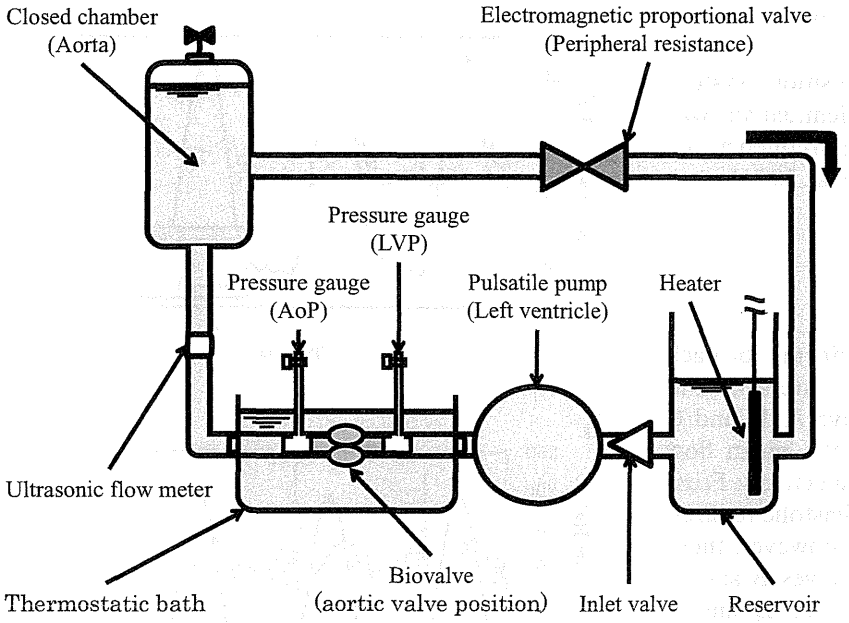

FIG. 3. Schematic drawing of the pulsatile mock circulation system that was used for the evaluation of valvular function and durability.

valvular function. This design included an outer casing (17 mm inner diameter) to prevent any growth of the sinuses of Valsalva.

Evaluation of valvular function

A pulsatile mock circulation system (LaboHeart NCVC, IWAKI Co., Ltd., Tokyo, Japan) (9) was used to simulate the systemic circulation to evaluate the valvular function of the five valves. Figure 3 shows a schematic drawing of the circulation circuit. The pulsatile mock circulation system consisted of the following elements: a pulsatile pump, a closed chamber, a reservoir, and an electromagnetic proportional valve to create fluid resistance. The mock circulation system can simulate various pulsatile conditions by adjusting the heart rate (HR) and stroke volume of the pulsatile pump, the fluid resistance of the electromagnetic proportional valve, and the compliance of the closed chamber. Each of the five valves was connected at the aortic position in the pulsatile mock circulation system. An inlet valve made of synthetic rubber was connected at the mitral valve position. The flow rate was measured using an ultrasonic flow meter (T106, Transonic Systems, Ithaca, NY, USA) attached between the valve and the closed chamber. Pressure at the aortic valve inlet reflected left ventricular pressure (LVP), and pressure at the outlet reflected aortic pressure (AoP). These two pressures were measured using a pressure gauge (PA-500, Nidec Copal Electronics Corporation, Tokyo, Japan). The stroke volume of the pulsatile pump, the compliance of the closed chamber, and fluid resistance were adjusted to maintain an average AoP of 100 ± 20 mm Hg as the HR of the pulsatile pump was varied from 70 to 120 bpm. Then, the flow rate, LVP, and AoP were measured at each HR. The regurgitation rate was calculated from the following equation: regurgitation rate (%) = (mean regurgitation/mean flow rate) × 100 (1). In this study, the regurgitation was defined as flow rate <0 L/min. The valvular functions of all valves were also measured with the same circuit, stroke volume, fluid resistance, and chamber compliance.

Long-term continuous operation

The circulatory model with biovalve C inserted at the aortic valve position was operated continuously for 40 days to evaluate the durability of the biovalve. The experiment was conducted under fixed conditions, and the pulsatile pump, closed chamber, and fluid resistance were adjusted to produce a mean flow rate of 5 L/min and mean AoP of 100 mm Hg when the HR of the pulsatile pump was set at 70 bpm. Several factors, including valve failure or fracture, valvular insufficiency, and changes in the pressure waveform, were evaluated.

Experimental conditions and data acquisition

Saline (viscosity, 0.001 Pa·s) at 37°C was used as the working fluid and the immersion fluid for all of the biovalves. A heater and a thermostatic bath were used to maintain a constant temperature. In the case of the long-term continuous operation experiment, a corrosion inhibitor (white7-SW, Yuai Kasei, Hyogo,

Japan) was added to prevent algae or limescale which are generated in the circuit.

For measurement, a data acquisition system (PowerLab; ADInstruments Japan, Aichi, Japan) was used. The analog-to-digital sampling frequency for each pressure and flow rate channel was 200 Hz.

RESULTS

Valvular function

The LVP, AoP, and flow rate waveforms for each biovalve (A, B, C) at a HR of 70 bpm are shown in Fig. 4a–c. The mean AoPs for biovalve A, B, and C were 80, 105, and 105 mm Hg, and the mean flow rates were 4.6, 5.5, and 5.5 L/min, respectively. From the flow rate waveforms, significant diastolic regurgitation was observed for biovalve A; however, there was almost no regurgitation for biovalves B and C. The regurgitation rates for biovalves A, B, and C were 46.3, 2.6, and 3.3%, respectively. Table 2 shows the pressures and flow rates for each biovalve and the two commercially available valves at each HR. In this experiment, the stroke volume of the pulsatile pump, the compliance of the closed chamber, and fluid resistance were adjusted to maintain an average AoP of 100 ± 20 mm Hg as the HR was increased. The mean flow rate for biovalve B and C ranged from 5.5 to 8.9 L/min at a mean AoP of 100 mm Hg as the HR was increased from 70 to 120 bpm. Equivalent pressures and flow rates were also obtained for the other two commercially available valves. The average regurgitation rate of biovalve B and C was approximately 3.0%. The regurgitation rate of biovalve B and C was equivalent to that obtained for the biological and mechanical valves at each HR.

