Application of a Fresh Decellularized Pulmonary Allograft for Pulmonary Valve Replacement in Japan

Hideto Ozawa, MD; Takayoshi Ueno, MD, PhD; Masaki Taira, MD; Koichi Toda, MD, PhD; Toru Kuratani, MD, PhD; Yoshiki Sawa, MD, PhD

Background: Tissue engineering has advanced the technique of decellularization of the heart valve. The valve is reseeded with the patient's own cells after implantation with suppression of immunologic reactions. The same advantage has been reported for fresh decellularized heart valves, and more than 10 years of excellent outcomes have been achieved. We began performing such heart valve implantations in 2013 as part of a clinical study at Osaka University. We report our evaluation of the safety and efficacy of heart valve implantation.

Methods and Results: Human pulmonary valves from the German Society for Tissue Transplantation (n=2) or from Japanese heart transplant recipient heart (n=4) were used to make decellularized heart valves; the decellularization process was the same as that used in Europe. Valves were implanted in 5 adults with pulmonary valve insufficiency after tetralogy of Fallot repair and in 1 infant with a double-outlet right ventricle with pulmonary stenosis. Postoperative echocardiography and cardiac magnetic resonance imaging revealed that the valve and ventricular function were significantly improved and maintained postoperatively.

Conclusions: Decellularized heart valves could be the new material used as artificial heart valves. Pulmonary allografts derived from the hearts of heart transplant recipients are considered to be useful material for decellularized heart valves. The application of this valve to Japanese clinical circumstances and using the hearts of heart transplant recipients is considered to be very significant.

Key Words: Decellularization; Heart valve replacement; Tissue engineering
we removed the RVOT from the heart of the recipient of a heart transplant. Exclusion criteria for RVOT removal were: (1) heart transplant recipient did not provide consent; (2) heart transplant recipient wore a ventricular assist device on the side of the heart valve to be removed; (3) heart transplant recipient had a history of active infection within 3 months; or (4) heart transplant recipient did not have suitable heart function and anatomy.

The decellularization procedure was the same as that reported by Lichtenberg et al because it avoids the need for cryopreservation, thereby minimizing the risk to the resulting valve scaffold. Valves created in this way are certified by transplant organizations in Germany (approval no.: PEI.G.11634.01.1). A decellularized human valve, termed the ESPOIR PV, is currently being used in an investigative trial (NCT 02035540, ClinicalTrials.gov) in Europe. For cases 3–6, we transported the removed pulmonary allografts to the Hannover Medical School in Germany under controlled temperature conditions (0–15°C). The decellularization treatment was performed according to the same protocol as that of the aforementioned European trial. Briefly, pulmonary allografts were treated under shaking conditions in a solution of 0.5% sodium deoxycholate and

Additionally, we do not agree with reports that the outcome after implantation differs according to the origin of the homograft. Therefore, for the next 4 patients, we used a heart valve removed from the heart of a heart transplant recipient at the time of cardiac transplantation to make a decellularized heart valve. For case 6, we used a small pulmonary allograft derived from a pediatric heart transplant recipient for primary repair of CHD. In this report, we describe the early results of these 6 cases of using a fresh decellularized heart valve in Japan.

**Methods**

This clinical study was performed with the approval of the Ethics Committee of the Osaka University Graduate School of Medicine in Osaka, Japan (no. 12318). All authors had full access to the data and take full responsibility for the integrity of the data. Informed consent was given by all heart transplant recipients and all patients who underwent PVR regarding use of the valves.

**Creating the Fresh Decellularized Heart Valve**

For pulmonary allografts, during heart transplantation, we removed the RVOT from the heart of the recipient of a heart transplant. Exclusion criteria for RVOT removal were: (1) heart transplant recipient did not provide consent; (2) heart transplant recipient wore a ventricular assist device on the side of the heart valve to be removed; (3) heart transplant recipient had a history of active infection within 3 months; or (4) heart transplant recipient did not have suitable heart function and anatomy.

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Decellularized Heart Valve Implantation in Japan

0.5% sodium dodecyl sulfate for 36h. Allografts were washed with NaCl 0.9% solution and stored at 4°C until implantation. The created decellularized heart valves were transported back to Japan under controlled temperature conditions (0–10°C) and transplanted within 4 months after creation (Figure 1A,B).

