RJ, Zdrahala IJ. Biomedical applications of polyurethanes: A review of past promises, present realities, and a vibrant future. *J Biomater Appl* 1999;14(1):67-90

## Figure Legends

Fig. 1 (A) Gross appearance of the assembly consisting of a crown-shaped scaffold made of polyurethane and a silicone rod mold. The latter was inserted into the former. Scale bar: 5 mm. (B) The Biovalve organized in subcutaneous spaces of rabbits for 4 weeks before trimming. The surface was completely encapsulated by connective tissues with rich neovascularization. Little adhesion to the surrounding native tissues enabled easy harvesting. Scale bar: 5 mm. (C) The Biovalves completed by trimming of the redundant membranous tissues formed between the 3 notches in the crown-shaped scaffold.

Fig. 2 Macroscopic observation of the Biovalve. (A) A longitudinal section of the boundary between the polyurethane scaffold and the leaflet tissues. (B) Closed form and (C) open form of the Biovalve observed from the bottom. Well-balanced coaptation and sufficient opening of the leaflets were observed. Scale bar: 1 mm.

Fig. 3 Histological observation of the circumferential whole (A) and partly (C) sections obtained from the Biovalve. The membranous leaflet tissues were firmly attached to the tissue growing into the interstices of the scaffold. The leaflets mainly consisted of fibroblasts and collagen-rich extracellular matrices (D). (B) A histological image of a polyurethane scaffold of the Biotubes. The micropores of the scaffold were fully occupied by connective tissue with rich neovascularization (arrows). Few inflammatory cells were observed. Histological staining: HE stain (A,B,C); Sirius red stain (D). Scale bar: A, 1 mm; B, 50  $\mu$ m; C, 200  $\mu$ m; D, 200  $\mu$ m.

Fig.4 Elastica-van Gieson staining (A) of the membranous tissue of the Biovalve leaflets showed the presence of collagen fibers. The cellular components showed immunoreactivity for  $\alpha$ -smooth muscle actin (B), vimentin (D); however, the cells were negative for desmin (D). Scale bars: 50  $\mu$ m.

Fig.5 The load-deformation relationships, obtained by causing expansion of the leaflets, comparing amongst the two different sizes of the Biovalves and porcine aortic valves of 20 mm in diameter (radial and circumferential directions). The relation curve of leaflets from the 20 mm Biovalves was equivalent to that from the porcine aortic valves in the radial direction.

Fig.6 Regurgitation test under static pressure against a closed Biovalve of 5 mm size using a flow loading circuit. No major regurgitation was observed under physiological diastolic blood pressure.