Long-term continuous operation

Figure 5 shows the result of the long-term, continuous operation experiment. Each plot shows the mean values of LVP, AoP, flow rate, and regurgitation rate, averaged for 1 min each day under steady-state circulatory conditions. The durability test demonstrated that even after biovalve C was pulsated more than four million times (HR, 70 bpm; mean flow rate, 5.0 L/min; mean AoP, 92 mm Hg), stable continuous operation was possible without excessive reduction of flow rate or bursting. The regurgitation rate on the first day was approximately 6%. The regurgitation rate was <10% in the early stages (after 2 days), but it increased gradually thereafter. The regurgitation rate after 40 days reached approximately 15%. Figure 6 shows the LVP, AoP, and flow waveforms on the first day and after 40 days. Although the pulsatility in each

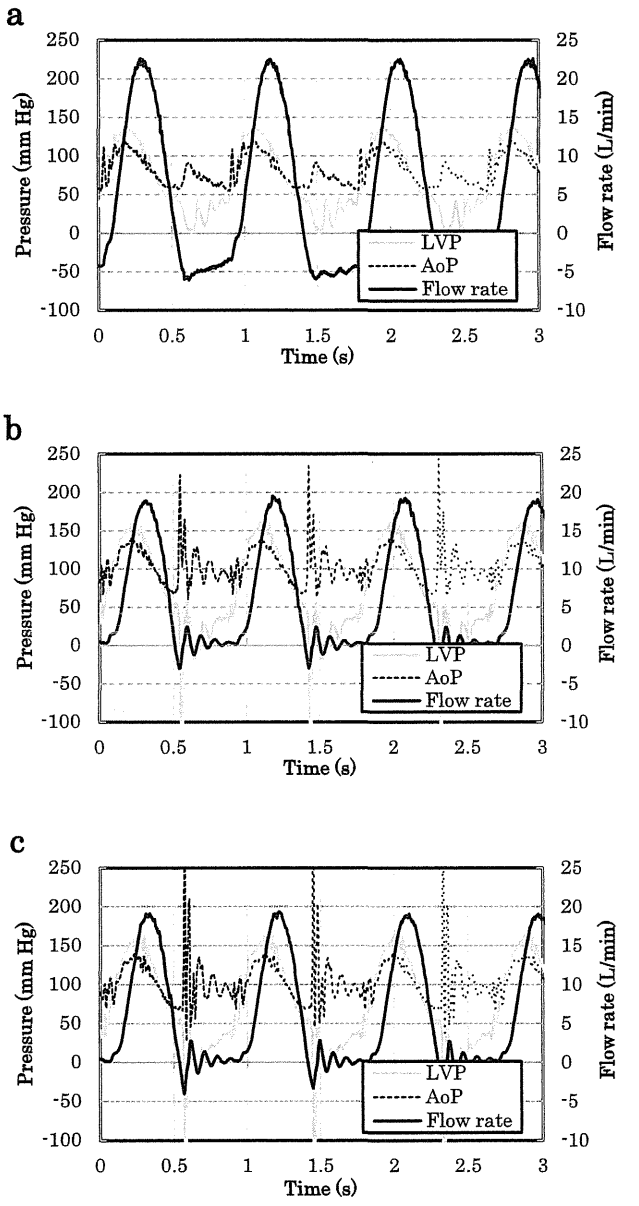

FIG. 4. LVP, AoP, and flow rate waveforms for each biovalve at 70 bpm in the pulsatile circulation circuit. (a) Biovalve A, (b) Biovalve B, (c) Biovalve C.

waveform did not change, the amount of diastolic regurgitation increased after 40 days.

DISCUSSION

A mock circulatory circuit was used to evaluate function and durability of the biovalve under the type of pulsatile load that exists in the systemic circulation. This in vitro test was performed to assess whether the biovalve can be used as the aortic valve. Recently, several valves such as prosthetic heart valves or porcine valves were evaluated using the

TABLE 2. *The HR characteristics of each biovalve, the biological valve, and the mechanical valve*

Parameters	Biovalve A	Biovalve B	Biovalve C	Biological valve	Mechanical valve
Heart rate (bpm)	70–120	70–120	70–120	70–120	70–120
Mean flow rate (L/min)	4.64–7.08	5.52–8.96	5.48–8.89	5.52–8.57	5.52–8.73
Mean LVP (mm Hg)	75.48 ± 6.07	74.86 ± 3.80	74.91 ± 3.08	88.56 ± 8.36	83.96 ± 8.31
Mean AoP (mm Hg)	82.17 ± 4.07	99.15 ± 8.40	98.54 ± 8.49	111.94 ± 3.04	115.84 ± 3.87
Mean regurgitation (L/min)	1.72 ± 0.26	0.18 ± 0.02	0.25 ± 0.05	0.15 ± 0.01	0.21 ± 0.01
Regurgitation rate (%)	26.12 ± 10.90	2.49 ± 0.22	3.40 ± 0.12	2.12 ± 0.36	2.97 ± 0.33

[Corrections added on 20 November 2013, after first online publication: Heart rate values in the first row have been changed from 7–120 to 70–120.]

pulsatile mock circulation system (10–13). Although a mock circulation loop does not replace in vivo trials, we believe that the use of the pulsatile mock circulation system in this study was an effective means of evaluating valve function before in vivo tests or clinical studies.

