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PRE-CLINICAL RESEARCH

Stem Cell–Based Transcatheter Aortic Valve Implantation

First Experiences in a Pre-Clinical Model

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Objectives This study sought to investigate the combination of transcatheter aortic valve implantation and a novel concept of stem cell-based, tissue-engineered heart valves (TEHV) comprising minimally invasive techniques for both cell harvest and valve delivery.

Background TAVI represents an emerging technology for the treatment of aortic valve disease. The used bioprostheses are inherently prone to calcific degeneration and recent evidence suggests even accelerated degeneration resulting from structural damage due to the crimping procedures. An autologous, living heart valve prosthesis with regeneration and repair capacities would overcome such limitations.

Methods Within a 1-step intervention, trileaflet TEHV, generated from biodegradable synthetic scaffolds, were integrated into self-expanding nitinol stents, seeded with autologous bone marrow mononuclear cells, crimped and transapically delivered into adult sheep (n = 12). Planned follow-up was 4 h (Group A, n = 4), 48 h (Group B, n = 5) or 1 and 2 weeks (Group C, n = 3). TEHV functionality was assessed by fluoroscopy, echocardiography, and computed tomography. Post-mortem analysis was performed using histology, extracellular matrix analysis, and electron microscopy.

Results Transapical implantation of TEHV was successful in all animals (n = 12). Follow-up was complete in all animals of Group A, three-fifths of Group B, and two-thirds of Group C (1 week, n = 1; 2 weeks, n = 1). Fluoroscopy and echocardiography displayed TEHV functionality demonstrating adequate leaflet mobility and coaptation. TEHV showed intact leaflet structures with well-defined cusps without signs of thrombus formation or structural damage. Histology and extracellular matrix displayed a high cellularity indicative for an early cellular remodeling and in-growth after 2 weeks.

Conclusions We demonstrate the principal feasibility of a transcatheter, stem cell–based TEHV implantation into the aortic valve position within a 1-step intervention. Its long-term functionality proven, a stem cell–based TEHV approach may represent a next-generation heart valve concept. (J Am Coll Cardiol Intv 2012;5:874–83) © 2012 by the American College of Cardiology Foundation

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The therapy options for patients with valvular heart disease are undergoing rapid changes and in addition to conventional, surgical valve replacement, representing the standard of care for several decades, transcatheter aortic valve techniques have entered the clinical routine, representing an efficient alternative for the treatment of elderly high-risk patients (1–4). Given sufficient long-term safety, it can be predicted that these minimally invasive techniques may have a major impact on the treatment strategy of patients with valvular heart disease.

However, despite this rapid technical progress, the currently available prostheses for transcatheter approaches are bioprosthetic with the known disadvantages comprising progressive calcification and degeneration. Furthermore, recent evidence suggests even accelerated degeneration resulting from structural damage due to the crimping procedures (5,6).

An autologous, living heart valve prosthesis created by tissue-engineering technologies with regeneration and repair capacities would overcome such limitations (7). A clinically relevant heart valve tissue-engineering (HVTE) concept would ideally comprise minimally invasive techniques for both cell harvest and valve implantation. We have demonstrated the technical feasibility of combining the concept of HVTE and transapical delivery into the pulmonary position of adult sheep (8). Thereafter, in a recent proof-of-concept study, we introduced a novel and clinically highly relevant concept of in vivo implantation of autologous bone marrow mononuclear cell (BMMC)-derived tissue-engineered heart valves (TEHV) in a primate model (9). Following recent U.S. Food and Drug Administration approval (10), techniques to generate tissue-engineered vascular grafts by using BMMC without any phase of in vitro culturing or expansion (11–15) are entering the clinical arena. Based on these techniques, we successfully generated and implanted autologous BMMC-derived TEHV in a 1-step intervention comprising cell harvest, in vitro engineering, and transapical delivery (9). However, except for an acute, technical feasibility study using a modified Hufnagel procedure (16) to implant TEHV into the descending aorta in an ovine model (17), chronic studies of successful TEHV implantations have only been reported for the low-pressure system in the pulmonary position (8,9,18).

