Clinical Application of Tissue Engineered Human Heart Valves Using Autologous Progenitor Cells

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Background—Tissue engineering (TE) of heart valves reseeded with autologous cells has been successfully performed in vitro. Here, we report our first clinical implantation of pulmonary heart valves (PV) engineered with autologous endothelial progenitor cells (EPCs) and the results of 3.5 years of follow-up.

Methods and Results—Human PV allografts were decellularized (Trypsin/EDTA) and resulting scaffolds reseeded with peripheral mononuclear cells isolated from human blood. Positive stain for von Willebrand factor, CD31, and Flk-1 was observed in monolayers of cells cultivated and differentiated on the luminal surface of the scaffolds in a dynamic bioreactor system for up to 21 days, indicating endothelial nature. PV reseeded with autologous cells were implanted into 2 pediatric patients (age 13 and 11) with congenital PV failure. Postoperatively, a mild pulmonary regurgitation was documented in both children. Based on regular echocardiographic investigations, hemodynamic parameters and cardiac morphology changed in 3.5 years as follows: increase of the PV annulus diameter (18 to 22.5 mm and 22 to 26 mm, respectively), decrease of valve regurgitation (trivial/mild and trivial, respectively), decrease of valve regurgitation (trivial/mild and trivial, respectively), decrease (16 to 9 mm Hg) or an increase (8 to 9.5 mm Hg) of mean transvalvular gradient, remained 26 mm or decreased (32 to 28 mm) right-ventricular end-diastolic diameter. The body surface area increased (1.07 to 1.42 m² and 1.07 to 1.46 m², respectively). No signs of valve degeneration were observed in both patients.

Conclusions—TE of human heart valves using autologous EPC is a feasible and safe method for pulmonary valve replacement. TE valves have the potential to remodel and grow accordingly to the somatic growth of the child. (Circulation. 2006;114[suppl I]:I-132–I-137.)

Key Words: congenital ■ heart defects ■ surgery ■ valves

The majority of pulmonary valve pathology are related to congenital heart diseases. Stenotic lesions include the hypoplasia of the right ventricular outflow tract (RVOT) and/or pulmonary arteries, etc. Pulmonary insufficiency is generally related to nonvalved transannular repair of RVOT, which leads to right ventricular failure, ventricular arrhythmias, and requires reoperations. Different types of valvular conduits including xenografts and allografts were implemented for reconstruction of RVOT in children and adults. Despite ongoing efforts to improve longevity of biological valve prostheses, the long-term results remain unsatisfactory. After implementation of cryopreservation techniques, human allograft valves have been widely used in reconstruction of the RVOT in congenital heart disease. However, preserved viability of the cells in allograft valvular tissue causes immunological responses and leads to subsequent graft failure. Clinical implantation of decellularized xenograft valves turned in early graft failure, possibly because of preserved matrix immunogenicity. All commercially available grafts for RVOT reconstruction necessitate subsequent replacement in pediatric patients, because of their unfeasibility to grow and remodel with concomitant growth of child organism.

The field of tissue engineering (TE) emerged as an alternative in search for an ideal valvular graft that could overcome all these limitations. Its principle is based on in vitro and in vivo repopulation of biological scaffolds with autologous cells that maintain continuous remodeling and growth of the implanted valvular prosthesis.

Our group already reported about successful in vitro creation of TE human heart valves based on decellularized allograft matrices reseeded with human venous endothelial cells. The present study involves the usage of autologous endothelial progenitor cells (EPCs) for tissue engineering
purposes. Moreover, here we report our first clinical experience of TE valves implantation in pediatric patients with pulmonary valve pathology and the results of 3.5 years of follow-up.

Materials and Methods

Statement of Responsibility

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

The study was performed in accordance to the Law on Health Care System (No. 411-XIII from 28.03.1995) of the Republic of Moldova. The Council of Experts of the Ministry of Health (No. 09 to 1/2 from 02.05.2002) as well as Ethical Medical Committee of the Ministry of Health (No. 01 from 17.05.2002) of the Republic of Moldova approved the study. In vitro studies were performed at the Leibniz Research Laboratory for Biotechnology and Artificial Organs and were approved by the state government (No. 108d-41401TPG from 17.01.2001) of Lower Saxony, Germany.