The influence of leaflet length on valvular function in the biovalves was examined. Regurgitation was observed during the diastolic phase of the cardiac cycle with biovalve A, whereas there was almost no regurgitation with biovalve B. We believe that biovalve B was able to maintain AoP during the diastolic phase because the coaptation area of leaflets was increased by extending the leaflets of the biovalve in an axial direction. The effectiveness of the extended leaflets in biovalve B against the pressures of the systemic circulation was confirmed. The influence of the presence or absence of sinuses of Valsalva in the biovalve under the pulsatile loading conditions that exist in the systemic circulation was also assessed. The results showed that there was no influence of the sinuses of Valsalva on the pressure or flow rate waveforms, or the mean regurgitation rate. Vortex flow in the sinuses of Valsalva plays an important role in the closure of native semilunar valves at the end of systole and in facilitating coronary flow during systole (14). Valves lacking the sinuses of Valsalva close only passively due to the backflow of blood (15). Although biovalve C did not have the sinuses of Valsalva, its regurgitation rate was not different from the regurgitation rate of biovalve B. Therefore, we believe that the sinuses of Valsalva may not be necessary if the coaptation area of leaflets in the biovalve is wide enough. Each biovalve was compared with conventional biological and mechanical valves over a practical range of HRs. There were no large differences in mean LVP, mean AoP, mean flow rate, or the mean regurgitation rate among four valves (biovalve B, biovalve C, biological valve, and mechanical valve) as the HR varied from 70 to 120 bpm. These results suggest that the biovalve B and C functioned as well as the two commercially available valves, at least on a short-term basis. In this study, evaluation of biovalve was performed

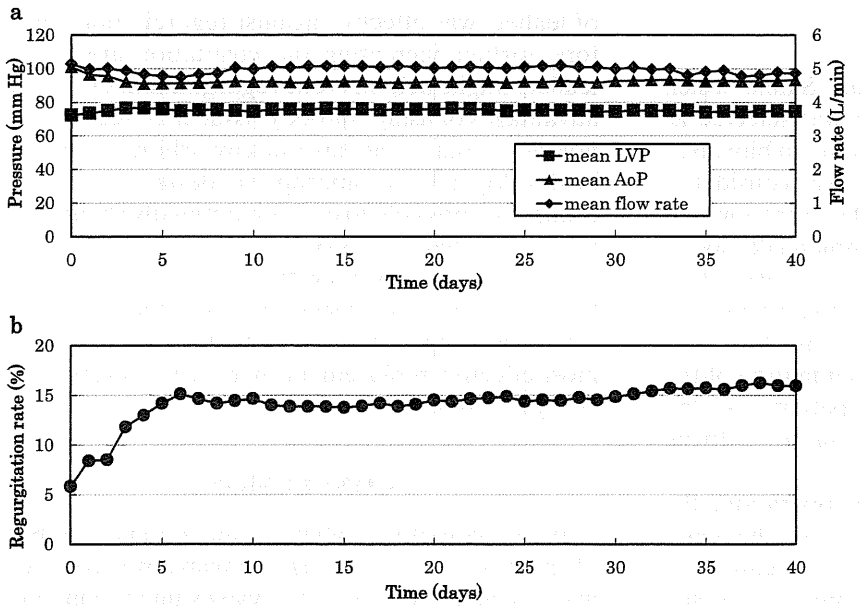

FIG. 5. Continuous operation of biovalve C for 40 days. (a) Mean LVP, AoP, and flow rate for biovalve C over time. (b) Regurgitation rate of biovalve C over time.

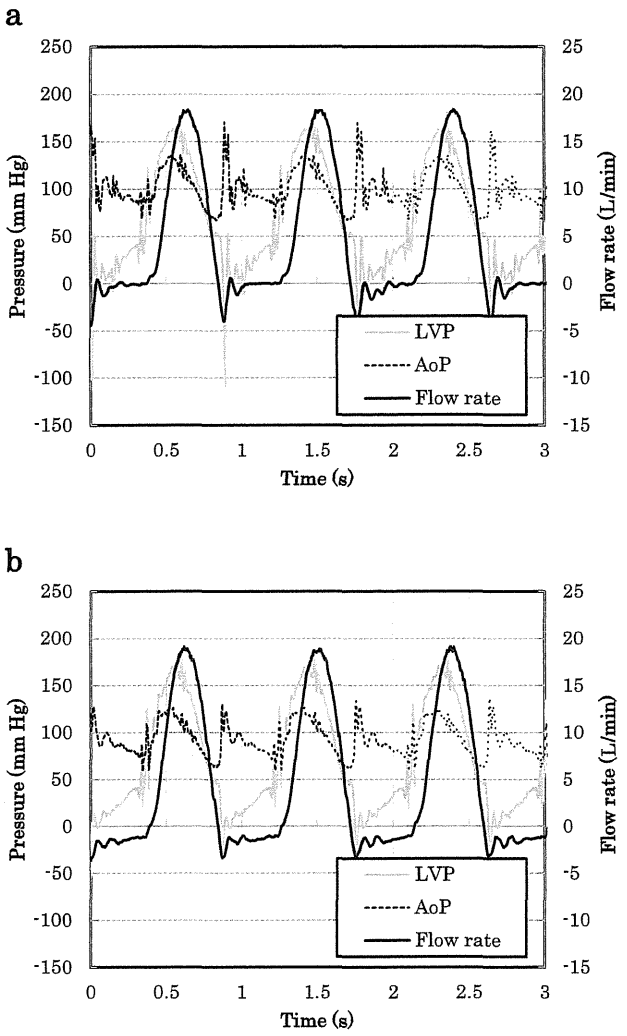

FIG. 6. LVP, AoP, and flow rate waveform on the first day and after 40 days in the long-term, continuous operation experiment in Fig. 5. (a) First day. (b) After 40 days.