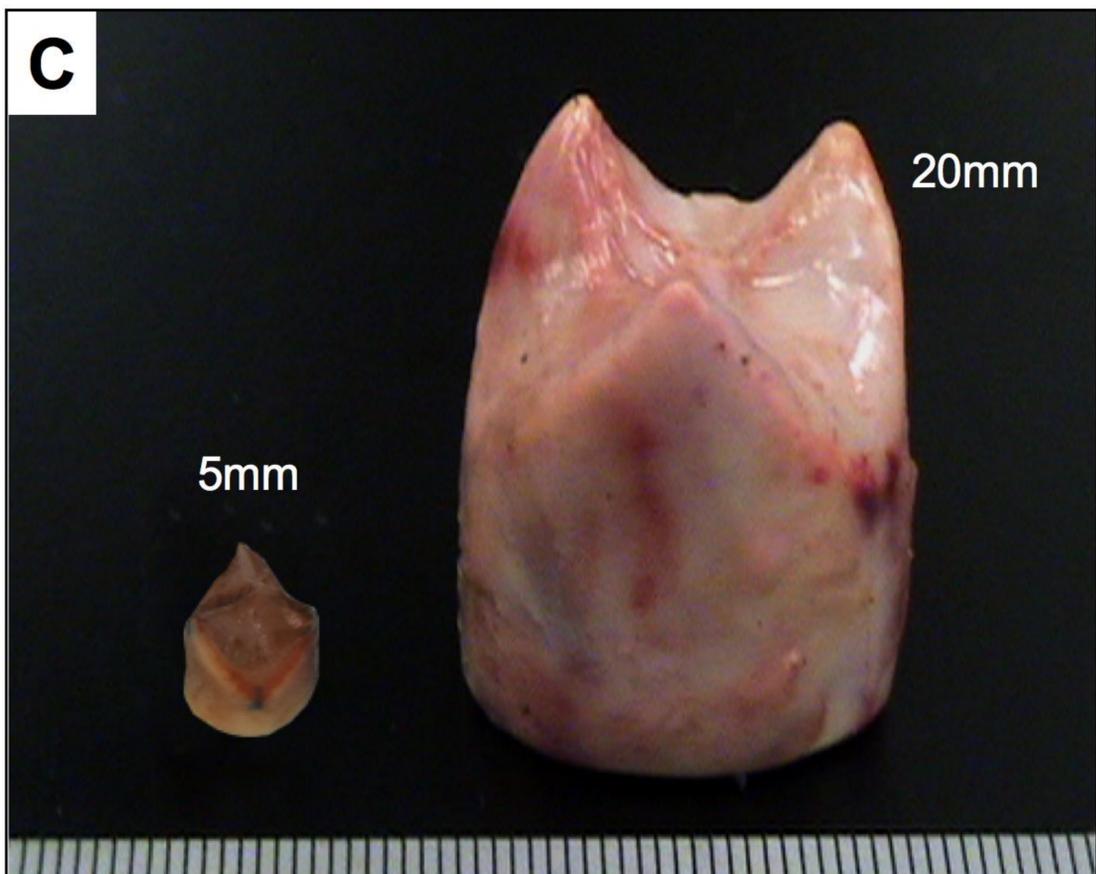
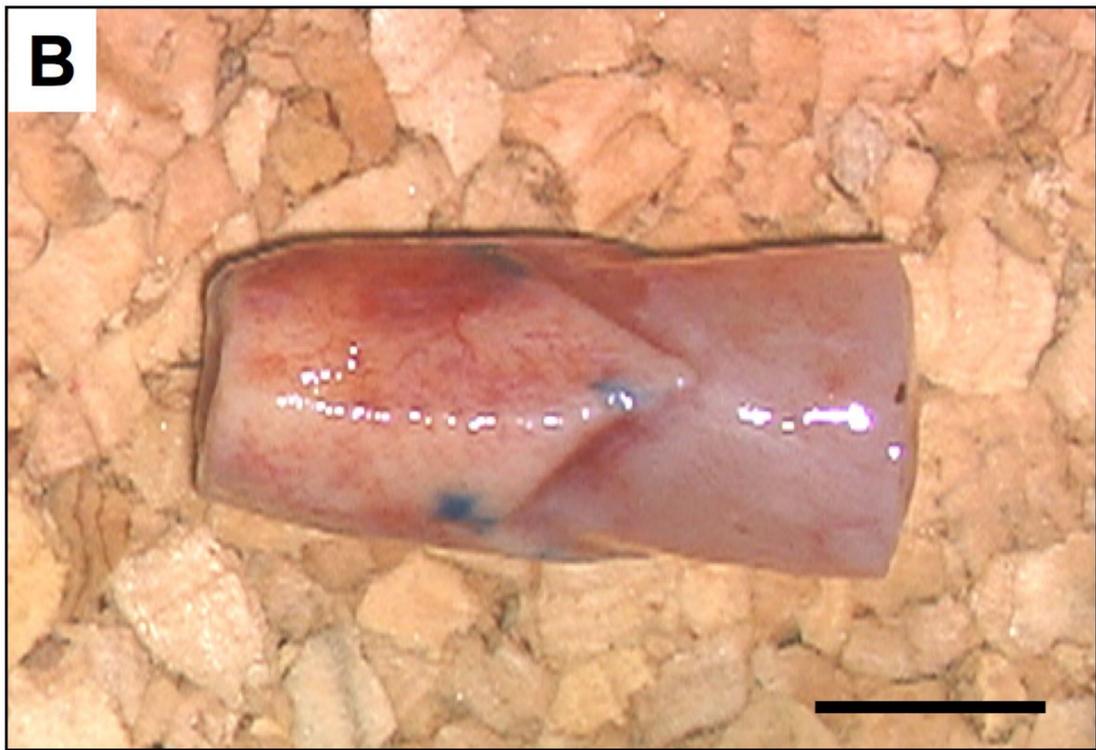
