Cell Transplantation and Tissue Regeneration

Development of a Completely Autologous Valved Conduit With the Sinus of Valsalva Using In-Body Tissue Architecture Technology

A Pilot Study in Pulmonary Valve Replacement in a Beagle Model

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**Background**—We developed autologous prosthetic implants by simple and safe in-body tissue architecture technology. We present the first report on the development of autologous valved conduit with the sinus of Valsalva (BIOVALVE) by using this unique technology and its subsequent implantation in the pulmonary valves in a beagle model.

**Methods and Results**—A mold of BIOVALVE organization was assembled using 2 types of specially designed silicone rods with a small aperture in a trileaflet shape between them. The concave rods had 3 projections that resembled the protrusions of the sinus of Valsalva. The molds were placed in the dorsal subcutaneous spaces of beagle dogs for 4 weeks. The molds were covered with autologous connective tissues. BIOVALVEs with 3 leaflets in the inner side of the conduit with the sinus of Valsalva were obtained after removing the molds. These valves had adequate burst strength, similar to that of native valves. Tight valvular coaptation and sufficient open orifice area were observed in vitro. These BIOVALVEs were implanted to the main pulmonary arteries as allogenic conduit valves \( n = 3 \). Postoperative echocardiography demonstrated smooth movement of the leaflets with trivial regurgitation. Histological examination of specimens obtained at 84 days showed that the surface of the leaflet was covered by endothelial cells and neointima, including an elastin fiber network, and was formed at the anastomosis sides on the luminal surface of the conduit.

**Conclusion**—We developed the first completely autologous BIOVALVE and successfully implanted these BIOVALVEs in a beagle model in a pilot study. (*Circulation. 2010;122[suppl 1]:S100–S106.*)

**Key Words:** prosthesis ■ regenerative medicine ■ tissue ■ Valsalva ■ valves

Tissue engineering combines the principles of engineering and biological sciences to develop viable structures that can replace diseased or deficient natural structures. Recently, autologous valve prostheses with enhanced maturation characteristics, such as anticoagulation, self-repair, tissue regeneration, and growth adaptability, have been developed using in vitro tissue engineering technology. Some investigators have successfully implanted in vitro engineered heart valves in animals and humans by using either decellularized natural tissues or biodegradable synthetic polymers as scaffolds.\(^1\)\(^–\)\(^3\)

However, these procedures require complicated cell-management protocols, including harvesting, seeding on appropriate scaffolds, and development of neotissues, by culturing cells in bioreactors under strictly sterile conditions; all of these procedures are time-consuming and expensive.

We developed autologous prosthetic tissues using “in-body tissue architecture” technology, which is a novel and practical approach of regenerative medicine based on the tissue encapsulation phenomenon of foreign materials in living bodies.\(^4\)

This technology has the following advantages. The tissue prostheses can be fabricated in a wide range of shapes and sizes to suit the need of individual recipients and, most importantly, these prostheses do not require complex in vitro cell management procedures or exceptionally clean laboratory facilities, which are expensive and time-consuming. This technology has been used for the development of cardiovas-
cular tissues such as vascular grafts, namely, BIOTUBE,4–7 or heart valves, namely, BIOVALVE.8–10

We report the development of the first autologous valved conduit with the sinus of Valsalva: (BIOVALVE). We performed a preliminary study in which we successfully implanted BIOVALVE of the allogeneic conduit valve in the pulmonary valve position in a beagle model.

Materials and Methods

Preparation of BIOVALVE

All animals received care according to the Principles of Laboratory Animal Care (formulated by the National Institutes of Health, Publication No. 56-23, received 1985), and the research protocol (No. 9044) was approved by the ethics committee of National Cerebral and Cardiovascular Center Research Institute. Specially designed concave-shape silicone rod (diameter, 14 mm; length, 25 mm; Figure 1A) and convex-shape silicone rod (diameter, 14 mm; length, 30 mm; Figure 1B) were assembled with a small aperture of 1 mm to prepare a mold for formation of trileaflet valve tissue (C). The concave-shape rod contains 3 removable projections (D) resembling the protrusions of the sinus of Valsalva. The mold was embedded for 4 weeks in a dorsal subcutaneous pouch in a beagle model (E). After 4 weeks, the mold encapsulated by the connective tissue (F) was harvested (G). H, Inside of the BIOVALVE after fixation with 10% formalin and cutting at longitudinal direction. BIOVALVE with 3 leaflets, which were formed on the inner side of the conduit with the sinus of Valsalva, was obtained after removing the molds from each end of the implant.

In Vitro Valve Motion

The motion of the BIOVALVE leaflets was recorded using a video camera at a frequency of 250 frames per second and was analyzed in conjugation with the circuit flow pattern by using a modified Windkessel pulsatile flow circuit model (working fluid, 0.9% saline; pulsatile rate, 90 bpm; flow rate, 230 mL/min).