Human Allografts

Seven human pulmonary heart valves (PVs) including short subvalvular myocardial cuff, valve annulus, valve leaflets, and pulmonary artery wall were harvested under sterile conditions from cadavers. Warm ischemic time was up to 12 hours (mean 7.0±2.9 hours) for 5 valves used for in vitro studies. For clinical application, 2 valves with ischemic time up to 6 hours (3.5 and 5.5 hours) have been selected. After removal of adherent fat, PV were flushed in phosphate-buffered solution (PBS) at 4°C and stored in Earle’s Medium 199 (PAA Laboratories GmbH) supplemented with 100 IU/mL penicillin–streptomycin (P/S). Valves were sterilized by γ-radiation for 30 minutes (100 Gray).

Valve donors were tested for transmissible diseases (AIDS, hepatitis, syphilis, tuberculosis).

Decellularization

Decellularization process has been previously described. In brief, pulmonary allografts were incubated under continuous shaking in 106 cells/H11003 including the EPC fraction was given into the lumen of the valve with CM. With a biweekly change of CM, the fluid exposure to rotation for 24 hours, then a continuous perfusion was started at 15 mL/min with fresh CM. Nonadherent cells were removed 3 days later by a thorough washing of the luminal part of the valve with CM. With a biweekly change of CM, the fluid circulation was maintained up to 21 days.

At days 2, 14, and 21, routine sterility tests were performed on used medium of the reseeded PV to exclude a bacterial or fungal infection.

Immunohistochemistry and Immunofluorescent Microscopy

Immunohistochemistry was performed on cryosections using the following antibodies: monoclonal anti-human antibodies against CD34 (clone JC70A; Dako, Hamburg, Germany), von Willebrand (clone F-8/86; Dako), and Flk-1 (clone A-3; Santa Cruz). Avidinbiotin-peroxidase or fluorescence methods were previously described.

Patients

In April 2002, 2 pediatric patients had been selected for clinical implantation of tissue engineered PV. Patient A (13 years old, female) had a diagnosis of tetralogy of Fallot with a hypoplasia of the main pulmonary artery and pulmonary valve annulus. The patient was cyanotic with frequent hypoxic accesses and accused on severe dyspnea (NYHA III-IV). Patient B (11 years old, male) underwent operation in 1996 for radical correction of tetralogy of Fallot with a transannular enlargement of the RVOT using autologous glutaraldehyde-treated pericardium. Postoperatively, a RVOT aneurysm and a severe pulmonary valve insufficiency developed. The patient showed dyspnea (NYHA III) and heart palpitations. According to these clinical findings, PV replacement was indicated in both children. The possibility of generating a TE PV using autologous cells was discussed with the children and their parents. The patients agreed to this type of procedure and the parents signed the informed consent to participate in the study.

At the time when decellularized pulmonary homografts became available, peripheral blood sample (30 mL) was obtained from each patient for autologous EPC isolation.

Surgical Implantation

Both operations were performed under general combined intravenous anesthesia through a median sternotomy using cardiopulmonary bypass with standard bicaval and aortic cannulation and mild hypothermia (32°C). Patient A underwent operation in cardiac arrest using intermittent antegrade cold blood cardioplegia. After transventricular closure of the ventriculoseptal defect (2.5 cm) with a Gore-Tex patch, the partial infundibulectomy and the autologous nontreated pericardial patch enlargement of RVOT was performed. The hypoplastic pulmonary artery was replaced with the TE PV. In the patient B, after initiation of cardiopulmonary bypass, PV replacement was performed on the beating heart. In both cases the RVOT was reconstructed with an interposition of the TE PV with a continuous suture for proximal as well as for the distal sutures lines. Postoperatively, aspirin therapy was administered to both patients for 1 month.