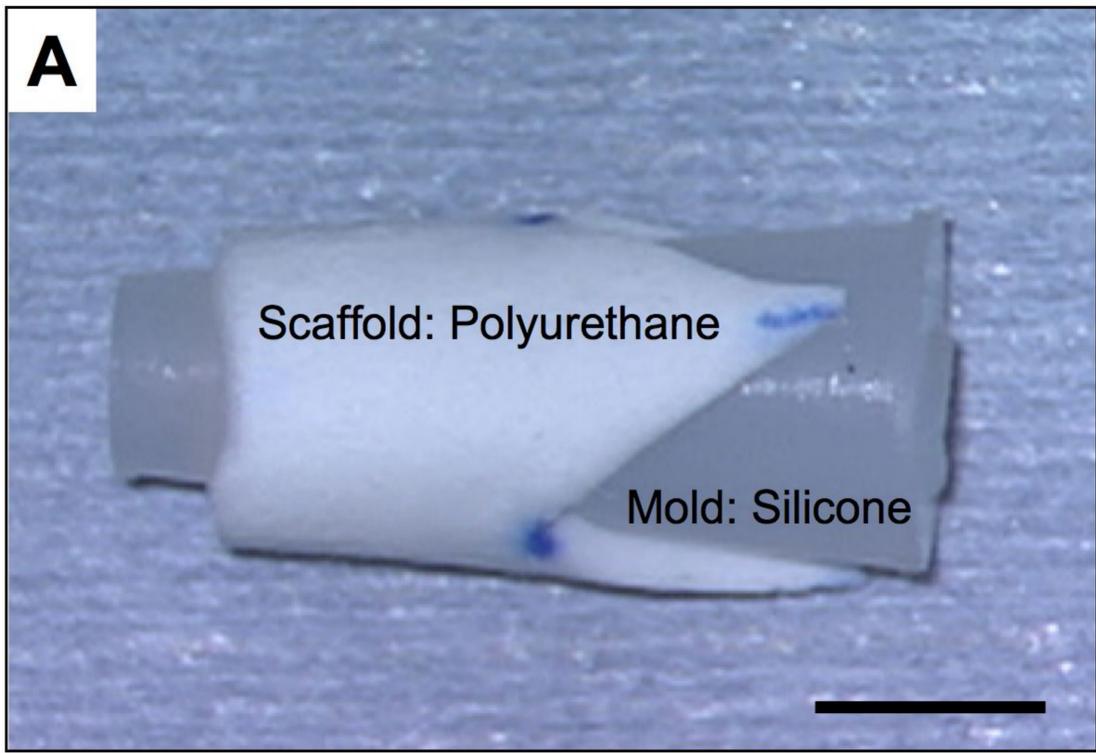


