

# In-Vivo Repopularization of a Tissue-Engineered Heart Valve in a Human Subject

Pascal M. Dohmen<sup>1</sup>, Steffen Hauptmann<sup>2</sup>, Alexander Terytze<sup>1</sup>, Wolfgang F. Konertz<sup>1</sup>

<sup>1</sup>Department of Cardiovascular Surgery, Charité, Medical University Berlin, Berlin, <sup>2</sup>Institute of Pathology, Martin-Luther University, Halle, Germany

A 63-year-old male with a massively calcified aortic valve showed an active lifestyle. Therefore, valve replacement was completed using the Ross procedure. During postoperative echocardiographic control, a ventricular septal defect was noted which was closed surgically. During this reoperation, a biopsy sample was taken from the wall of the tissue-engineered heart valve which was used to reconstruct the

right ventricular outflow tract. A persistent monolayer of endothelial cells and host recellularization of the deeper layer was demonstrated histologically. The postoperative course was uneventful, and the patient rapidly recovered. After six years, he remains in excellent health.

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As most bioprotheses are glutaraldehyde-fixed and so constitute non-viable valve structures, autografts represent the only viable heart valves available for cardiac valve surgery. In contrast, homografts are only partially viable and carry a high bioburden (1) since, due to their allogeneic origin, there is a possibility of immunologic reactions, resulting in degeneration and calcification of the valves (2). In recent years, however, tissue engineering technology had allowed the creation of a viable heart valve with potential for regeneration, repair, and growth (3,4).

## Case report

A 63-year-old male patient suffering from degenerative aortic valve disease had, until very recently, had enjoyed an active lifestyle. As the patient had rejected postoperative anticoagulation, a mechanical valve did not represent a viable option, and consequently a Ross procedure was preferred for heart valve replacement.

After having obtained approval from the ethics committee of the Humboldt University, the native pulmonary valve was used to replace the diseased aortic valve and a tissue-engineered (TE) heart valve transplanted to reconstruct the right ventricular outflow tract (RVOT). Details of the creation of the TE heart valve have been published

previously (5). In brief, a peripheral vein of approximately 10 cm length was harvested to isolate autologous vascular endothelial cells. The cells were characterized and expanded to achieve a monoculture of  $5.0 \times 10^6$  viable endothelial cells (Fig. 1). A 27-mm cryopreserved pulmonary allograft was decellularized at 37°C in a mixture of 1% deoxycholic acid (Sigma Chemical Co., St. Louis, MO, USA) and 70% ethanol to produce a cell-free scaffold comprising only collagen and elastin. Prior to the seeding procedure, the decellularized scaffold was precoated with fibronectin (PAA Laboratories GmbH, Coelbe, Germany) during a 3-h procedure at 37°C to increase the binding capacity of the endothelial cells. One week later, when the seeding and conditioning phases were complete, histological examination was conducted to confirm the efficacy of re-endothelialization (Fig. 2a and b). After verifying the sterility of the heart valve, it was implanted in June 2000 using a 'no-touch' technique. At surgery, the aortic valve deterioration was seen to be extreme, with calcification extending into the left ventricular outflow tract. A complete decalcification was performed with great care, at which time there was no evidence of any ventricular septal defect. Although, postoperatively, no valve-related complications were observed, the patient showed a grade III atrioventricular block, which was treated with a pacemaker. After three months, echocardiographic evidence was obtained of a ventricular septal defect, which required closure.

At reoperation to close the connection, and after having obtained informed consent, a tiny biopsy of the TE heart valve wall was taken intraoperatively. Histological examination of the biopsy material showed the presence

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Address for correspondence:

P. M. Dohmen MD, PhD, Department of Cardiovascular Surgery, Charité Hospital, Medical University Berlin, Luisenstraße 13, D-10117 Berlin, Germany  
e-mail : pascal.dohmen@charite.de