Measurement of Burst Strength

BIOVALEs, native pulmonary valves, and native aortic valves (n=3) were used as samples. The native pulmonary valve leaflets were obtained at BIOVALE implantation, and the native pulmonary and aortic valves were obtained at euthanization for removing the implanted BIOVALEs. The burst strength was determined by using a specially designed apparatus. These valves were separated into 3 parts—a leaflet, a sinus, and a conduit—each with area of 3 mm². These specimens were fixed on a sample folder with a hole (diameter, 2 mm) at its center. Saline solution was

![Figure 1. Specially designed concave-shape silicone rod (A) and convex-shape silicone rod (B) were assembled with a small aperture of 1 mm to prepare a mold for formation of trileaflet valve tissue (C). The concave-shape rod contains 3 removable projections (D) resembling the protrusions of the sinus of Valsalva. The mold was embedded for 4 weeks in a dorsal subcutaneous pouch in a beagle model (E). After 4 weeks, the mold encapsulated by the connective tissue (F) was harvested (G). H, Inside of the BIOVALE after fixation with 10% formalin and cutting at longitudinal direction. BIOVALE with 3 leaflets, which were formed on the inner side of the conduit with the sinus of Valsalva, was obtained after removing the molds from each end of the implant.](https://circ.ahajournals.org/doi/fig/10.1161/CIRCULATIONAHA.106.637538)
introduced into this apparatus at a rate of 50 mm Hg per second. 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).

**Pulmonary Valve Replacement**

BIOVALVEs were implanted into the main pulmonary artery of the beagles (n=3; average age, 296.7 days; average body weight, 7.2 kg) as allogeneic conduit valves. Anesthesia was induced with 0.3 mg/kg of midazolam and maintained with continuous infusion of 0.3 mg/kg per minute of propofol and 1% isoflurane. The heart was exposed through a left thoracotomy at the fourth intercostal space. Using common carotid artery and internal jugular vein cannulation, a moderately hypothermic cardiopulmonary bypass was established. The ascending aorta was clamped, and cardioplegic solution (10 mL/kg; Miotecter; Mochida Pharmaceutical) was infused through the aortic root cannula to achieve cardiac arrest. Cardioplegia was repeated every 30 minutes. After, the native pulmonary leaflets were excised. BIOVALVE was implanted into the main pulmonary artery by end-to-end anastomosis with a 7-0 nylon suture. Then, the aorta was declamped, and normal heart rhythm was restored. Finally, the beagle was weaned off cardiopulmonary bypass. Systemic anticoagulation was achieved by subcutaneous injection of low-molecular-weight heparin (50 U/kg/d) for 1 week after implantation. The beagles were followed-up to 84 days after implantation, and their valve function was evaluated using Doppler echocardiography.

**Histological Evaluation**

The beagles were euthanized at 2, 21, or 84 days after implantation. Subsequently, the BIOVALVE implants were removed. The BIOVALVE specimens acquired after implantation were fixed with 10% formalin, embedded in paraffin, sliced into longitudinal sections, and finally stained with hematoxylin-eosin, Masson trichrome, or Elastica-van Gieson. In addition, few sections of BIOVALVE were also stained for α-smooth muscle actin and factor VIII by immunohistochemical techniques; these proteins were detected using monoclonal antibodies (Dako Japan).

**Results**

**Preparation and Properties of BIOVALVE**

The assembled molds (Figure 1C) embedded in the subcutaneous pouches of the beagles for 4 weeks showed complete encapsulation with the connective tissue and marked neovascularization (Figure 1G). The implants could be easily harvested because the developed BIOVALVE and the subcutaneous tissues were connected only by very fragile, irregular, and redundant tissues, which could be dissected easily. The convex and concave rods could be smoothly removed from each end of the implant because there was no adhesion between the substrates and the tissues of BIOVALVE. The conduit had 3 protrusions, which were formed because of the shape of the concave substrate resembling the sinus of Valsalva (Figure 1H). A membranous tissue with the shape of a closed trileaflet valve was formed at the aperture of the combined rods, as intended by its design.

Analysis of the video data showed that the BIOVALVE leaflets closed rapidly and tightly in synchronization with the backward flow in the diastolic phase (Figure 2). In the transition phase of the flow direction, the valve opened smoothly without flapping or hitting the conduit wall. The opening ratio of the BIOVALVEs was 42%, which was equivalent to the ratio of the inscribed triangle of the circle, ie, 41%.

The burst pressure of the BIOVALVE leaflets was 2842±1238 mm Hg (mean±standard deviation), which was approximately equal to that of the beagle’s native pulmonary valve leaflets (Figure 3). The robustness of the sinus and conduit of the BIOVALVE were approximately equal to those of the native sinus of Valsalva and the native aorta, respectively.