Figure 1

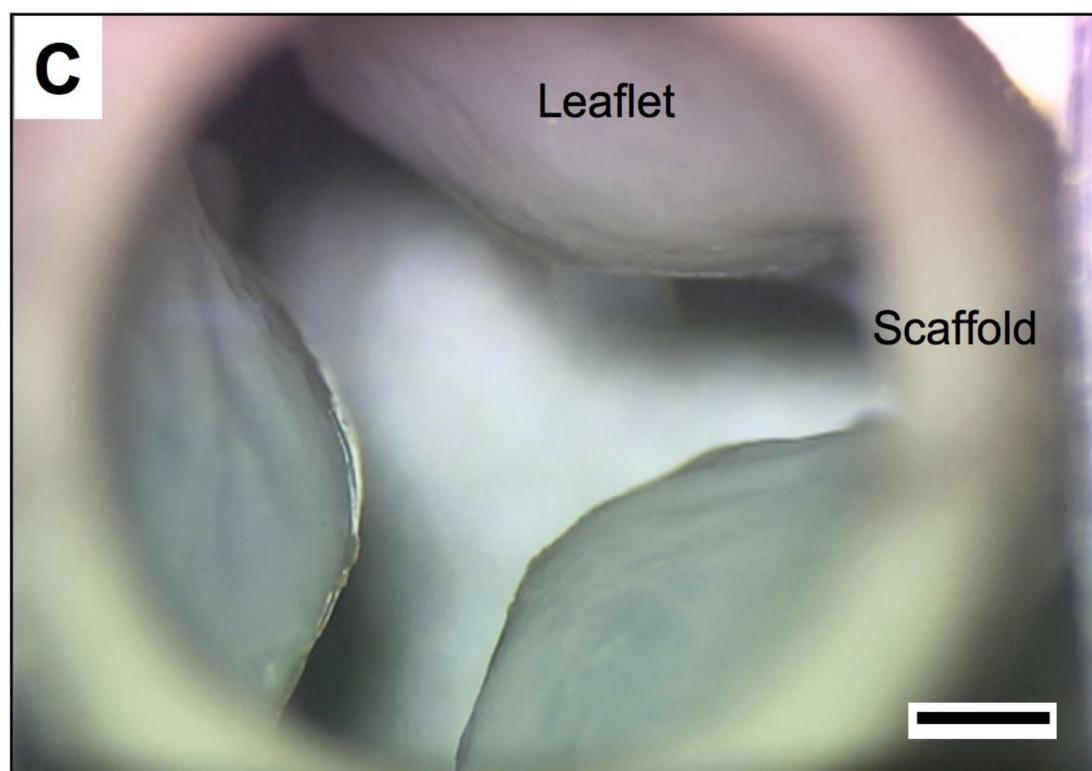
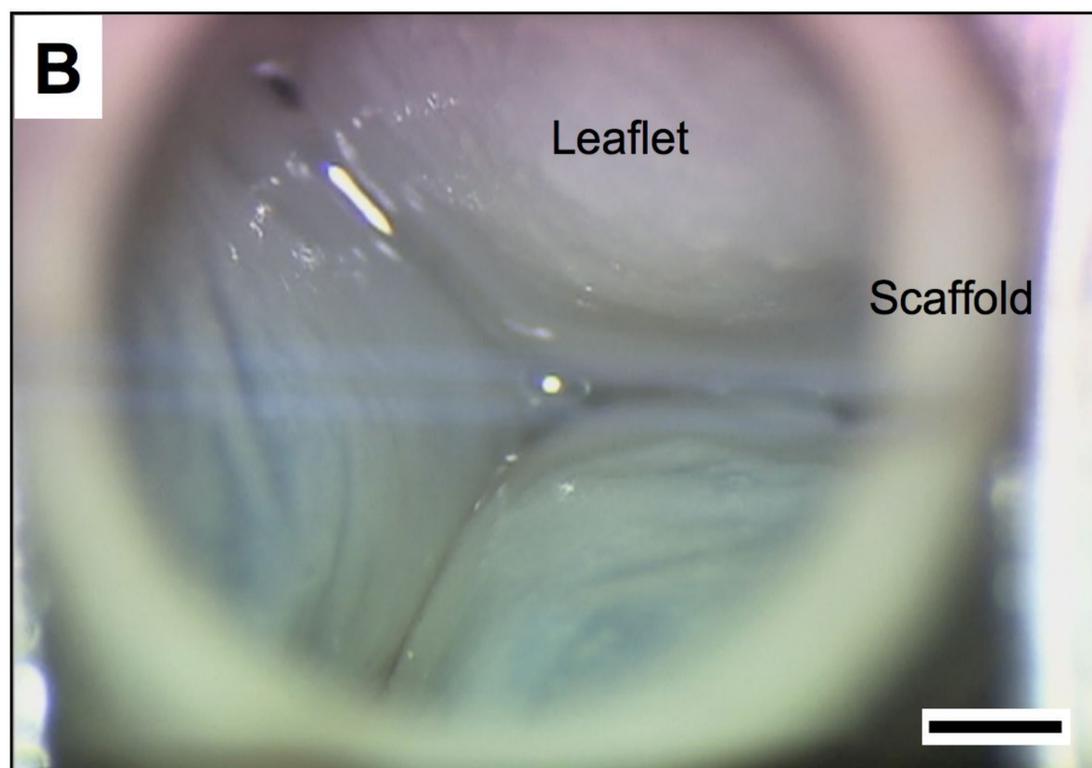
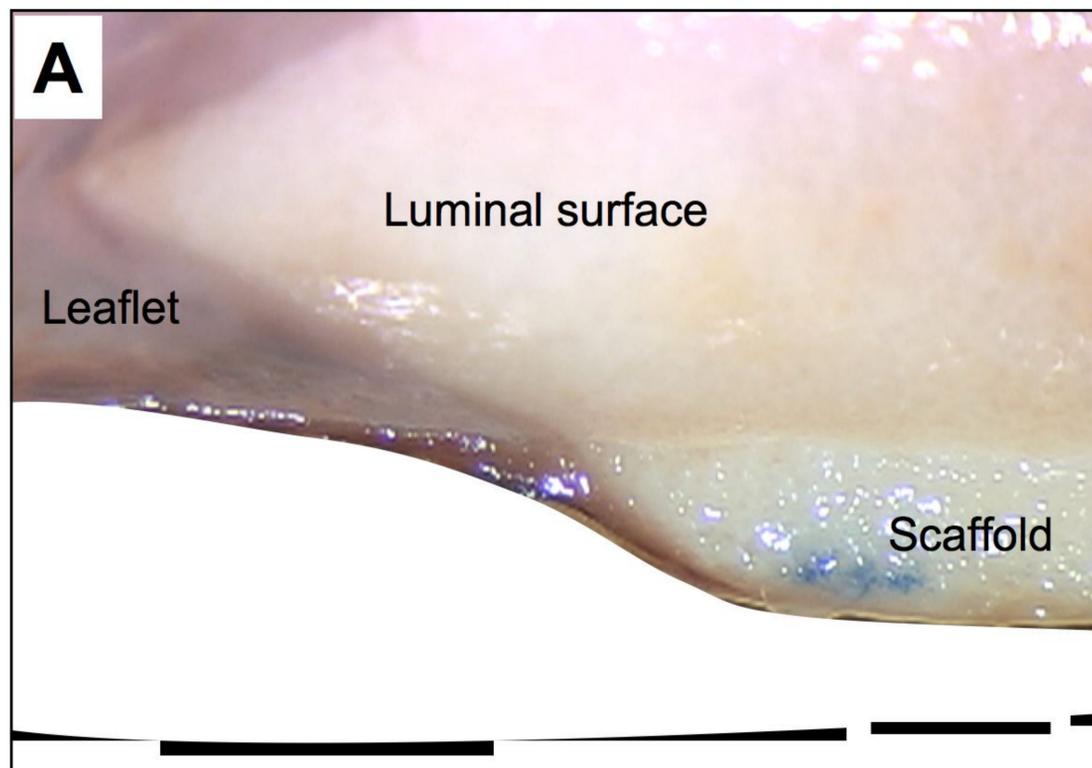