**Patient Selection for PVR**

Patients with existing indications for PVR or RVOT reconstruction with cardiac dysfunction accompanied by PV dysfunction were the targets of this procedure. Exclusion criteria were: (1) consent from the patient or parent was not given; (2) active infective endocarditis (within 3 months); (3) calcium metabolic abnormality (chronic renal failure, hyperparathyroidism, etc.); (4) pregnancy; (5) patient requiring any surgical treatment for a mitral valve, tricuspid valve, or aortic valve; (6) malignant disease or active systemic disease; (7) allergies to sodium deoxycholate, sodium dodecyl sulfate, and human collagen; and (8) congenital aortic wall abnormalities (cystic medial necrosis, Marfan syndrome, etc.).

**Implantation Procedure**

For patients requiring re-operation after intracardiac repair for CHD with RVOT reconstruction (cases 1–5), we performed decellularized heart valve implantation as follows. After repeat median full sternotomy, cardiopulmonary bypass was established using aortic and bicaval cannulation. The pulmonary trunk was opened longitudinally, and the distal site of the main pulmonary trunk was resected. If the original PV was present, the leaflets were committed type of ventricular septal defect was closed with a polytetrafluoroethylene (ePTFE) patch using a 6-0 Prolene mattress suture. Using the water test, the decellularized heart valve function was determined to be good, and a 10-mm bougie could be passed though the allograft. The proximal site of the allograft was anastomosed with s6-0 Prolene continuous and interrupted suture. After peripheral site anastomosis, we could confirm the condition of the 3 PV cusps was good (Figure 1E). The proximal site of the allograft was anastomosed at the native PV annular position with 5-0 Prolene continuous and interrupted sutures. Augmentation with an ePTFE patch was performed on the anterior surface of the right ventricle (Figure 1F). Weaning from cardiopulmonary bypass was uneventful.

We assessed the presence of infectious diseases using blood collected at the time of heart transplantation for patients who underwent heart valve excision and for those who underwent decellularized heart valve implantation at 3 months postoperatively. No immunosuppressant drugs were used postoperatively for adult re-operation cases, but 2 types of antiplatelet drugs were used for primary repair patients with an intraventricular ePTFE patch.

**Postoperative Evaluation of the Performance of the Implanted Decellularized Heart Valves**

To evaluate the safety of the transplanted human PV, the following factors were evaluated: (1) the presence and extent of surgical complications, especially handling during transplantation and hemostatic ability to prevent bleeding from anastomosis and tissue; (2) body surface area, blood pressure, heart rate, ECG, and blood test results (i.e.,

<table>
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<th>Body weight (kg)</th>
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<tr>
<td>Case 6</td>
<td>DORV</td>
<td>RVOT stenosis</td>
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The decellularized heart valves for patients 1 and 2 were provided by the German Society for Tissue Transplantation (Hannover, Germany). Case 6 was a pediatric patient and the decellularized heart valve was used for primary repair of congenital heart disease. DCM, dilated cardiomyopathy; DORV, double-outlet right ventricle; LVAS, left ventricular assist system; PV, pulmonary valve; RVOT, right ventricle outflow tract; TF, tetralogy of Fallot.

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Preoperative Demographic Data of Patients Receiving an Implant

Demographic data of the patients who received an implant are shown in Table 1B. 5 of the patients (cases 1–5) were adults and had PV insufficiency after intracardiac repair of tetralogy of Fallot and there was 1 pediatric patient with a double-outlet right ventricle with severe RVOT stenosis. All patients met the criteria for conventional PVR or RVOT reconstruction and agreed to participate in this clinical study.

Surgical Complications

In case 5, symptoms of cerebral infarction, which are thought to be associated with heart–lung machine manipulation, were transiently recognized, but had completely

Results

Origin of the Decellularized Heart Valves

The origins of the pulmonary allografts used for creating the decellularized valves are shown in Table 1A. In cases 1 and 2, pulmonary allografts were provided by the German Society for Tissue Transplantation (Hannover, Germany), so information was limited.

Preoperative Demographic Data of Patients Receiving an Implant

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In case 5, symptoms of cerebral infarction, which are thought to be associated with heart–lung machine manipulation, were transiently recognized, but had completely