The aim of this study was to assess the principal feasibility of implanting stem cell–based TEHV into the aortic valve position using a transcatheter, 1-step interventional approach. Beside the technical feasibility of the deployment of a TEHV in the aortic position, the major endpoints of this study were the assessment of the valve functionality and initial tissue-remodeling phenomena of TEHV in the aortic position.

Methods

Experimentation approval. All animals received humane care in compliance with the "Principles of Laboratory Animal-Care" as well as with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (19). All procedures were approved by the Institutional Ethics Committee (approval no. 11-15/2011).

Study design. The study aim was to assess the principal feasibility of implanting stem cell–based TEHV into the orthotopic, aortic valve position using a transcatheter, 1-step interventional approach (Fig. 1, Online Fig. 1). The study animals (adult sheep, n = 12) were divided into 3 groups: Group A (follow-up: 4 h, n = 4); Group B (follow-up: 48 h, n = 5); and Group C (follow-up: 1 and 2 weeks, n = 3) (Fig. 2).

In the first set of experiments, the animals of Group A (n = 4) were used in an acute fashion (follow-up: 4 h) to establish the technical feasibility of transapical TEHV implantation into the orthotopic, aortic position with a specific focus on technical aspects, such as device insertion, stepwise delivery, and optimal positioning considering the anatomical condition of the aortic annulus, the aortic root, and the coronaries. To assess TEHV stability and functionality as well as early tissue reaction and remodeling in the systemic pressure system, the other animals were planned to be followed up for either 48 h (Group B, n = 5) or 1 and 2 weeks (Group C, n = 3) (Fig. 2).

Abbreviations and Acronyms

BMMC = bone marrow mononuclear cell(s)

HVTE = heart valve tissue engineering

TEHV = tissue-engineered heart-valve(s)

TTE = transthoracic echocardiography

Scaffold fabrication. Trileaflet heart valve scaffolds were fabricated as previously described (9,17) (Online Appendix).

Isolation, characterization, and differentiation of ovine BMMC. See Online Figures 1 and 2.

Seeding of ovine BMMC. Fibrin was used as a cell carrier to seed BMMC onto both sides as well as on the conduit of the stented heart valve scaffolds ($1.73 \pm 0.47 \times 10^6$ cells/cm^2 valve leaflets) (Sigma-Aldrich, St. Louis, Missouri), before the stented TEHV was loaded into the delivery device by crimping the outer diameter from 25 mm down to 8 mm (Online Appendix).

Pre-operative assessment, planning, and sizing. Before implantation, all sheep underwent transthoracic echocardiography (TTE) to adapt the ideal stent size to each animal. Before starting the implantation procedure, the following parameters were measured by intraoperative angiography to reconfirm the ideal stent size: diameters of the aortic annulus, sinus portion, the sinus-tubular junction, and the brachiocephalic trunc, as well as the distance to the brachiocephalic trunc and the height of the sinus portion (Table 1). Animals with an annulus between 22 mm and 23 mm received a 25 mm stented TEHV (n = 8), whereas animals with an annulus of 24 mm to 25 mm received a 27 mm stented TEHV (n = 4).