using the pulsatile circulation circuit. Saline which has a different viscosity from blood was selected as the working fluid for prevention of injury in biovalves made of natural tissues. However, it was considered that the evaluation of the valvular functions of biovalves in comparison to two commercially available valves to the pulsatile pressure loads that simulated the systemic circulation conditions was achieved in this circulation circuit. In the near future, we will perform a detailed examination of the valve behavior or internal flow pattern of the biovalve under flow conditions similar to a living body.

In terms of continuous biovalve operation, the regurgitation was approximately 6% in the early phase, and it increased gradually thereafter to approximately 15%. Normally, the connective tissue of the biovalve becomes infiltrated with cells, and the wall thickness of the conduit and sinus of Valsalva significantly increases after implantation in vivo. In this study, the biovalve was evaluated under very severe conditions because cell-free saline was used as the working fluid in the mock circulation system. We believe that the regurgitation of biovalve C increased after 40 days because the mechanical strength of the biovalve decreases without cell invasion during continuous operation. It was reported that pulsatile flow loading in vitro for 2 days caused the sparse, randomly oriented collagen fibers in biotubes to become dense and oriented in the regular circumferential direction (16). The biotubes used in that study were fabricated with the same technology as the biovalves used in the present study, in terms of collagen structures and mechanical properties (16). Therefore, if cells infiltrate the connective tissue of the biovalve in the early phase of low regurgitation, it may be possible to prevent the increase in regurgitation. In future studies, evaluation of biovalve durability including cell invasion will be necessary to validate the usefulness of the biovalve. In the present study, the developed biovalve maintained a regurgitation rate of about 15% without cell invasion into the connective tissue. However, long-term durability of the biovalve is insufficient compared with mechanical valves or biological valves in the case where cells do not invade. Therefore, not only the improvement of mechanical properties which depend on cell invasion but also the improvement in the mechanical durability of the biovalve itself is required in order to obtain the durability equivalent to the mechanical valves or biological valves. The results of valvular function testing showed that the expansion of coaptation area of leaflets was effective against regurgitation. Therefore, further increasing the coaptation area of the leaflets might help prevent regurgitation. One of the advantages of using "in-body tissue architecture technology" is that a biovalve of any arbitrary form can be developed by the appropriate design of the molds. It might be possible to develop optimally shaped leaflets in a biovalve to produce good valvular function and durability, even without cell invasion. The biovalve developed using "in-body tissue architecture technology" has the potential to become the most effective replacement therapy for severe valvular heart disease.

CONCLUSIONS

We evaluated the valvular function and durability of the biovalve in vitro. The biovalve was compared with commercially available valves under conditions

that simulated the systemic circulation. The leaflet size, which is responsible for the coaptation area, is important to prevent increases in biovalve regurgitation during long-term operation, and the sinuses of Valsalva may be unnecessary for optimal biovalve function. The developed biovalve demonstrated good valvular function and durability and may be potentially useful for aortic valve replacement.

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Preparation of an autologous heart valve with a stent (stent-biovalve) using the stent eversion method

Takeshi Mizuno,[1,2] Yoshiaki Takewa,[3] Hirohito Sumikura,[3] Kentaro Ohnuma,[3] Takeshi Moriwaki,[1] Masashi Yamanami,[1] Tomonori Oie,[1] Eisuke Tatsumi,[3] Masami Uechi,[1,2] Yasuhide Nakayama[1]

[1]Division of Medical Engineering and Materials, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan
[2]Department of Veterinary Medicine, Veterinary Cardiovascular Medicine and Surgery Unit, Laboratory of Veterinary Internal Medicine, College of Bioresource Sciences, Nihon University, Kanagawa, Japan
[3]Department of Artificial Organs, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan

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Abstract: We designed a novel method for constructing an autologous heart valve with a stent, called a stent-biovalve. In constructing completely autologous heart valves, named biovalves, which used in-body tissue architecture technology, tissues for leaflets were formed via ingrowths into narrow apertures in the preparation molds, frequently leading to delayed or incomplete biovalve preparation. In this technique, self-expandable nitinol stents after everting were mounted on an acrylic column-shaped part and partially covered with an acrylic cylinder-shaped part with three slits. This assembled mold was placed into subcutaneous abdominal pouches in beagles or goats for 4 weeks. Upon removing the acrylic parts after harvesting and trimming of capsulated tissues, a tubular hollow structure with three pocket-flaps of membranous tissue rigidly fixed to the stent's outer surface was obtained. Then, the stent was turned inside out to the original form, thus moving the pocket-flaps from outside to the inside. Stent-biovalves with a sufficient coaptation area were thus obtained with little tissue damage in all cases. The valve opened smoothly, and high aperture ratio was noted. This novel technique was thus highly effective in constructing a robust, completely autologous stent-biovalve with adequate valve function. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2013.