| Table 2. Preoperative and Postoperative Blood Sampling Results |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Baseline         | Discharge        | 3 months         | 6 months         | 12 months        | 24 months        | 36 months        |
| Case 1           |                  |                  |                  |                  |                  |                  |                  |
| WBC              | 8.03             | 6                | 5.57             | 5.15             | 6.31             | 7.9              | 4.47             |
| Free Hb          | 16.3             | 11               | 16.6             | 16.7             | 15.1             | 14.1             | 15.8             |
| Haptoglobin      | 48.6             | 221              | 96               | 66               | 96               | 319              | 95               |
| CRP              | 0.04             | 1.37             | <0.04            | <0.04            | 0.13             | 11.05            | 0.06             |
| LDH              | 187              | 161              | 168              | 142              | 205              | 201              | 155              |
| Case 2           |                  |                  |                  |                  |                  |                  |                  |
| WBC              | 5.6              | 8.22             | 7.59             | 6.29             | 7.85             | 6.99             |
| Free Hb          | 14.6             | 11.2             | 14.2             | 14.8             | 14.2             | 14.7             |
| Haptoglobin      | 76               | 280              | 96               | 84               | 88               | 81               |
| CRP              | <0.04            | 4.44             | <0.04            | <0.04            | <0.04            | <0.04            |
| LDH              | 141              | 232              | 138              | 139              | 139              | 150              |
| Case 3           |                  |                  |                  |                  |                  |                  |                  |
| WBC              | 8.16             | 9.27             | 9.09             | 10.56            | 10.2             |
| Free Hb          | 14               | 10.6             | 13.2             | 14.2             | 14.4             |
| Haptoglobin      | 159              | 414              | 220              | 185              | 196              |
| CRP              | 0.21             | 3.45             | 0.29             | 0.28             | 0.16             |
| LDH              | 244              | 222              | 241              | 231              | 233              |
| Case 4           |                  |                  |                  |                  |                  |                  |                  |
| WBC              | 8.33             | 10.3             | 6.99             | 5.81             | 7.14             |
| Free Hb          | 16               | 11.1             | 13.9             | 15.7             | 15.1             |
| Haptoglobin      | 92               | 291              | 126              | 97               | 60               |
| CRP              | <0.04            | 3.35             | <0.04            | <0.04            | <0.04            |
| LDH              | 164              | 233              | 184              | 216              | 185              |
| Case 5           |                  |                  |                  |                  |                  |                  |                  |
| WBC              | 7.62             | 9.97             | 6.55             | 6.94             |
| Free Hb          | 15.5             | 11.3             | 14.3             | 14.8             |
| Haptoglobin      | 66               | 183              | 73               | 60               |
| CRP              | 0.08             | 1.25             | 0.19             | 0.13             |
| LDH              | 175              | 215              | 164              | 167              |
| Case 6           |                  |                  |                  |                  |                  |                  |                  |
| WBC              | 10.4             | 11.7             | 17.53            | 9.02             |
| Free Hb          | 12.4             | 10.1             | 10.4             | 10.6             |
| Haptoglobin      | 82               | 157              | 51               | 128              |
| CRP              | <0.04            | 0.56             | <0.04            | 0.06             |
| LDH              | 284              | 334              | 314              | 344              |

CRP, C-reactive protein (mg/L); Free Hb, free hemoglobin (g/dL); Haptoglobin, g/L; LDH, lactate dehydrogenase (U/L); WBC, white blood cells (10⁹/μL).
resolved at patient discharge. In all 6 cases, there were no complications related to decellularized heart valve implantation. Additionally, the handling of the valve was sufficient for anastomosis, bleeding from the anastomosis site could be controlled easily, and weaning from cardiopulmonary bypass was uneventful. In case 5, deformity of the annulus of the decellularized valve was a concern if direct anastomosis was performed on the central side. Therefore, augmentation with a woven graft patch was performed (Figure 1D).

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<th>Table 3. Results of Cardiac Magnetic Resonance Imaging</th>
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<td>Annulus diameter</td>
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Annulus diameter, mm; LVEDVI, left ventricular end-diastolic volume index, mL/m²; LVESVI, left ventricular end-systolic volume index, mL/m²; RVEDVI, right ventricular end-diastolic volume index, mL/m²; RVESVI, right ventricular end-systolic volume index, mL/m²; Valve flow, m/s.
Postoperative Outcomes
The follow-up periods and results are shown in Table 2 and Table 3. All patients experienced a smooth clinical course after transplantation. Additionally, no increase in arrhythmia was observed on ECG. Moreover, during the follow-up periods, evidence of hemolysis was not apparent in the blood chemistry results (Table 2).

Cardiac Function Evaluation After Decellularized Heart Valve Implantation
The results of cardiac MRI are shown in Table 3. Implanted PV function was maintained in all patients during the follow-up periods. Regarding PV insufficiency, significant valve regurgitation was not detected during the follow-up period in any of the patients (Figure 2). In case 3, the regurgitant fraction in the PV was 25% at 3 months postoperatively, but no exacerbation occurred during the follow-up period. Regarding valve stenosis in the adult cases, the blood flow velocity at the site of the transplanted PV did not increase. In addition, regarding enlargement of the right ventricle, we confirmed improvements in right ventricular expansion in all cases.

