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Injectable Tissue-Engineered Pulmonary Heart Valve Implantation Into the Pig Model: A Feasibility Study

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Background: Transcatheter pulmonary valve replacement is currently performed in clinical trials, but is limited by the use of glutaraldehyde-treated bioprostheses. This feasibility study was performed to evaluate delivery-related tissue distortion during implantation of tissue-engineered (TE) heart valves.

Material/Methods: The injectable TE heart valve was mounted on a self-expanding nitinol stent (n=7) and delivered into the pulmonary position in 7 pigs, (weight 26 to 31 kg), performing a sternotomy or limited lateral thoracotomy. Prior to implantation, the injectable TE heart valves were crimped and inserted into an applicator. Positioning of the implants was guided by fluoroscopy, and after careful deployment, angiographic examination was performed to evaluate the correct delivered position. Hemodynamic measurements were performed by epicardial echocardiography. Finally, the animals were sacrificed and the injectable TE heart valves were inspected by gross examination and histological examination.

Results: Orthotopic deliveries of the injectable TE heart valves were all successful performed, except in 1 where the valve migrated due to a discrepancy between pulmonary valve annulus size and injectable TE valve size. Angiographic evaluation (n=6) showed normal valve function, supported by epicardial echocardiography in which no increased flow velocity was measured, neither trans- nor paravalvular regurgitation. Histological evaluation demonstrated absence of tissue damage from the delivery process.

Conclusions: Transcatheter implantation of an injectable TE heart valve seems to be possible without tissue distortion due to the delivery system.

MeSH Keywords: **Cardiac Catheters • Pulmonary Valve • Tissue Engineering**

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Background

The incidence of complex congenital heart disease is approximately 8.21/1000 live births in Canada, with involvement of the right ventricular outflow tract (RVOT) of approximately 10% [1]. Generally, initial RVOT reconstruction is performed by patch, but due to the absence of a sufficient functioning pulmonary valve, the right ventricle will be volume-overloaded and dilate over time. The volume overload of the right ventricle is usually well tolerated, but over time it might lead to decreased exercise tolerance, dyspnea, arrhythmia, symptoms of heart failure, and, eventually, sudden death [2,3]. At the time of ventricle dilatation, a valve conduit is chosen to reconstruct the RVOT, since today's available valve conduits degenerate over time in absence of regeneration or growth potential [4-6].

The ideal would be a living autologous valve with regeneration, remodelling, and growth potential, implanted with minimal invasiveness, without tissue distortion or compromising valve function. Tissue engineering techniques allows us to construct valves with remodelling, regeneration, and growth potential, but these valves have only limited availability for clinical use and are conventionally implanted [7,8].

In 2000 Bonhoeffer et al. [9] began using glutaraldehyde-fixed bovine jugular veins mounted on balloon-expandable stainless stents to be percutaneously implanted. These valves were initially implanted in patients with pulmonary valve stenosis, but nowadays pulmonary valve regurgitations can also be treated with these valves if the current annulus size is still suitable and not over-dilated [10]. General disadvantages of the current clinically available transcatheter valves are availability for selected cases, high rate of stent fractures [11], and increased risk of endocarditis during follow-up [12]. Glutaraldehyde treatment is not an optimal option in congenital heart disease treatment because there is lack of growth potential as well as increased risk for early deterioration [13,14]. The aim of the present study was to evaluate the implantation of a newly designed injectable TE pulmonary heart valve with a self-expandable nitinol stent to reconstruct the RVOT.

Material and Methods

All experiments were performed in accordance to the "Principles of Laboratory Animal Care" prepared by the National Society of Medical Research, and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH, revised 1996). The study was approved by the institutional review and ethics committee at the University of Leipzig.