Patient Follow-Up

Clinical follow-up included a regular physical examination of the patients (physical status, measurements of body height and weight, systemic blood pressure, ECG, NYHA), as well as echocardiographic evaluation of the TE PV status by M-mode, 2-dimensional, color-flow, pulsatile and continuous wave Doppler using “Sonolayer SSH-140A” (Toshiba), and “Vivid-3 Pro” (General Electric Ultrasound) equipment. Transthoracic echocardiography was performed in standard longitudinal and cross-sectional views and was recorded on videotape. Maximum velocities across the pulmonary valve were calculated by a continuous-wave Doppler imaging transducer. Semi-quantitative assessment from grade 0 to 4 of pulmonary regurgitation (0 absence, 1+ trivial, 2+ mild, 3+ moderate, 4+ severe) was based on the length and width of the regurgitant jet and the distance that it reaches into the RVOT on the parasternal short-axis view. The transvalvular pressure gradient was determined using the Bernoulli equation. Both patients underwent echocardiographic examinations by the same in-house cardiologist preoperatively, before hospital discharge and at 3, 6, 12, 18, 24, 30, 36, and 42 months postoperatively.

Results

TE Valvular Graft

Human mononuclear cells containing the EPC fraction were isolated from peripheral blood and successfully seeded on decellularized scaffolds. After 21 days of cultivation, the cell monolayer on the valvular surface expressed CD31 and von
Willebrand factor (Figure 1A,1C,1D), as well as vascular endothelial growth factor receptor FLK-1 (Figure 1B), indicating an endothelial nature of adherent cells.

All cultured PVs were free of any infection.

Clinical Results

TE PV showed acceptable mechanical properties for surgical implantation and provided enough resistance and stability for suture lines. Neither the pericardial strip nor the pledgeted sutures were used for reinforcement of proximal or distal anastomosis. In patient A there was a mismatch between native and TE valve annulus size (10 mm versus 21 mm). After release of RVOT stenosis through longitudinal incision and RVOT enlargement with pericardial patch, the proximal anastomosis entailed to take wider bites on the ventricular margin of the graft to ensure the correct seating of the valvular ring. The PV annulus was then measured and corresponded to \( \approx 18 \) mm.

In patient B there was a minimal mismatch between native and TE valve annulus size (23 mm versus 22 mm). After performing the proximal anastomosis, the valvular ring corresponded to \( \approx 22 \) mm in diameter.

Both patients recovered uneventful after operation. No important elevations of C-reactive protein or body temperature occurred in postoperative period. After operation, both patients were in functional class NYHA II. After 1 year and during the remaining follow-up period, both children are clinically well in functional NYHA class I (Table 1). No any other limitations regarding general physical activity, neurological or psychological status; no cardiac arrhythmias were observed in the concerned patients in follow-up. Beginning with postoperative month 6, both patients showed distinct somatic growth that corresponded to the normal growth of healthy subjects. During the follow-up, the body surface area (BSA) increased from 1.07 to 1.42 m² in patient A and from 1.07 to 1.46 m² in patient B (Figure 2a).

**Figure 1.** Representative pictures of reseeded allograft valve leaflets immunohistochemically stained against (A) CD31 (red), (B) FLK-1 (red), and (C) von Willebrand factor (vWF) (green) as well as pulmonary artery wall stained against vWF (green) (D). Bars =50 \( \mu \)m

| Echocardiographic and Clinical Data of Both Patients Before the Operation and During 3.5 Years of Follow-Up |
|---------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                           | Before OP | After OP  | 3 months  | 6 Months  | 12 Months | 18 Months | 24 Months | 30 Months | 36 Months |
| RV-EDD (mm)               |           |           |           |           |           |           |           |           |           |
| Patient A                 | 26        | 27        | 28.3      | 28.3      | 28.8      | 28.8      | 28         | 27        | 26        |
| Patient B                 | 31.7      | 27.7      | 29        | 29.4      | 29        | 29        | 29.5       | 28.8      | 29        |
| RV-FS %                   |           |           |           |           |           |           |           |           |           |
| Patient A                 | 22        | 25        | 29        | 31        | 31        | 39        | 34         | 33        | 43        |
| Patient B                 | 27        | 27        | 30        | 28        | 30        | 29        | 26         | 26        | 28        |
| LV-EF %                   |           |           |           |           |           |           |           |           |           |
| Patient A                 | 55        | 72        | 60        | 59        | 65        | 65        | 63         | 65        | 63        |
| Patient B                 | 76        | 73        | 69        | 71        | 72        | 66        | 68         | 72        | 69        |
| PV insufficiency (0–4)    |           |           |           |           |           |           |           |           |           |
| Patient A                 | 0         | 2         | 2         | 2         | 1–2       | 1–2       | 1–2        | 1–2       | 1–2       |
| Patient B                 | 4         | 2         | 1–2       | 1–2       | 1–2       | 1–2       | 1          | 0–1       | 0–1       |
| PV morphology             |           |           |           |           |           |           |           |           |           |
| Patient A                 | Hypoplasia Normal Normal Normal Normal Normal Normal Normal Normal Normal |
| Patient B                 | Hypoplasia Normal Normal Normal Normal Normal Normal Normal Normal Normal |
| TVG (mm Hg) (max/mean)    |           |           |           |           |           |           |           |           |           |
| Patient A                 | 100/52    | 32/16     | 20/9.1    | 16/8.5    | 21/9.2    | 18/9.1    | 17/8.5     | 17/8.3    | 18/8.8    |
| Patient B                 | 18/7.9    | 18/7.7    | 17/7.4    | 16/7.5    | 15.5/7.2  | 20/10     | 17.2/7.7   | 16.8/7.1  | 17.5/7.7  |
| NYHA (I-IV)               |           |           |           |           |           |           |           |           |           |
| Patient A                 | III-IV    | II        | II        | II        | II        | II        | II         | II        | II        |
| Patient B                 | II        | II        | II        | II        | II        | II        | II         | II        | II        |