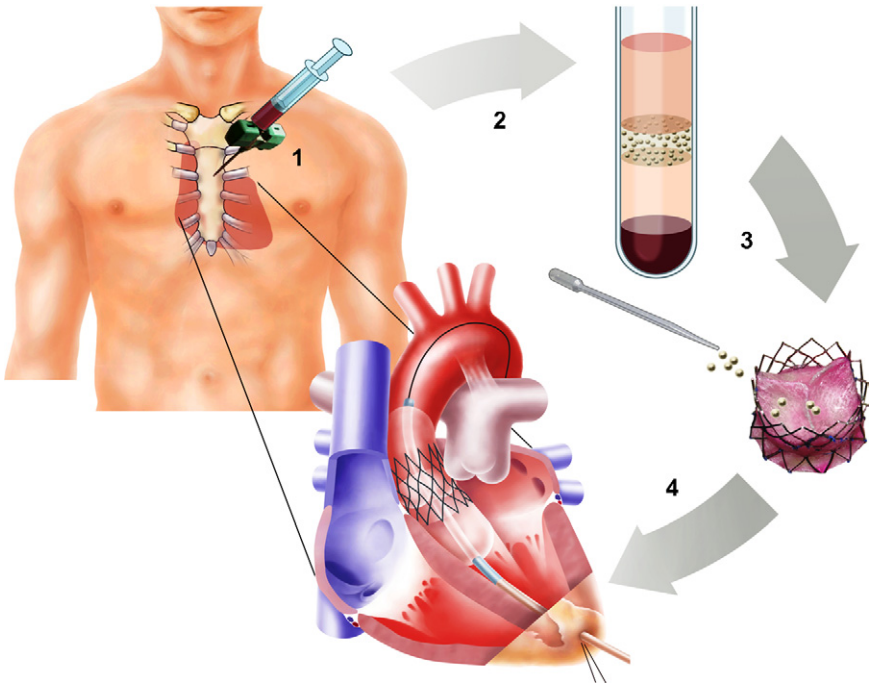

Figure 1. Stem Cell–Based, TEHV Implantation Into the Aortic Valve Position via a Transcatheter, 1-Step Interventional Approach

Bone marrow is aspirated from the sternum into a heparinized syringe **(1)** and bone marrow mononuclear cells (BMMC) are obtained by centrifuging the samples on a histopaque density gradient **(2)**. The BMMC are seeded onto the stented heart valve scaffolds using fibrin as a cell carrier **(3)**. Thereafter, the tissue-engineered heart valve (TEHV) is loaded into the delivery device by crimping the outer diameter down to 8 mm and transapically delivered **(4)**. The mean duration of the entire procedure, starting from cell harvest until TEHV-implantation takes approximately 2 h.

Transcatheter implantation of stem cell–based TEHV into the aortic valve position. TEHV were transapically delivered into the aortic valve position via a mini sternotomy (Fig. 1). The valves were crimped and loaded onto a custom-made, guidewire-inducing system (outer diameter = 8 mm). The apex of the left ventricle was punctured after 5/0 Prolene (Ethicon Inc., Norderstedt, Germany) pledged, purse-string sutures were placed. TEHV were delivered into the aortic valve position under fluoroscopic control (OEC 9900 Elite, GE Healthcare, Fairfield, Connecticut). After the stepwise

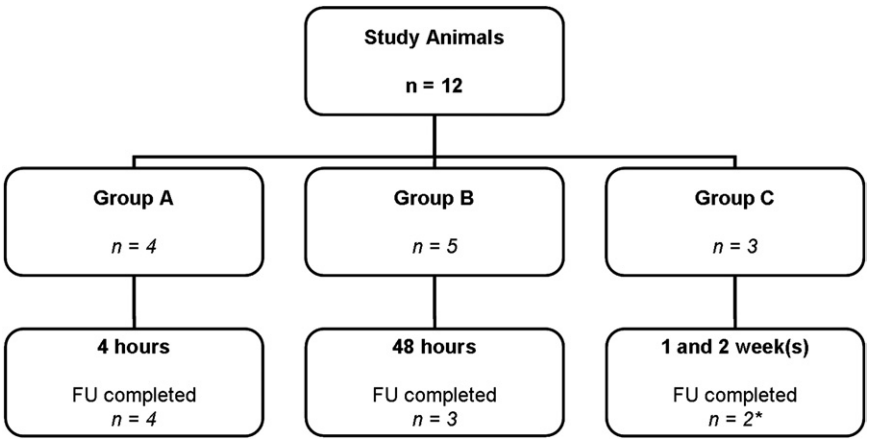

Figure 2. Study Design

Animal distribution and follow-up (FU). *In Group C, 1 animal completed 1 week FU and 1 animal completed 2 weeks FU.