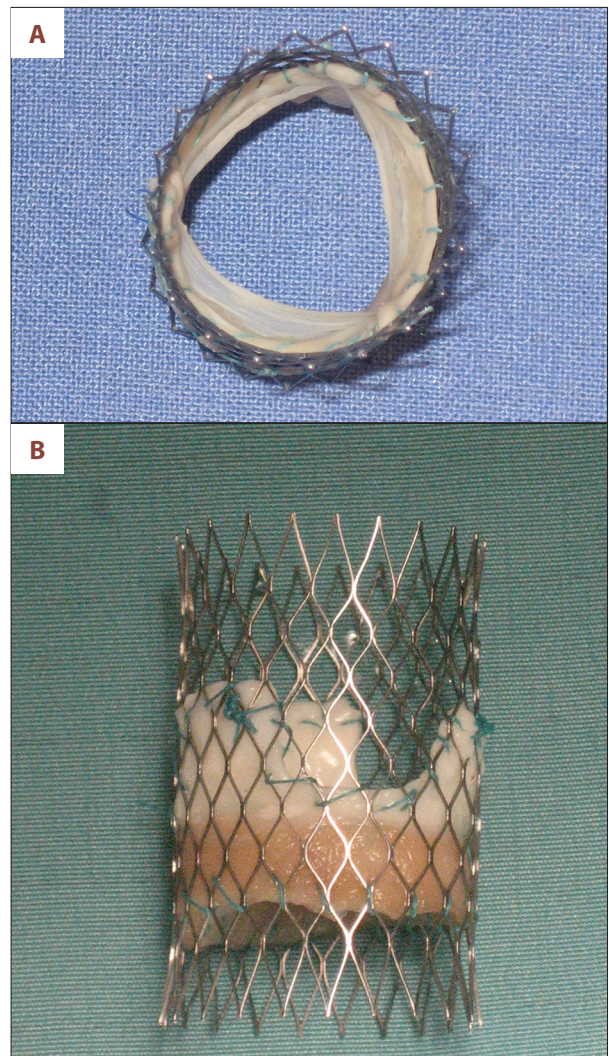


Figure 1. (A) View inside the outflow tract of the injectable TE heart valve. (B) Side view of the injectable TE heart valve mounted on a self-expanding nitinol stent.

Valve design

Details on decellularization of porcine pulmonary heart valves were previously reported [15]. In brief, fresh porcine pulmonary heart valves were obtained from the slaughterhouse. After preparation and carefully trimming the muscle tissue to a minimum, the valves were placed in antibiotic solutions until decellularization with deoxycholic acid (Sigma Chemical Co, St. Louis, MO) was started. Extensive rinsing and ethanol treatment was performed before final sterilization. The decellularized pulmonary valves were sutured into a self-expandable nitinol stent (PFM Medical AG, Koeln, Germany) with a diameter of 16 mm (Figure 1A, 1B). This size was chosen because the diameter of the pulmonary valve annulus was preoperatively measured, adding 10% to ensure optimal fitting of the injectable TE heart valve into the pulmonary valve annulus.



Figure 2. Crimping procedure of the injectable TE heart valve prior to delivery.

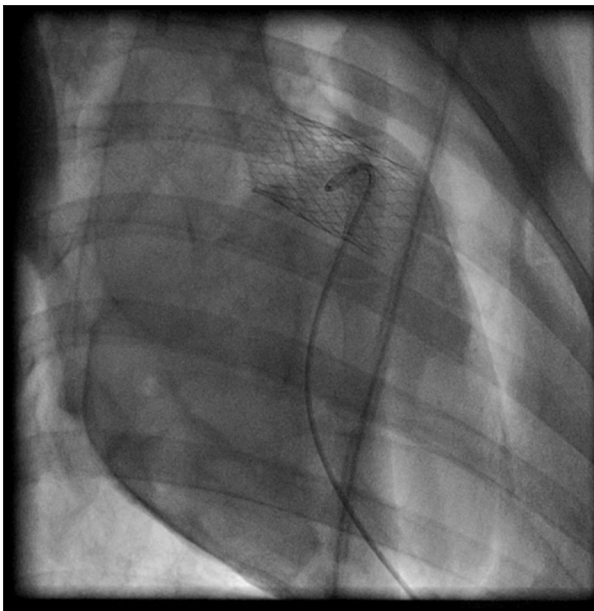


Figure 3. Angiographic examination shows the optimal position of the injected TE heart valve.

Implantation procedure

Seven healthy pigs between 6 and 9 months of age with a median weight of 28 kg (range 26–31 kg) were included in this study. All animals were pre-medicated and, after general anesthesia was induced, mechanical ventilation was started. A median sternotomy or limited lateral thoracotomy was performed to implant the injectable TE pulmonary heart valve. A bolus of 5,000 IU heparin intravenously was administered and the pericardium was opened. One purse-string suture was placed at the level of the right ventricular outflow tract. After crimping the valve for at least 30 min, an introducer was used to implant the injectable TE pulmonary heart valve (Figure 2). The