Key Words: heart valve, autologous tissue, stent, biovalve

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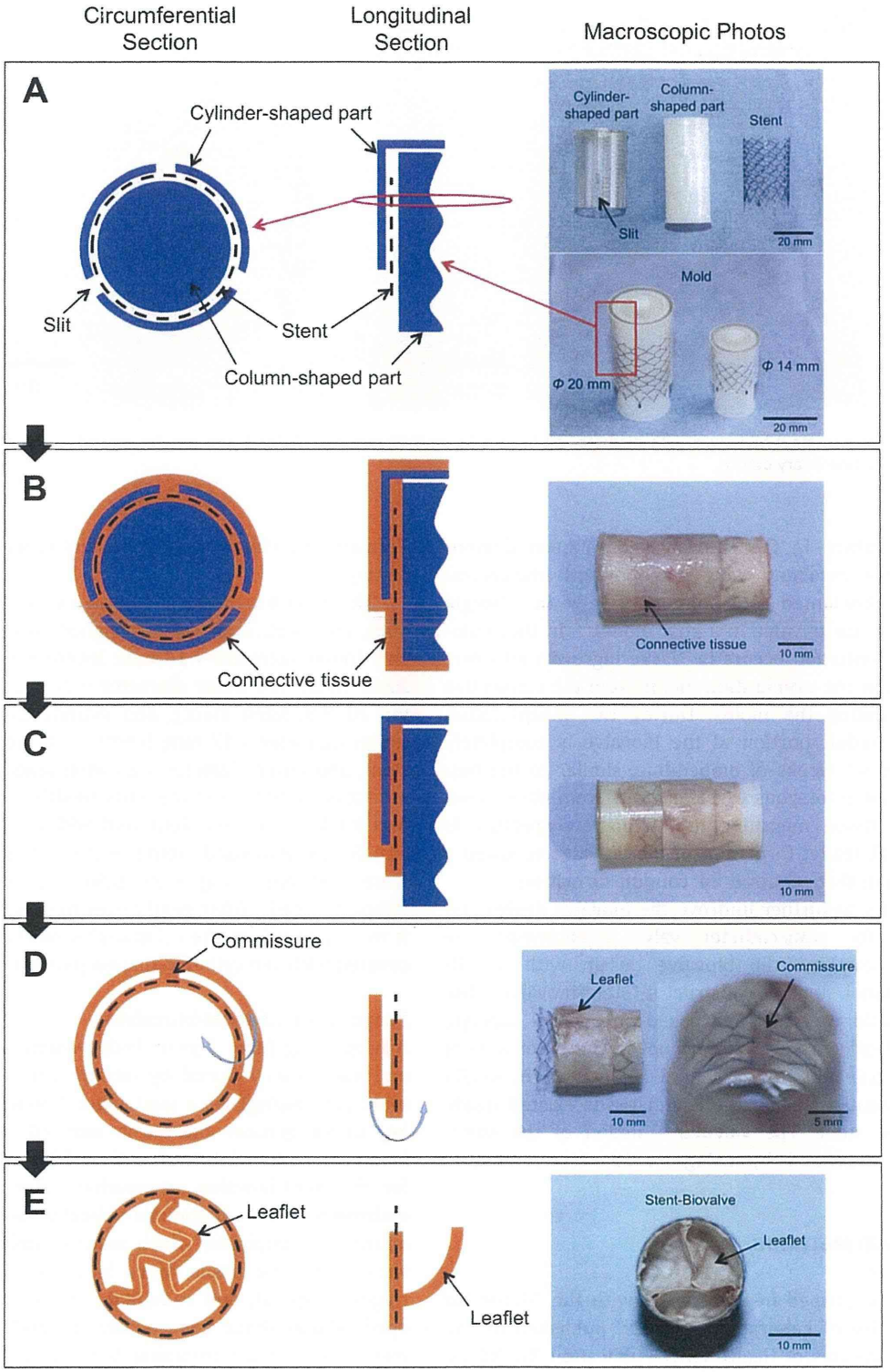
INTRODUCTION

Aortic valve disease is one of the most major heart diseases in humans. Once symptoms develop, the average survival of patients with aortic stenosis is reduced to <5 years.[1–3] Patients with severe valvular insufficiency or stenosis typically require valve repair or valve replacement. Surgery for end-stage valvular heart disease consists of two basic alternatives, mechanical and biological prostheses, both of which have significant limitations.[4] While mechanical valves have a functional life span of at least 25 years, they are associated with the need for life-long anticoagulation treatment and the concomitant risks of thromboembolism and bleeding. Biological prostheses generally have better hemodynamic characteristics and do not require long-term aniticoagulation therapies, but are associated with progressive tissue deterioration. Since surgical valve replacement is a highly invasive surgery involving thoracotomy and cardiopulmonary bypass, elderly patients and those with extensive comorbidities cannot undergo this surgery. Since 2002,[5] the less invasive transcatheter aortic valve implantation (TAVI) has been introduced for inoperable and high-risk patients. Recently, with technological advancements, the clinical application of TAVI has extended to intermediate-risk patients.[6] TAVI is expected to evolve further and become more commonly used in the future. However, using a bioprosthetic valve for TAVI has certain disadvantages, since it undergoes progressive degeneration and calcification as it contains no living cells.[7,8]

To overcome these limitations, living heart valves—created by tissue engineering—have been under development. Some heart valves created by tissue engineering have been successfully implanted in animals.[9,10] However, these valves require complicated cell management protocols with cell culture in bioreactors under strictly sterile conditions; this procedure is time-consuming and expensive.