Figure 2

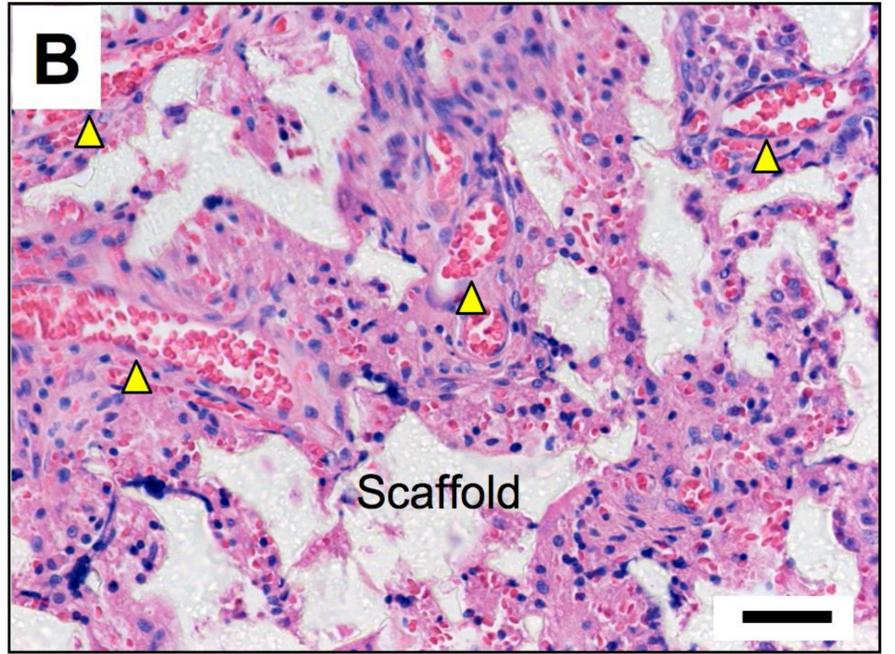
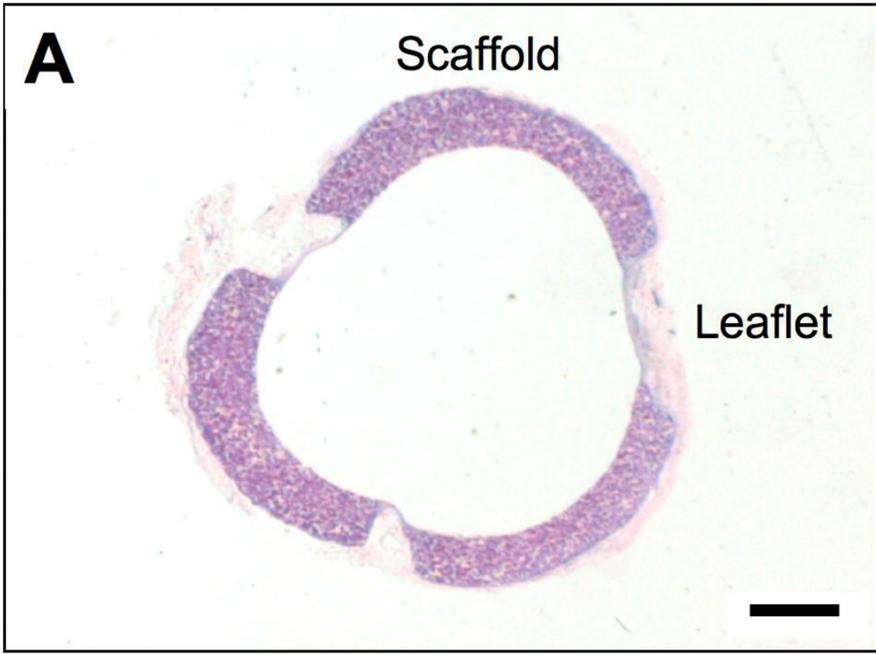