LV-EF indicates left ventricle ejection fraction; NYHA, New-York Heart Association classification of physical activity; OP, operation; PV, pulmonary valve; RV-EDD, right ventricle end-diastolic diameter; RV-FS, right ventricle fraction of shortening; TVG, transvalvular gradient.
TE Valve Dimensions and Function

The echocardiographic evaluation of implanted TE valves is shown in Figure 2, Figure 3, and Table 1. The annulus diameter of TE valves increased in both patients. In patient A the valve annulus increased by 25% (from 18 to 22.5 mm), and by 18% in patient B (from 22 to 26 mm) during 3.5 years of follow-up (Figure 2b, 2c; Table 1). At hospital discharge, mild regurgitation was detected in both patients. During follow-up, pulmonary regurgitation decreased in both patients after 1 year and currently corresponds to trivial/mild grade in patient A, and trivial in patient B (Figure 3C, 3D; Table 1).

At hospital discharge, mild regurgitation was detected in both patients. During follow-up, pulmonary regurgitation decreased in both patients after 1 year and currently corresponds to trivial/mild grade in patient A, and trivial in patient B (Figure 3C, 3D; Table 1). In patient A, the initial postoperative mean transvalvular gradient was 16 mm Hg and decreased to 9 mm Hg after 3 months and remains stable during the follow-up. In patient B, the gradient was 8 mm Hg postoperatively and slightly increased to 9.5 mm Hg at the most recent follow-up. The end-diastolic diameter of the right ventricle remained 26 mm in patient A and decreased from 32 to 28 mm in patient B (Table 1). In both children, no signs of pulmonary dilatation or stenosis, valve degeneration, cusp thickness, or reduction of cusp’s mobility were observed during 3.5 years of follow-up.

Discussion

Pulmonary valve failure is a common pathology especially in pediatric patients with congenital heart diseases and requires valve replacement. Homografts were initially used as valved conduits for reconstruction of the RVOT.1,12 However, cryopreserved allografts represent viable tissue with enhanced immunogenicity and releases immunological response with subsequent valve degeneration especially after implantation in children.6 Glutaraldehyde-preserved xenogeneic porcine and bovine valvular conduits became available in a variety of sizes and were largely used for pulmonary valve replacement.13,14 Although glutaraldehyde crosslinking reduces immunogenicity, the fixation of cellular debris causes progressive calcification of the valve.15,16 Available prostheses do not grow and require reoperations in growing children.

TE might bear promising solutions to overcome the limitations of biological heart valves substitutes.9,17 The concept of TE is based on usage of decellularized biological matrices, because the removal of cellular components might reduce immunological reactions which are regarded to be responsible for accelerated graft deterioration. We already reported on successful in vitro creation of TE pulmonary heart valves based on decellularized human allograft valve tissue using an enzymatic treatment with Trypsin/EDTA. Decellularization with Trypsin/EDTA converts pulmonary valves in a cell-free scaffold with 98% reduction of DNA content. Histology revealed a well-preserved 3-dimensional network of collagen fibers in extracellular matrix.10
Implantation of decellularized xenogenic valved conduits as a root in the RVOT has already reached clinical trials. However, a report of SynerGraft decellularized porcine valves implantation in pediatric patients resulted in catastrophic failure associated with strong immunologic response. Probably, the decellularization process does not adequately remove all xenoantigens from the extracellular matrix of the porcine tissue.