In the infant case of primary repair (patient no. 6), cardiac MRI revealed that the valve annulus size was 11 mm in diameter (Figure 3A) and the maximum velocity through the PV was 2.5 m/s. The regurgitant fraction of the PV was 0% (Table 3). Echocardiography showed that the
valvar annulus size was 10 mm in diameter and the maximum velocity through the PV was 2.3 m/s without regurgitation at 6 months after surgery (Figure 3B–D).

Discussion
This is the first report of using a fresh decellularized heart valve derived from a heart transplant recipient heart prepared using pulmonary allografts in Japan. The decellularization technique used in this report is a method approved for tissue transplants by an organization in Germany; however, we applied this method in Japan.

The application of this German idea to Japanese clinical circumstances and using the hearts of Japanese heart transplant recipients is significant because the lack of both organ donation and tissue donation is a serious problem in Japan. Sarikouch et al reported good clinical results for more than 10 years with a fresh decellularized heart valve. According to that report, compared with other valves such as homografts or Contegra®, which are commonly used in Europe and the USA for patients with CHD accompanied mainly with RVOT dysfunction, the recurrence avoidance rate is significantly lower with fresh decellularized heart valves. Moreover, in Japan, for patients with CHD who have RVOT dysfunction, reconstruction is performed during infancy using artificial materials such as a transannular patch if the annulus cannot be preserved. For such patients, and even for patients with annulus preservation, surgical intervention for PV dysfunction is often necessary during the postoperative period. At our hospital, approximately 34% of patients during a postoperative follow-up observation period of 40 years required surgical intervention for PV dysfunction. A procedure using a biological valve is generally used as a replacement surgery for such patients. However, for this type of valve, the postoperative operation avoidance rate is 60% during 10 years, and it has been reported that its durability is poor, especially in younger patients. Although the mechanism has not been elucidated, a biological valve has been reported as one of the reasons why the immune response is high when the patient is young.

As described previously, it is considered that a fresh decellularized heart valve can be a durable, superior artificial valve with less immune reaction. However, because the supply of allografts is limited, it is important to acquire them whenever possible. We believe that the valves from the hearts of recipients that are removed during heart transplantations in Japan can be a useful source of decellularized heart valves. However, for patients with an artificial heart, it is difficult to use these heart valves because abnormalities, such as thickening of the valve leaflets and meniscal valve and the adhesion of commissures on the implanted side, have been reported. However, we believe that we can use PVs even if an artificial heart has been implanted on the left side.

Another important point regarding the decellularized heart valve is that it has the potential for self-regeneration by autologous cells in the transplanted tissue. Miyazaki et al reported the usefulness of fan-shaped ePTFE valve conduits with bulging sinuses during mid-term follow-up. ePTFE valves should be covered by a thin fibrous tissue and show focal regions of endothelialization. The ePTFE valve cannot be expected to develop adaptive growth during the follow-up period. However, Cebotari et al reported that when this valve was implanted in a child, the diameter of the valve annulus was maintained normally according to the patient’s growth and valve function was maintained. Therefore, it is suggested that this valve is likely to grow as the patient grows after transplantation.

In case 6, the implanted PV annulus diameter at 6 months postoperatively was the same as at the time of surgery without valve insufficiency. It is considered that a certain period of time is required for the growth of the PV after transplantation, during which the recipient’s cells produce a new extracellular matrix. Therefore, it might be necessary to implant PVs with a somewhat larger annulus diameter than the recipient’s physique at the time of transplantation.

In conclusion, we have described the early results of PVR using fresh decellularized heart valves in Japan. Long-term follow-up is necessary, but PV function postoperatively has been excellent and accompanied by improvements in right ventricular function. Furthermore, pulmonary allografts removed from heart transplantation recipients’ hearts is considered to be viable as material for decellularized heart valves. The application of this German idea to Japanese clinical circumstances and using the hearts of Japanese heart transplant recipients is considered to be very significant. We plan to perform an investigational clinical trial to obtain more information. The results are similar to those from clinical trials performed in Europe and we hope that will lead to approval of insurance reimbursement for such procedures in Japan.

Acknowledgments
We earnestly thank Axxel Haverich, MD, PhD (Professor of the Division of Cardiothoracic, Transplantation, and Vascular Surgery, Hannover Medical School, Hannover, Germany) and his colleagues for their cooperation in providing the decellularized fresh pulmonary allografts.

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Conflicts of Interest Statement
H.O. and other co-authors have no conflicts of interest.

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