Figure 3

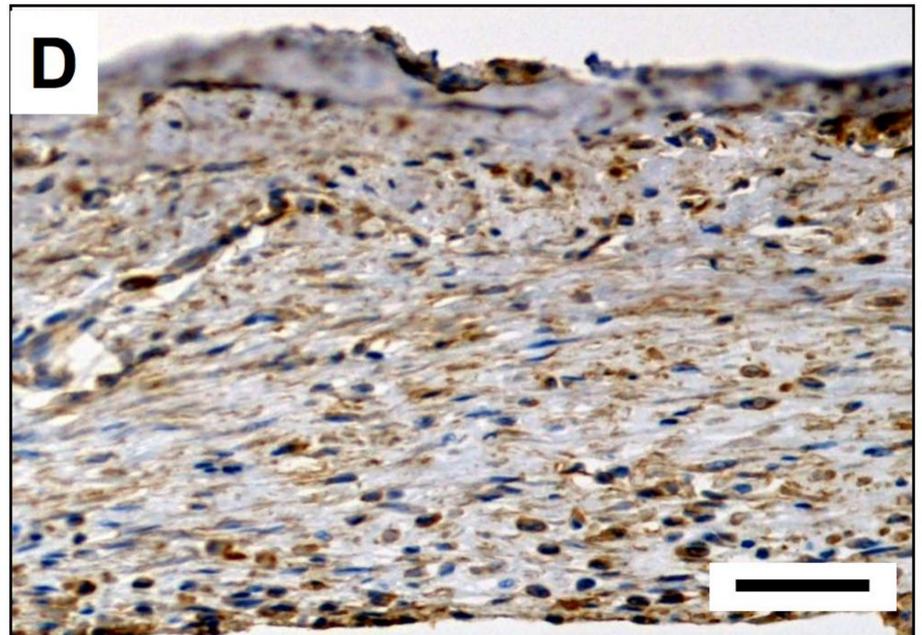
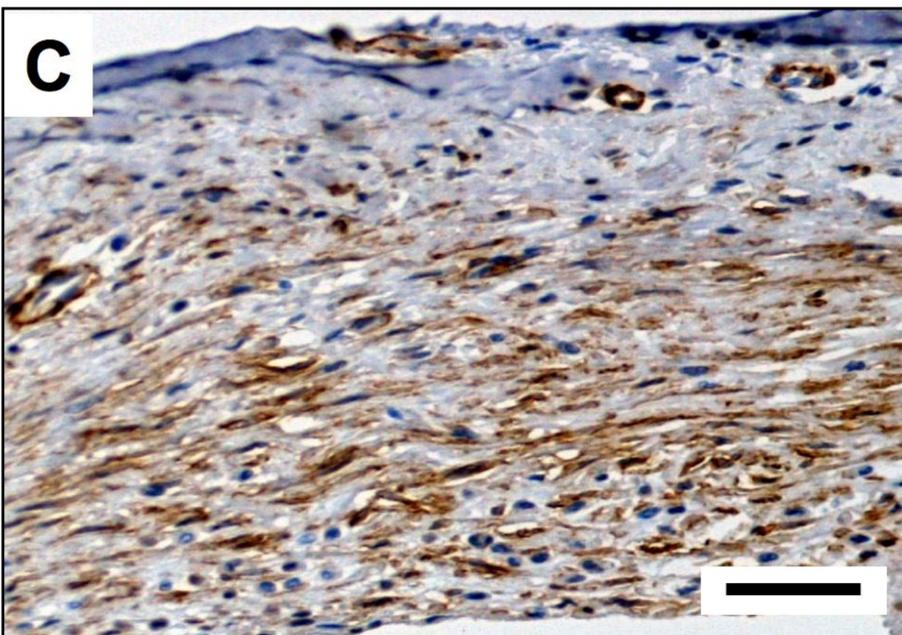
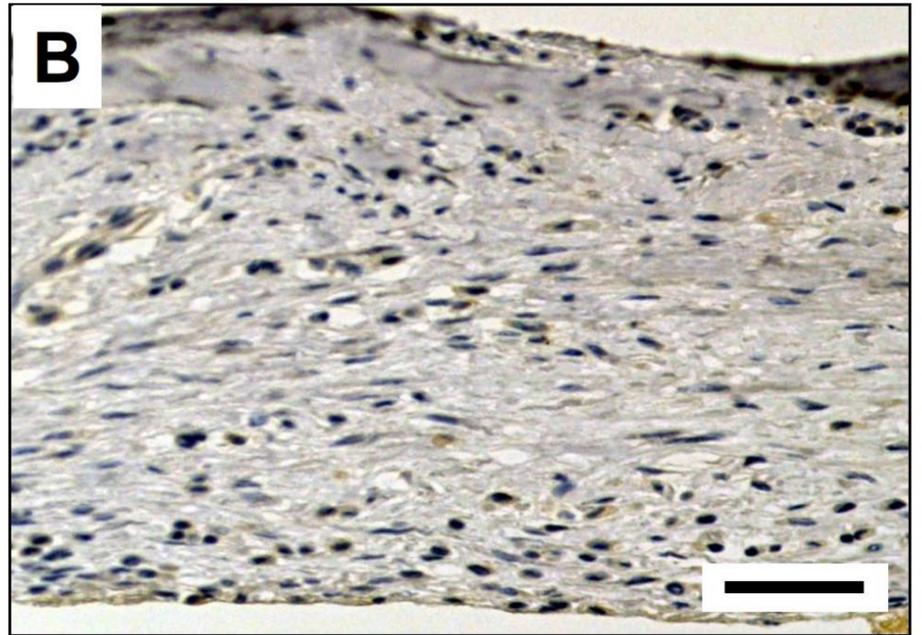
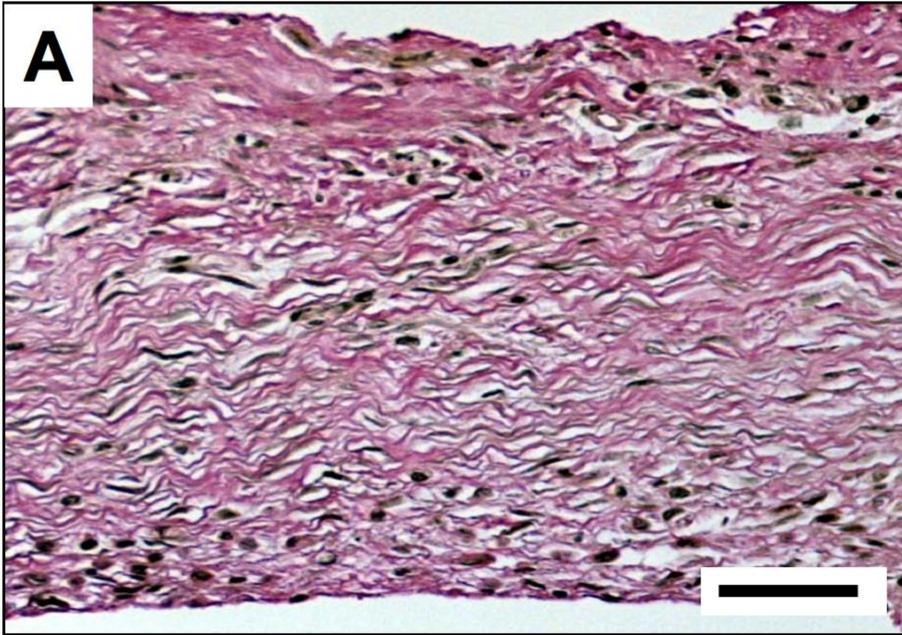