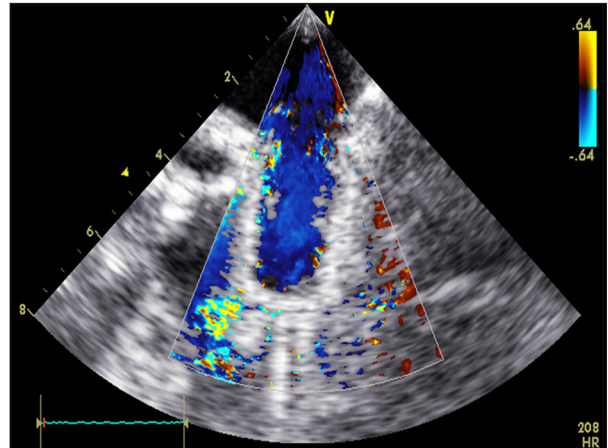


Figure 4. Echocardiographic examination shows the absence of para- and transvalvular regurgitation.

release procedure was guided by fluoroscopy and performed under rapid pacing. The implanted valve was studied by epicardial echocardiographic examination (Vivid; GE Healthcare, Munich Germany), invasive pressure, and angiographic measurements. Finally, 4–6 h after implantation, the animals were sacrificed and the injectable TE heart valves were explanted, rinsed, and subjected to gross examination and histopathological analysis.

Results

The injectable TE heart valve was guided by fluoroscopy (Figure 3) and successfully delivered in all animals, except in 1 where the valve migrated due to the discrepancy between the implanted valve and the native pulmonary valve annulus. Angiography and epicardial echocardiography proved that all injectable TE heart valves were competent, and no trans- or paravalvular leakage was seen in any of the 6 successful implantations (Figure 4). Invasive pressure measurement showed a median maximum pressure gradient of 4 mm Hg (range 3–5 mm Hg) and median maximum pressure gradient of 8 mm Hg (range 6–12 mm Hg), confirmed by epicardial echocardiographic examination. During follow-up, none of the valves migrated and hemodynamic behavior of the implanted valves was stable.

Gross examination of the injectable TE heart valve showed pliable leaflets without tears or bleeding. The leaflets were all intact and showed no thrombus formation (Figure 5A, 5B).

Histopathological examination showed a preserved extracellular matrix with absence of any tissue damage due to the delivery system or implantation process (Figure 6A–6D).

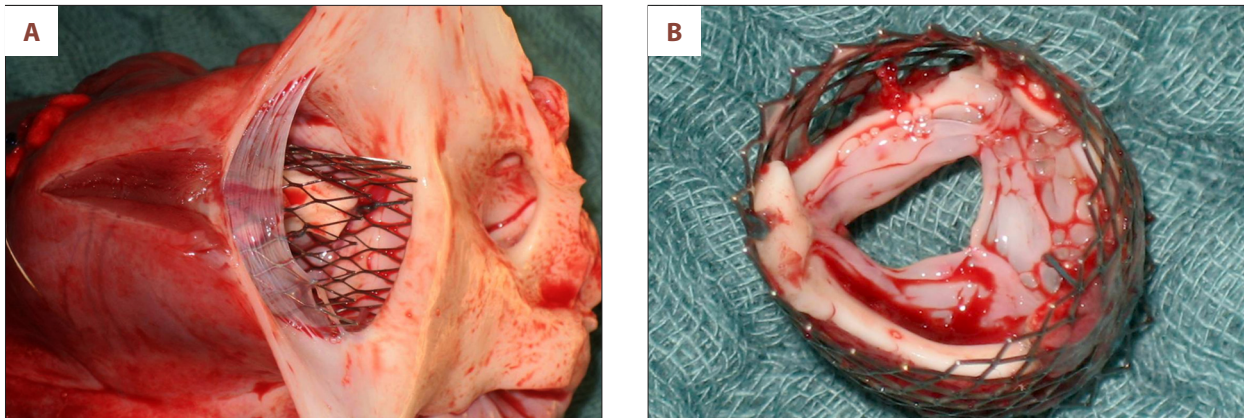


Figure 5. (A) Gross examination of the injectable TE valve implanted into the native pulmonary valve. (B) Pliable leaflets of the injectable TE heart valve without tissue distortion or biofilm.