**In Vivo Application**

BIOVALVEs were implanted into the main pulmonary arteries by end-to-end anastomosis. The anastomosis of BIOVALVEs was performed with little stress and was similar to the anastomosis present in the native pulmonary arteries (Figure 4A). After declamping, the implanted valve pulsed with little bleeding (Figure 4B). Echocardiographic examination of BIOVALVE revealed protrusions similar to the sinuses of Valsalva and rapid opening (Figure 5A) and closure (Figure 5B) of the leaflets. Doppler echocardiography did not yield substantial evidence of stenosis and pulmonary regurgitation (Figure 5C). Further, echocardiographic examinations revealed that the size and shape of BIOVALVEs were maintained throughout the implantation period.

BIOVALVEs were observed in the beagles euthanized after 84 days after implantation. The protrusions resembling...
those of the sinus of Valsalva did not change in size or shape after implantation (Figure 6A). The macroscopic appearance of the BIOVALVE leaflets after 84 days was similar to that of the native heart valve leaflets. No thrombus was observed on any luminal surfaces of the valved conduits (Figure 6B).

At all observation periods, the whole body of BIOVALVE, including the valve leaflets and the conduit, was mainly composed of collagen-rich tissue (Figure 7A-2, 7B-2). On the surface of the leaflet, few endothelial cells were observed at 2 and 21 days after implantation, and endothelial lining was formed at 84 days (Figure 7A-5). However, at the luminal surface of the conduit at 84 days, several kinds of cells, including endothelial cells (Figure 7B-5) and/or smooth muscle actin-positive smooth muscle cells or myofibroblasts (Figure 7B-4), and vascular extracellular matrix structure formation, including an elastic fiber network, were observed (Figure 7B-3).

**Discussion**

Valvular insufficiency is one of the major causes of morbidity and mortality in heart disease. Although the currently used homografts and replacement valves, including mechanical valves and bioprosthetic xenograft valves, are efficacious, they still require further development. In the case of mechanical valves, lifetime anticoagulation therapy is required because thrombus formation around the valve can lead to its insufficiency or serious embolisms, whereas in the case of bioprosthetic valves, the structure of xenogeneic tissues deteriorates gradually with calcification. Moreover, because
of their inability to grow, the prosthetic valves of both types are unsuitable for pediatric patients. Cryopreserved homograft valves contain viable fibroblasts, but they are subject to rejection. In addition, the scarcity of homograft donors remains a key clinical limitation.\textsuperscript{13,14}

Tissue engineering applies the principles and methods of engineering to biological science to create viable structures that can be used as replacements for diseased or deficient natural structures. Recent developments in tissue engineering technology and the production of autologous valve prostheses can solve the aforementioned problems. Autologous valves can be engineered to possess the characteristics of natural counterparts, including anticoagulation, self-repair, tissue regeneration, and growth adaptability. However, this approach uses recipient-derived cells such as bone marrow cells or vascular-derived cells as sources, and it involves complicated cell-management protocols, including harvesting, seeding, and culturing in sterilized bioreactors. Particularly while using homografts or xenografts as scaffolds, complete decellularization is indispensable because it is necessary to exclude all immunologic sources to avoid acute and chronic rejections and to avoid subsequent calcification in the valve implants.\textsuperscript{15}

Therefore, all of these procedures are time-consuming and expensive.

For the development of autologous prosthetic tissues, we focused on the in-body tissue architecture technology, which

\begin{figure}
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\includegraphics[width=\textwidth]{figure6}
\caption{The size and shape of the protrusions resembling those of the sinus of Valsalva (black arrow) did not change at 84 days after implantation (A). The overall appearance of the luminal surface of the BIOVALVE leaflets (yellow arrows) resembled that of the native leaflets (B; PA, pulmonary artery; RV, right ventricle).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7}
\caption{The longitudinal cross-sections of BIOVALVE leaflets (A1–5) and conduit (B1–5) obtained at 84 days after implantation and stained with hematoxylin and eosin (A-1, B-1), Masson trichrome (A-2, B-2), and Elastica-van Giesson (A-3, B-3). The full thicknesses of BIOVALVE leaflets and conduit were 291.0±29.7 μm and 681.2±23.6 μm, respectively (mean±standard deviation; n=3). The valve leaflet specimen stained with Masson trichrome revealed that it was composed of collagen-rich tissue. Elastica-van Giesson staining revealed an elastic fiber network in the conduit of the neointima. Immunohistochemical staining for α-smooth muscle actin (A-4, B-4) and factor VIII (A-5, B-5) revealed endothelial lining at the luminal surfaces of valvular tissues, including the leaflet and conduit, and α-smooth muscle actin-positive cells at the neointima (magnification×400; scale bar, 100 μm). Left side is the pulmonary artery side and right side is the right ventricle side in all photos.}
\end{figure}
is a novel concept of regenerative medicine based on the tissue encapsulation phenomenon of foreign materials in living bodies. This technology involves the use of living bodies as a reactor, and it is simple, safe, and cost-effective. The concept of constructing tissues within a recipient’s body was pioneered by Sparks, who constructed arterial bypass grafts in subcutaneous spaces of recipients. In 1972, the silicone mandril method was clinically applied to femoropopliteal bypass in temporality.