We have previously developed autologous prosthetic tissues by using the "in-body tissue architecture (IBTA)" technology, which is a novel and practical approach for regenerative medicine based on the tissue encapsulation phenomenon of foreign materials in living bodies.[11] This

Correspondence to: Y. Nakayama (e-mail: ny@ncvc.go.jp)



technology involves the use of living bodies as a reactor, and does not need expensive facilities or complicated manipulations. We have reported the construction of completely autologous trileaflet heart valves, named biovalves, prepared using this technology,[11–15] which may resolve the abovementioned problems encountered with

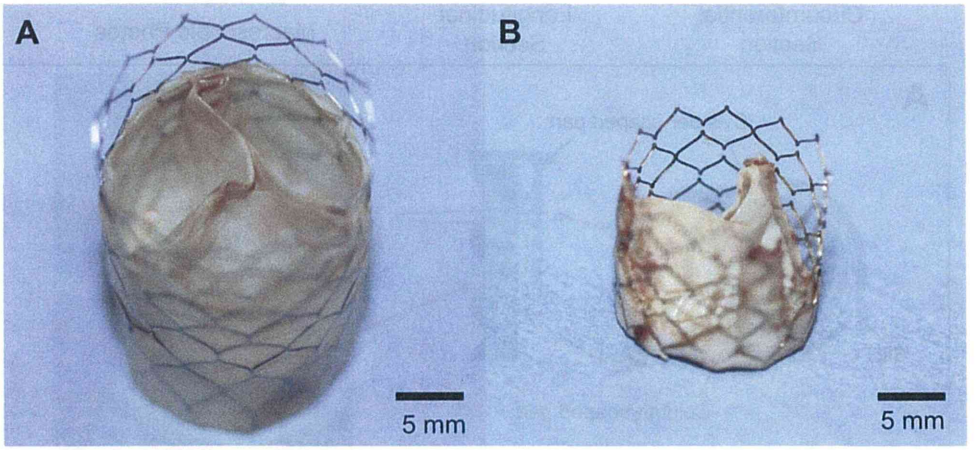


FIGURE 2. The obtained stent-biovalves with diameter of 20 mm (A) and 14 mm (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

bioprosthetic valves. In fact, biovalves have been demonstrated to have excellent valve function and histological changes when implanted as a pulmonary valve in a beagle model[16] and an aortic valve in a goat model.[17] In these biovalves, leaflet formation occurs by tissue ingrowth into narrow apertures in the preparation molds from the connective tissues surrounding the molds. During the encapsulation process, the conduit portion of the biovalve is completely formed within ~4 weeks of embedding, similar to the biotubes, which are autologous vascular grafts from IBTA. However, because tissue migration into a narrow aperture is slow in general, leaflet formation in the biovalve required a longer time than that required for conduit formation.

In this study, to further improve the biovalve design and to adapt it for transcatheter valve replacement, we attempted to combine the biovalve design with a self-expandable nitinol stent to construct a "stent-biovalve." The preparation mold was designed based on a novel concept, in which the leaflet tissue was formed on the outer side of the mold; the trileaflet-shaped valve was obtained by finally everting the stent such that the leaflet tissue existed inside surface of the stent. The valvular function of the stent-biovalve was examined by using an *in vitro* circulation circuit.

MATERIALS AND METHODS

Animal studies

Studies were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) under a protocol approved by the National Cerebral and Cardiovascular Center Research Institute Committee (No. 12002).

Mold assembling

Two kind of stents used were self-expandable (diameter- = 14 mm; length = 15 mm or diameter = 20 mm; length- = 30 mm), obtained from shape rememory of E-LUMINEXX (diameter = 12 mm; length = 100 mm; Bard, Karlsruhe, Germany) by Piolax Medical Devices (Yokohama, Japan) and cutting.

The mold for the stent-biovalve was obtained by assembling the stent, a specially designed column-shaped acrylic part (outer diameter = 14 mm; length = 32 mm for 14 mm-sized stent, and outer diameter = 20 mm; length = 46 mm for 20 mm-sized stent), and cylinder-shaped acrylic part (outer diameter = 17 mm; length = 18 mm for 14 mm-sized stent, and outer diameter = 23 mm; length = 34 mm for 20 mm-sized stent) with three slits (width = 1 mm; length = 10 mm for 14 mm-sized stent, and width = 1 mm; length = 15 mm for 20 mm-sized stent) [Figure 1(A)]. All acrylic parts were prepared using a 3D printer (CONNEX 260, Objet, Rehovot, Israel). After gently everting the stent in ice water, it was mounted on the column-shaped acrylic part and then covered with the cylinder-shaped part for the final mold.

Preparation of stent-biovalves

A beagle dog (age: 1 year; body weight: 10 kg) under general anesthesia induced by intramuscular injection of ketamine (20 mg/kg), or a goat (age: 1 year; body weight: 50 kg) under general anesthesia induced with 10 mg/kg of ketamine and maintained with 1–3% isoflurane was used for the stent-biovalve preparation. After 4 weeks of mold embedding in the abdominal subcutaneous pouches of each animal, the implants, which were completely encapsulated with connective tissue, were harvested [Figure 1(B)]. The fragile, irregular, and redundant tissues around the developed tubular tissue were gently cut, and three leaflet parts were obtained by trimming the capsulated tissue [Figure 1(C)]. The acrylic cylinder- and column-shaped parts were removed. The stent, now embedded in connective tissue, also showed three flaps of membranous connective tissue on its outer surface [Figure 1(D)]. The stent was then everted to its original form in ice water to obtain the stent-biovalve, with a tri-leaflet valve on its inner surface [Figure 1(E)]. The stent-biovalves with diameter of 20 mm [Figure 2(A)] were prepared from goats and those of 14 mm [Figure 2(B)] were from beagles.