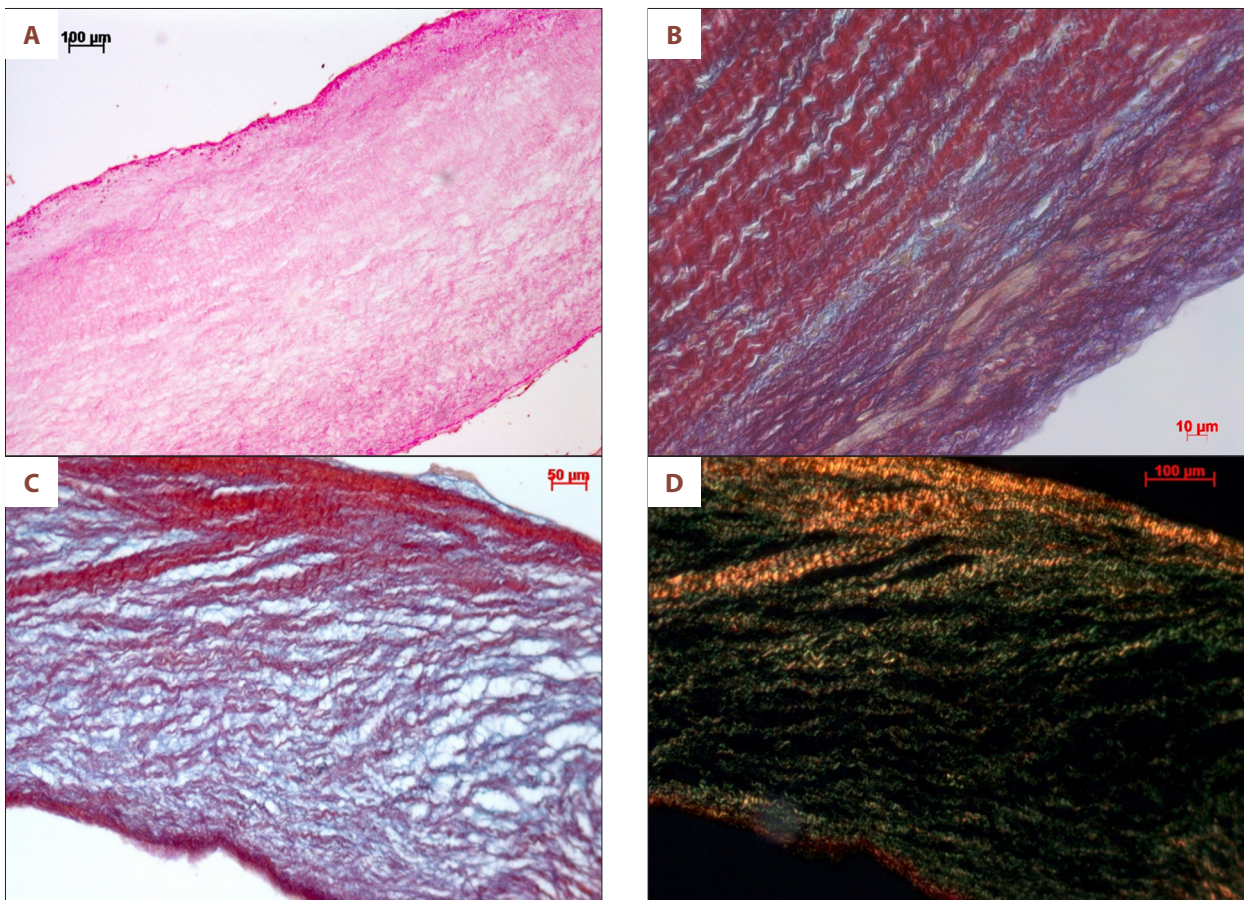


Figure 6. (A) Global histological tissue examination shows an intact extracellular matrix after crimping and catheter delivery (hematoxylin & eosin staining). (B, C) Detailed examination shows absence of tissue distortion of the extracellular matrix (picrosirius red staining). (D) Detailed examination of staining of the intact collagen I (red in polarized light) and collagen III (green in polarized light) of the injectable TE heart valve.