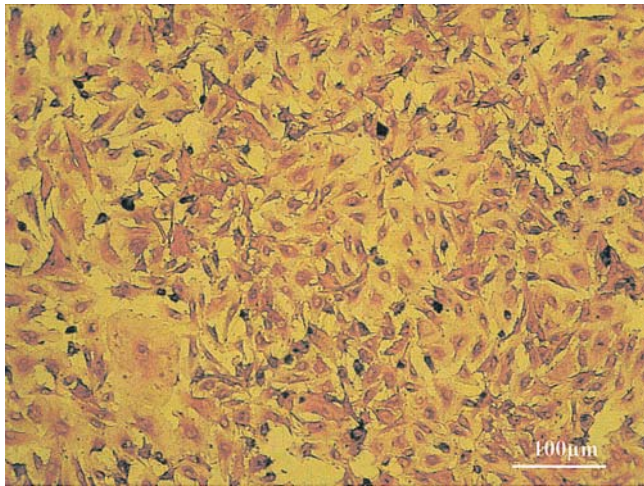


Figure 1: Anti-factor VIII staining showing a mono cell culture.

of a persistent monolayer of endothelial cells and ingrowth of interstitial cells into the scaffold (Fig. 2c-e).

Postoperatively, the patient made a rapid recovery and is currently in NYHA class I, with no signs of valve dysfunction. At six-year follow up, the neo-aortic and pulmonary valve functions were within the normal range, with mean flow velocities of 0.7 m/s and 0.8 m/s, respectively.

## Discussion

When implanted in young patients, glutaraldehyde-preserved heart valves degenerate more rapidly than in older patients (6). Unfortunately, this problem can be only partly overcome by using antimineralization treatments, as there is an increased calcium turnover in the young (7).

O'Brien et al. (8) reported excellent long-term results with cryopreserved aortic allografts implanted in the aortic position in patients aged >60 years, and a freedom from reoperation after a 20-years follow up of 94 %. Results in patients aged <40 years were poor, however, with only