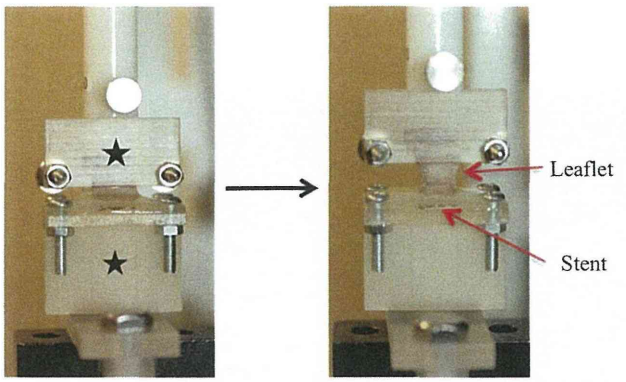


FIGURE 3. Photographs of sample folder in the apparatus for connective strength measurement before (A) and after (B) stretching. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Histological evaluation

The leaflets of the stent-biovalve were fixed in 10% formalin solution and embedded in paraffin. Then leaflet sections were cut into pieces 3–5 μm thick for hematoxyline and eosin staining or masson's trichrome stain. The wall thickness of the leaflets was measured by microscopic observation of the sections.

Measurement of mechanical properties

The burst strength of the leaflet tissues of stent-biovalve was determined by using a specially designed apparatus. The specimens were fixed on a sample holder with a hole (diameter = 2 mm) at its center. Saline solution was introduced into this apparatus at a rate of 50 mmHg/s. The burst strength was determined by measuring the water pressure at the instant of the tissue rupture using a pressure transducer (N5901; Nihon Denki Sanei, Tokyo, Japan).

The elastic modulus of the biotubes was examined using a custom designed tensile tester. Tubular samples were cut circumferentially and opened. Tissue specimens, 10 × 10 mm^2, were tested under humid conditions. The load was recorded until the samples ruptured, with a tissue-extension rate of 0.05 mm/s. Elastic modulus values were obtained from the maximum slope of the deformation-force relationships.

Measurement of the connective strength between leaflet and stent of the stent-biovalve was performed by use of a uniaxial tensile-testing apparatus (Rheoner II; Yamaden, Tokyo, Japan) [Figure 3]. The connective strength between native aortic valve and conduit of goat were also measured in same way. Each sample was fixed in a sample folder that was specially designed by use of a 3D printer (Projet HD3000; 3D Systems, Rock Hill, SC). The testing speed was 0.05 mm/s until failure, that is, tissue rupture. Ultimate tensile strength was calculated from the stress-strain curves.

In vitro valve function

Valve function was examined using a pulsatile circulation circuit (LaboHeart NCVC, IWAKI; working fluid, 0.9% saline; mean arterial pressure, 100 mmHg; mean flow rate, 5–6 L/min, Figure 4). The flow rate, left ventricular pressure, and aortic pressure were measured using an ultrasonic flow meter and pressure meter. The regurgitant ratio and mean flow rate at every 10 bpm from 70 to 120 pulsatile rates were evaluated.

RESULTS

Preparation of stent-biovalves

The two different sized assembled molds [outer diameter of stents, 14 or 20 mm; Figure 1(A)] that were embedded in the subcutaneous pouches of the beagle or the goat for 4 weeks showed complete encapsulation with autologous connective tissue [Figure 1(B)]. The implants could be easily harvested because the developed capsulated tissues and the surrounding subcutaneous tissues were connected only by very fragile, irregular, and redundant tissues, which could be easily removed. The capsulated tissues were dissected to remain the tissue for the leaflets [Figure 1(C)]. The molds could be smoothly removed from both ends of the implant because there was no adhesion between the molds and the tissues covering the stent [Figure 1(D)]. The leaflet tissues were strongly fixed at the three commissures. The stents were iced and then inverted inside out. The tissue flaps, which originally existed outside the stent, were thereby converted to inner leaflets; the stent-biovalves were thus completely prepared [Figure 1(E)]. During the inversion, no or little damage occurred to the leaflet tissues and to the connecting tissues between the leaflet and the stent. Two sized stent-biovalves with a sufficient coaptation area were thus obtained with outer diameter of 14 mm from beagles or 20 mm from goats [Figure 2]. The success rate of the stent-biovalve preparation was 100% (8/8) for each size.

The histological photographs showed that the valve leaflets of the stent-biovalve mainly consisted of collagen fibers [Figure 5]. The wall thickness of the valve leaflet was 285 ± 96.2 μm.

Mechanical properties

The burst strength of the leaflets of the stent-biovalves was over 7600 mmHg, which was closed to that of aortic valve leaflets (6200 ± 1400 mmHg).[16] Elastic modulus of the leaflets of the stent-biovalve was 2.6 ± 1.1 MPa, whereas that of goat aortic valve leaflets was 1.1 ± 0.4 MPa. To evaluate the

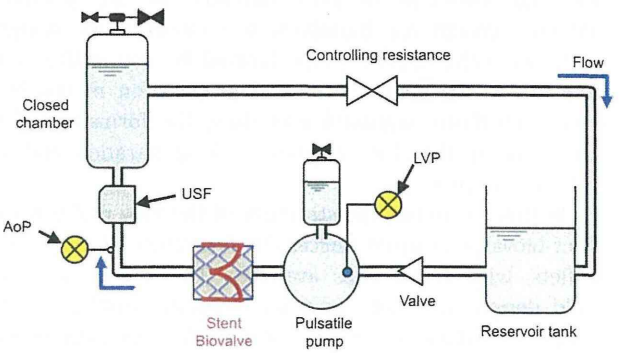


FIGURE 4. A pulsatile circulation circuit model designed for the evaluation of valve function. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