Discussion

Today's treatment options for complex congenital RVOT disease are not yet optimal and need to be improved. Allografts

are still an excellent option, but availability is limited; therefore, alternative materials are used such as glutaraldehyde-fixed conduits to correct congenital malformation of the RVOT in neonates and infants. Homann et al. [6] showed similar survival

rates for patients treated with allografts and xenografts, but freedom of re-operation was different for both groups, at 28% for xenografts versus 62% for allografts ($p < 0.01$). These xenografts showed serious deterioration during long-term follow-up, such as sclerosis and calcification. In a more recent study by Vitanova et al. [16], different types of conduits for RVOT reconstruction were compared in patients below the age of 1 year. They showed similar rates of freedom for reoperation for allografts, Contegra, and Hancock conduits at $38.1 \pm 8.3\%$, $38.0 \pm 11.3\%$, and $20.3 \pm 7.5\%$, respectively ($p = 0.4$). The Hancock conduit, however, was associated with a significantly higher rate of thrombosis ($P = 0.01$) and the patients receiving a Contegra conduit developed faster conduit regurgitation or stenosis than patients with allografts ($p = 0.01$). Nevertheless, all these heart valve substitutes have no growth potential.

New technologies are therefore needed that are free of glutaraldehyde, to allow tissue remodelling. Tissue engineering could overcome this problem, which clinically started with autologous endothelial cell seeded grafting [17]. Several TE heart valves have been developed and are now clinically used. The reported clinical outcomes, however, strongly varied [7,8,18–21]. One reason for this could be the different origins of tissue used for the extracellular matrix [22,23]. Excellent clinical results are achieved with human-origin tissue [24,25]. Another issue is the performed decellularization process itself, which can be very different and has enormous influence on the extracellular matrix [8,19,20]. Conventional surgical implantation techniques have been used for these TE heart valves [7,8,18], but it would be desirable to implant these valves minimally invasively by using a catheter.

Percutaneous pulmonary valve implantation (PPVI) was started in 2000 by Bonhoeffer et al. [9] in patients with degenerated conduits within the RVOT, showing initially limited benefit [26]. Nordmeyer et al. [27] reported a high number of stent fractures, which can be dramatically reduced by performing pre-stenting using a strong stent [28] or performing a valve-in-valve procedure [29]. After modifications were done, this treatment option become promising, but only for selected patients.

An additional patient group – those with pulmonary valve regurgitation of different etiology – could also benefit from this method. Prospective randomized studies on the optimal timing for intervention of pulmonary valve regurgitation are not currently available. Today, cardiac magnetic resonance imaging (cMRI) is the criterion standard for diagnosing ventricular function, mass, and volumes [30], which is also important in evaluating the postoperative development of right ventricular volume reduction and cardiac improvement, resulting in better exercise tolerance [31]. The improvement of right ventricular function after pulmonary valve implantation, however, did

not decrease death in recent studies [32]. cMRI examination will also provide additional information of value in avoiding fatal complications of percutaneous pulmonary valve implantation by left coronary occlusion, which can occur immediately after or hours after the procedure has been completed [33]. McKenzie et al. [34] showed comparative data of surgical pulmonary valve replacement to PPVI, showing freedom of reoperation was 94%, and in the expanded Melody valve trial [35] freedom of re-intervention was 53%. If PPVI is advocated in patients because it is less invasive and is suggested to be done at an earlier stage, modification of materials is needed to achieve this. Kiefer et al. [36] showed changes of the extracellular matrix, which were related to the crimping time. However, in a subcutaneous rat model, which is a passive screening model for calcification, there were no sign of increased tissue calcification. Therefore, examinations in a large circulating animal model are needed to investigate the impact of tissue crimping on leaflet durability and calcification during long-term follow-up. The present study was performed by using a decellularized porcine extracellular matrix in the porcine model, which simulates the situation of implanting a decellularized allograft in humans. To implant this injectable TE heart valve into the pulmonary position using a limited thoracotomy, the valve does not need to be extensively crimped. To implant an injectable TE heart valve percutaneously, the material of choice is pericardial tissue. In a previous study [37], we showed that decellularized pericardium withstands systemic circulation pressure without any effects. Additionally, because the decellularization of the pericardial tissue stays very pliable, and due to the absence of glutaraldehyde to cross-link, no tissue rupture can occur. The present feasibility study shows that injectable TE heart valves can be implanted safely and that crimping for the delivery process can be safely performed without tissue distortion. Long-term follow-up will be needed to evaluate functionality and absence of tissue distortion to eventually expand the use of this less invasive approach in humans.

Limitations of the study

This feasibility study was performed to evaluate the influence of delivery procedure on an injectable TE heart valve. However, due to the limitation of the acute setting of these experiments, long-term implantation studies will be needed to evaluate the injectable TE heart valve behavior under permanent burden.

Conclusions

Transcatheter implantation of an injectable TE heart valve seems to be feasible, without tissue distortion due to the delivery procedure.

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