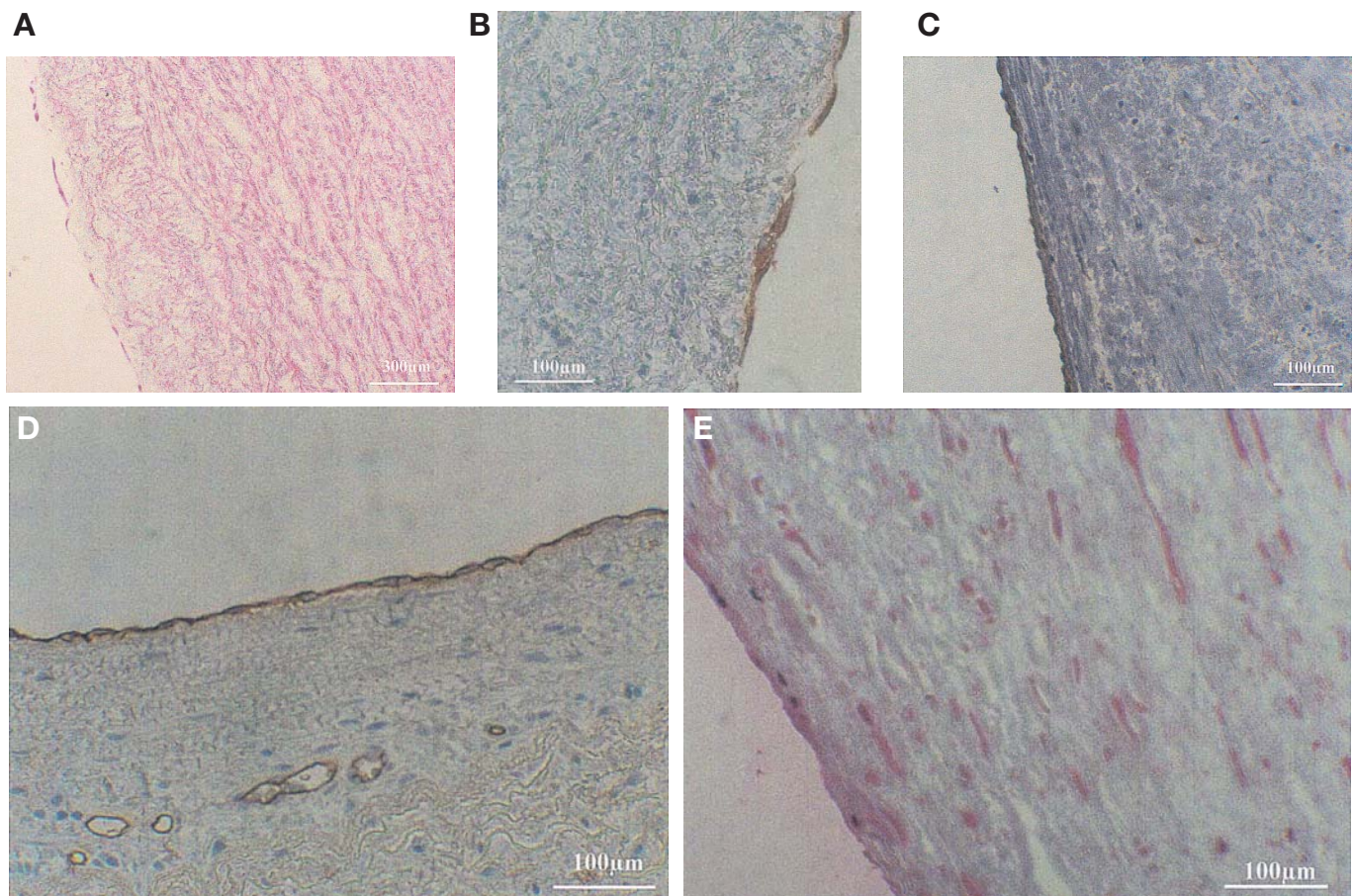


Figure 2: a) Histological examination (hematoxylin and eosin staining) of pulmonary wall after seeding and prior to implantation, showing a normal extracellular matrix and a monolayer of endothelial-like cells. b) CD 31 staining confirming the presence of endothelial cells. c) von Willebrand Factor staining showing a monolayer of endothelial cells at three months after implantation. d) CD 31 staining confirming the monolayer of endothelial cells and ingrowth of interstitial cells. A vasa vasorum is visible in the deeper layers. e) Anti-fibroblast factor staining showing positive cells in the deeper layers of the extracellular matrix at three months after implantation.

37% freedom from reoperation at this time.

The Ross procedure appears to be the best option for younger patients, as operative complications are rare and the long-term results encouraging, with excellent hemodynamic behavior. Freedom from autograft explantation was surprisingly high at 90% at 10 years and 84% at 20 years follow up (9), although 54% of these autografts could be saved by valve repair. The high rate of autograft failure could be overcome by changing the implantation technique.

Chambers et al. (10) reported that most reoperations were required due to failure of the non-viable heart valve prosthesis used to reconstruct the RVOT, with 20% failure occurring after 20 years due to stenosis or valve degeneration. Oury et al. (11) reported excellent hemodynamic behavior at maximum exercise in athletes who had either undergone the Ross operation or who were controls, with mean aortic valve pressure gradients of 16.30 mmHg and 14.61 mmHg, and maximal velocities of 190.00 and 190.23 cm/s, respectively. This study highlighted the possibility of reconstructing the RVOT by using a viable heart valve, with its associated advantages over the non-modified, cryopreserved allograft.

An increased transpulmonary flow velocity often occurs even following the use of oversized cryopreserved allografts in patients. For example, Carr-White et al. (12) demonstrated an increased transpulmonary flow velocity with maximum pressure gradient up to  $46 \pm 18$  mm Hg, and a peak flow velocity of approximately 3.39 m/s in 21% of patients after a two-year follow up. In the present patient, the basic mean flow velocity was lowered immediately after implantation and also during follow up, to within the physiological range. Such an occurrence, which may be related to the immunologic reaction of the host against the transplanted donor heart valve, does not occur after heart transplantation as valvular rejection is prevented by immunosuppressive medication (13).

Previously, decellularization without in-vitro seeding of the decellularized allografts has been shown to cause a significant reduction in immunological reaction compared to that with regular allografts (14). The recellularization potential in human was also demonstrated histologically, using an in-vitro endothelial cell-seeded decellularized and cryopreserved allograft. However, the impact of this recellularization potential on valve durability and hemodynamic behavior must be monitored during a longer follow up period.

*In conclusion*, tissue engineering appears to offer an excellent means of improving the function of regularly cryopreserved allografts. Biopsy-proven evidence is also available that the repopularization of acellular tissue, which previously was demonstrated only in animal models, does indeed occur in humans. However, the key factor for recellularization to occur seems to be complete decellularization, the removal of all cellular debris, and preservation of the collagen-elastin matrix.

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