Figure 4

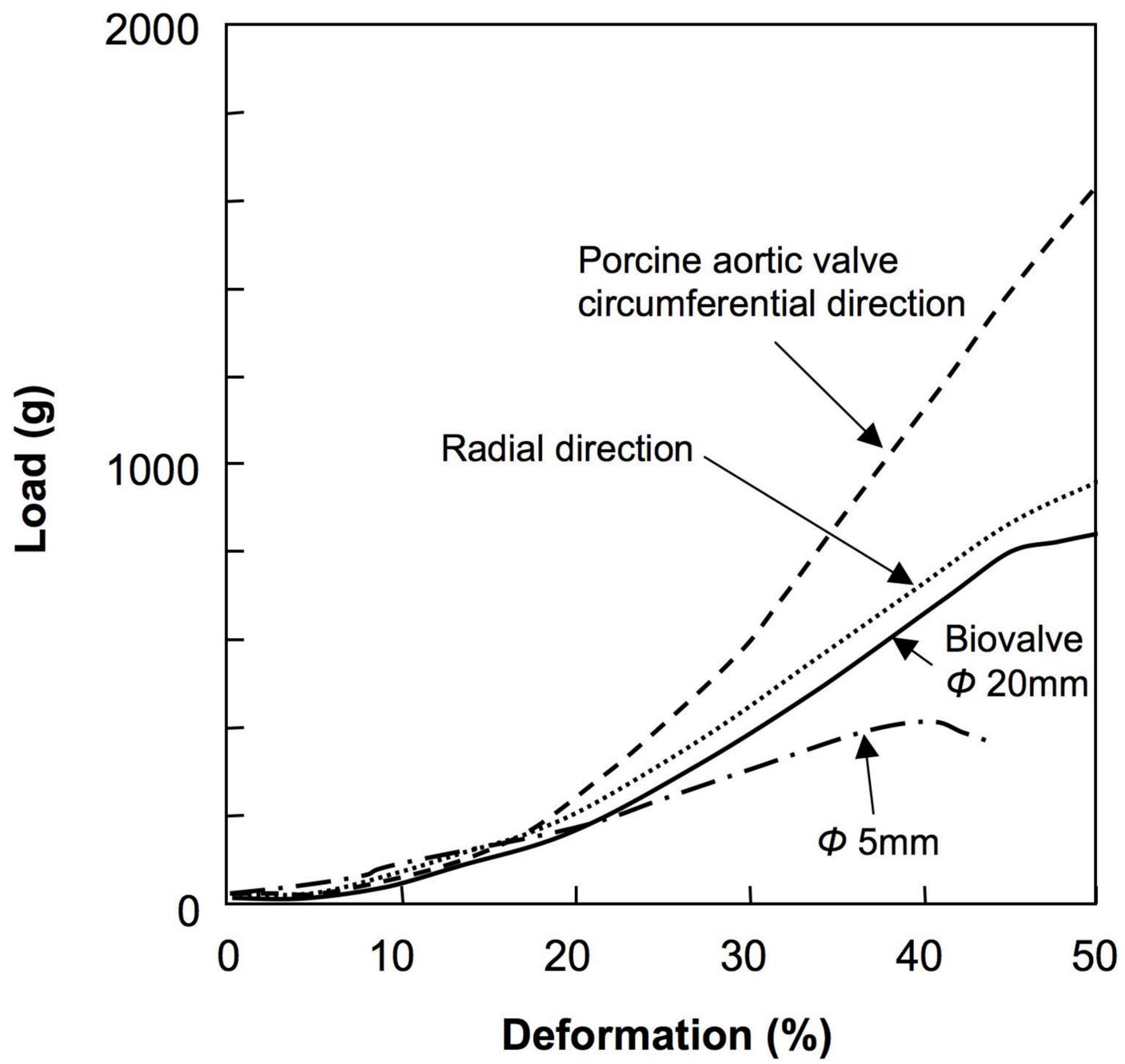


Figure 5

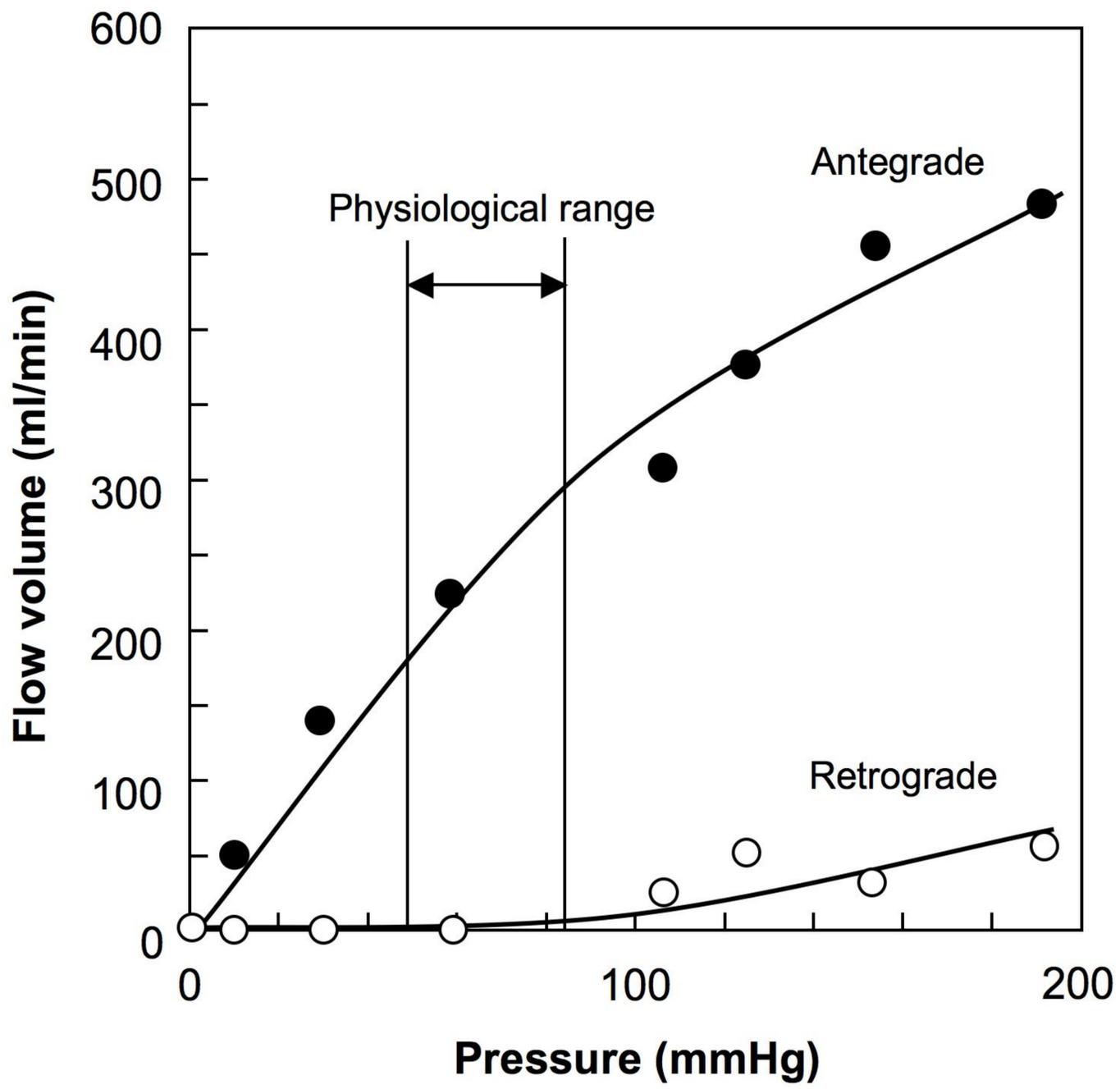


Figure 6

**Table 1.** Physical properties of the Biovalve leaflets

	Thickness $\mu\text{m}$	Tensile strength gf	Elongation at max stress, %	Apparent elastic modulus, gf/mm
Biovalve				
5 mm (n=30)	197.5 $\pm$ 60.4	307.8 $\pm$ 66.5 <sup>*,**</sup>	43.1 $\pm$ 5.4 <sup>**</sup>	251.9 $\pm$ 90.6 <sup>*</sup>
20 mm (n=42)	667.1 $\pm$ 100.6	802.5 $\pm$ 202.2 <sup>*</sup>	53.7 $\pm$ 8.4	415.4 $\pm$ 58.8 <sup>*</sup>
Porcine aortic valve				
Circumferential (n=6)	409.4 $\pm$ 252.4	1475.0 $\pm$ 367.9	68.0 $\pm$ 35.3	837.5 $\pm$ 220.9
Radial (n=6)	484.4 $\pm$ 125.1	824.8 $\pm$ 411.2	65.0 $\pm$ 13.5	410.4 $\pm$ 196.9

All values are mean  $\pm$  standard deviation. Max, maximum; \* $p < 0.05$  versus porcine aortic valve of circumferential direction; \* \* $p < 0.05$  versus porcine aortic valve of radial direction. The specimens from Biovalves were sampled from 4 rabbits.