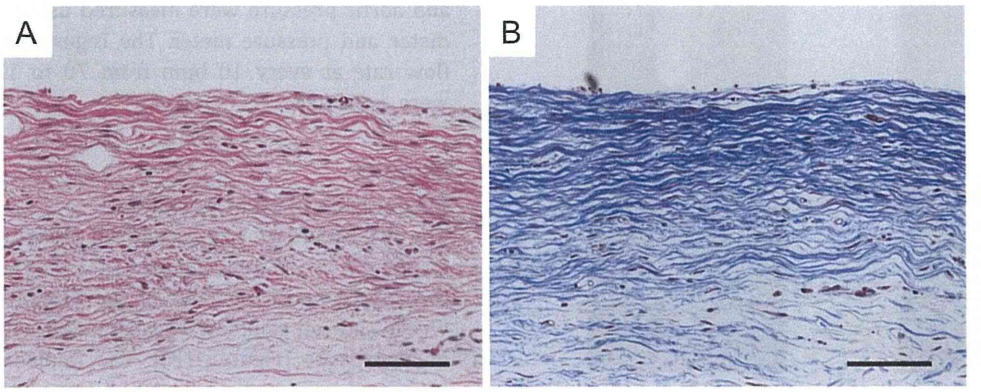


FIGURE 5. Histology of the leaflet of stent-biovalve stained with Hematoxylin and eosin staining (A) and Masson's and trichrome staining (B). bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

connective strength between biovalve leaflet and stent, the tensile strength of the biovalve leaflet and stent was measured by using specially designed sample folder [Figure 5]. The connective strength between biovalve leaflets and stent was comparable to between aortic valve and conduit of goats. The mean values of ultimate tensile strength in each sample were as follows, biovalve leaflet-stent: 833.8 ± 215.5 gf; aortic valve-conduit of goat: 949.7 ± 186.1 gf.

In vitro valve function

The movement of the leaflets in a pulsatile flow circuit [Figure 4] was examined using videography. The stent-biovalve leaflets closed rapidly and tightly in synchronization with the backward flow in the diastolic phase. In the transition phase of the flow direction, the valve opened smoothly and the aperture ratio of the valve was 89% [Figure 6(A)], and coaptation of valve leaflets was optimal [Figure 6(B)].

Figure 6(C) shows the flow rate waveforms of the stent-biovalve at 70 bpm. Regurgitation in the diastolic phase was almost completely prevented. The mean flow rate was ~5–6 L/min [Figure 7(A)], and the regurgitation ratio was ~4% for each heart rate tested [Figure 7(B)].

DISCUSSION

Here, we report the successful development of novel construction method for a stent-biovalve with robust valve leaflets and favorable *in vitro* function. In the previously reported design for biovalves, the molds were designed such that valve leaflets were formed by connective tissue penetrating the apertures in the preparation molds. However, such tissue ingrowth was slow; the formation of the valve leaflets therefore required a long duration and was not always robust.

In this technique, the structure of the new molds for the stent-biovalve ensured successful formation of three valve leaflets, with broad flaps available. The key point of this mold design was that the outer circumferential connective tissue was used to form each valve leaflet. Typically, in a tri-leaflet valve with a diameter of 14 mm, the horizontal leaflet length for adequate coaptation in the closed valve is 14 mm. Using this method, the leaflet was formed along the acrylic cylinder-shaped part, which had a length of 17 mm, on the outer side of the stent that had a diameter of 14 mm. Thus, the formed leaflet had a length of 17 mm, which is ~1.2 times longer than that required for definitive coaptation. Since the area of the leaflet tissues was sufficient large, an extremely low regurgitation rate (~4%) was noted when testing *in vitro* valvular function, much lower than that noted for the previous biovalve (type IV, regurgitation rate 20%).

Moreover, since the valve leaflets were technically formed in the opening state, each valve leaflet opened smoothly in the systolic phase, resulting in a high aperture ratio of 89%. In addition, the leaflet tissues were very

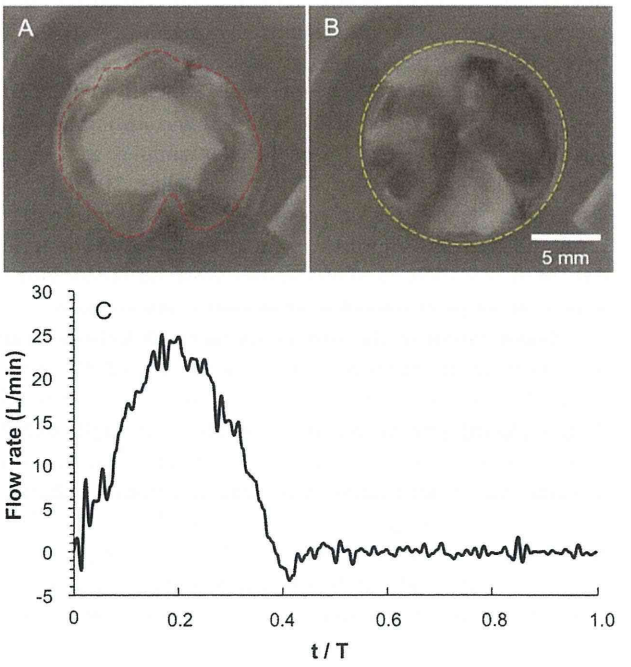


FIGURE 6. Macroscopic photos of the opening (A) and closing (B) form of the stent-biovalve in the circuit as shown in Figure 3. The pulsatile flow was 70 bpm, and the mean flow rate was 5–6 L/min. The aperture ratio was 89%. Pulsatile flow waveform in a single cycle of the stent-biovalve. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]