Recent publication regarding RVOT reconstruction using decellularized versus cryopreserved human allograft valves reported about important reduction of the immunogenic response in decellularized group and their normal function in vivo up to 18 months of follow-up. However, our preclinical studies on decellularized pulmonary valve implantation in sheep model showed that, besides of progressive matrix recellularization with interstitial cells, these valves suffer from enhanced thrombogenicity and pathological neointima formation, comparing with in vivo pre-endothelialized grafts. Based on these data, we believe that repopulation of EPCs in children make them especially attractive in TE of pulmonary valve.

Here, we report our first clinical experience with 3.5 years of follow-up in implantation of TE PV reseeded with autologous EPCs. Dynamic cultivation of EPCs during a period of 21 days resulted in a monolayer on the valve surface that expresses an endothelial phenotype. In 1997, Asahara et al identified in adult human peripheral blood a small population of CD34 circulating mononuclear hematopoietic progenitor cells which was capable of providing endothelial characteristics in culture. These cells are normally isolated from mononuclear cell pools of peripheral blood and grow in the presence of endothelial growth factors. Enhanced plasticity of EPCs in children make them especially attractive in TE of heart valves in pediatric population. Moreover, omitting the surgical vein harvesting for endothelial cells isolation, non-invasive harvesting of progenitor endothelial cells from peripheral blood represents an undoubted benefit for the patient.

TE valves, constructed using this principle, were implanted in 2 pediatric patients, both with pulmonary valve pathology. During the 3.5 years of follow-up, TE valves showed excellent performance and normal hemodynamics. In patient A with pulmonary hypoplasia, the mean transvalvular gradient decreased and became normal at 3 months postoperatively and remained stable during the entire follow-up. Interestingly, the size of pulmonary valve annulus increased together with somatic growth of the child; however the valve regurgitation did not progress. The child did not have enlargement of right ventricular cavity, although her BSA increased 33%. The same increase of valve annulus we documented in patient B. In contrast, pulmonary regurgitation decreased from “mild” postoperatively to “trivial” at 3.5 years. In this case, we documented a reduction of the right ventricle end-diastolic diameter and a relatively stable, almost physiological, transvalvular gradient over the whole follow-up period. However, midterm results (3.2 years) of cryopreserved allograft implantation in RVOT in children and young adults reported by Ward et al showed a decrease of the homograft annulus size by 15% in 88% of the patients. Moreover, transvalvular pressure gradient increased significantly from 10 to 17 mm Hg because of calcification and contracture of the homograft wall. Intermediate-term echocardiographic follow-up of pediatric patients who underwent RVOT reconstruction with cryopreserved homografts reported by Chan et al revealed progression of homograft regurgitation >2 grades in 35% of the patients, as well as progression of homograft stenosis with gradient >25 mm Hg in 51% of implanted homografts. These phenomenon may be related to early postoperative inflammatory reaction to the pulmonary homograft that leads to extrinsic compression and/or shrinkage or immunologic homograft valve deterioration.

None of these complications were observed in our patients; during the entire follow-up period there were no signs of graft stenosis, valve degeneration, progression of valve regurgitation, cusps thickenss, or reduction of cusp’s mobility. We interpret the increase of TE pulmonary valve annulus diameter and diminution of valve regurgitation, together with physiological development of patient’s BSA during 3.5 years of follow-up as normal physiological growth of the TE valve. These results underline the importance of autologous valve creation using methods of TE to create viable, self-remodeling valvular substitutes. These valves are especially needed in pediatric patients because of their ability to grow in parallel to the somatic growth.

Conclusions

TE of heart valves based on decellularized allografts reseeded with autologous EPC is a feasible and safe method for pulmonary valve replacement. These valves have the potential to remodel and grow along with the somatic growth of the child as demonstrated in our first successful clinical application in pediatric patients.

Disclosures

None.

References


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