Using the in-body tissue architecture technology, we developed the first BIOVALVE with 3 triangular, collagen-rich leaflets that were tightly attached to a crown-shape, tissue-occupied, microporous polyurethane scaffold. Subsequently, we introduced some improvements in our BIOVALVEs. In this study, we combined the design of molds consisting of the 2 polymeric rods and used the in-body tissue architecture technology to successfully develop, for the first time to our knowledge, a completely autologous valved conduit with the sinus of Valsalva as the fifth model of BIOVALVE. The most recent BIOVALVE was easily obtained by using a mold having 3 projections resembling the sinuses of Valsalva. The vortex flow in the sinus of Valsalva plays an important role in the closure of native semilunar valves and coronary flow. Valves lacking the sinus of Valsalva close only passively because of the backflow of the blood. Miyazaki et al reported that expanded polytetrafluoroethylene valved conduit with protruding sinuses similar to those of the sinus of Valsalva showed good midterm clinical results in right ventricular outflow tract reconstruction.

BIOVALVEs created using in-body tissue architecture technology offer many potential advantages. They overcome the drawbacks of graft rejection and donor organ scarcity. Our results have confirmed the formation of functional endothelial cells and fibroblasts in these implanted leaflets. These cells generated collagen and factor VIII and attained properties similar to the cells of the native leaflets. After 84 days after implantation, the biochemical and histological characteristics of BIOVALVEs evolved to resemble those of the native pulmonary valve leaflet. Therefore, BIOVALVE leaflets appear to be viable structures with high durability, because the tissue can use naturally existing mechanisms for repair and remodeling. Hence, these tissues may exhibit growth potential in pediatric patients.

This study is quite preliminary and should be considered only as addressed. One of the unresolved aspects of this study is that the BIOVALVEs are not rejected even though they are implanted as “allogeneic” conduit valves. Shin’oka reported that the implantation of tissue-engineered valve leaflets as allografts into the pulmonary valve position in lambs failed to exhibit adequate valve function after 1 week, possibly because of rejection of the valve by the recipient. However, it is unclear why BIOVALVE allografts in our study were not rejected. Therefore, it is necessary to investigate this issue by implanting BIOVALVEs as “autografts.” Another concern is whether BIOVALVE will be able to withstand hemodynamic stresses for the long-term. In this study, we implanted BIOVALVEs in a region with low-pressure pulmonary circulation. BIOVALVEs showed adequate robustness in vitro before implantation, and their performance was found to be equivalent to that of native aortic valves. In our previous experiments with BIOTUBE vascular grafts, which were fabricated by in-body tissue architecture technology, we implanted BIOTUBE into rabbit carotid arteries. BIOTUBE were patent without degenerative changes for at least 3 months. However, it still remains unclear for now whether BIOVALVE leaflets can tolerate systemic pressures.

Conclusion

We are the first to our knowledge to report the successful development of autologous valved conduits with the sinus of Valsalva (BIOVALVEs) by in-body tissue architecture technology. BIOVALVEs were successfully implanted as allogeneic conduit valves in the pulmonary valve position in a beagle model. Although many issues remain to be addressed, the results of the preliminary studies appear to be promising. The exact physiological characteristics and long-term fate of these implanted BIOVALVEs require further investigation. Development of physiologically identical and natural heart valves by in-body tissue architecture technology can have potential applications in the treatment of patients with valvular disease. This technology could be used to develop rejection-proof replacement valves for humans using autologous cells in the future.

Acknowledgments

The authors thank Takeshi Mizuno, DVM, Masashi Mizuno, DVM, Shuhei Uchida, DVM, Masaki Endo, DVM, and students of Uechi Laboratory for performing surgeries. The authors also thank Tonomori Oie, PhD, Kyoko Hayashida, MD, PhD, and Keiichi Takamizawa, PhD, for their participation in this study.

Sources of Funding

This study was funded in part by a grant-in-aid for Scientific Research (B19390368, B21360123) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Disclosures

None.

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_Circulation_. 2010;122:S100-S106
doi: 10.1161/CIRCULATIONAHA.109.922211

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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