Table 1. Pre-Operative Data and Angiographic Measurements

Body weight, kg	53.3 ± 8.1
Diameter AA on pre-op TTE, mm	22.1 ± 0.7
Diameter AA, mm	22.7 ± 1.0
Diameter BCT, mm	15.2 ± 1.4
Distance to BCT, mm	45.1 ± 2.7
Diameter SP, mm	28.3 ± 2.0
Diameter STJ, mm	24.5 ± 1.4
Height of SP, mm	13.3 ± 2.0

Values are mean ± SD.
AA = aortic annulus; BCT = brachiocephalic trunc; pre-op = pre-operative; SP = sinus portion; STJ = sinus-tubular junction; TTE = transthoracic echocardiography.

opening and positioning of the distal part of the stent in the aortic root under fluoroscopic control, the proximal part of the stent was instantly delivered. The appropriate placement and functionality of the implanted valve was confirmed by contrast angiography before the device was carefully removed and the purse-string sutures were tightened.

Assessment of stent positioning and TEHV functionality. Stent positioning was controlled using angiography and computed tomography (Siemens, Munich, Germany). In vivo functionality was evaluated using intraoperative, epicardial 2- and 3-dimensional echocardiography (iE33W xMATRIX Ultrasound, Philips Healthcare, Best, the Netherlands). TTE was serially performed until the animal was sacrificed. Three-dimensional computed tomography reconstruction and volume rendering were performed using the OsiriX Image Processing Software (OsiriX Mac OSX, version 3.8.1).

Post-operative care and follow-up. Anticoagulation was done with aspirin 100 mg/day and a clinical checkup was performed daily until sacrifice. The animals were sacrificed applying potassium euthanization and exsanguination. Thereafter, the hearts were harvested and the TEHV were excised for in vitro analysis.

Histology and scanning electron microscopy. Tissue samples of the explanted TEHV were analyzed qualitatively via histology and scanning electron microscopy (Online Appendix).

Quantitative explant tissue analysis. Explanted TEHV were lyophilized and analyzed by biochemical assays for total deoxyribonucleic acid and glycosaminoglycans content (Online Appendix).

Statistical analysis. Quantitative data are presented as mean ± SD (SPSS, version 17.0, IBM, Somers, New York).

Results

Cell isolation, characterization, and preparation of TEHV. Ovine BMMC were evaluated by flow cytometric analysis. Surface marker expression of CD29 (59.0 ± 7.0%) and CD44 (61.0 ± 16.7%) was detected. Less than 4% of the cells were positive for CD31 (3.7 ± 1.7%), CD34 (3.7 ± 1.0%), and CD166 (3.5 ± 4.4%) and no expression of Stro-4 was observed (Online Fig. 2A). Ovine mesenchymal stem cells were positive for CD29 (92.3 ± 2.1%), CD44 (24.3 ± 11.9%), CD166 (73.0 ± 14.2%), and Stro-4 (93.7 ± 14.2%). In contrast, low expression of the hematopoietic lineage markers CD31 (1.3 ± 0.9%) and CD34 (2.1 ± 1.8%) was observed (Online Fig. 2B). The results of the ovine mesenchymal stem cells phenotype were confirmed using immunofluorescence (Online Fig. 2C). Ovine mesenchymal stem cells displayed a characteristic spindle-shaped fibroblastic morphology (Online Fig. 2D). Their differentiation potential was demonstrated by inducing cells to specific lineages: adipogenic; osteogenic; and chondrogenic (Online Fig. 2D).

Transcatheter aortic valve implantation of stem cell–based TEHV. DELIVERY, POSITIONING, AND INTRAOPERATIVE COMPLICATIONS. Transcatheter aortic valve implantation of stem cell–based TEHV could be performed successfully in all animals (n = 12) (Fig. 3, Online Video 1). The aortic root was visualized (Fig. 3A) before the loaded delivery device was inserted and positioned (Fig. 3B). Under instant fluoroscopic control, the TEHV was stepwise delivered beginning with the deployment of the distal part (Figs. 3C and 3D) followed by the proximal part (Fig. 3E). Immediately after full deployment, coronary perfusion (Fig. 3F) and valve functionality was confirmed in the fluoroscopy (Fig. 3G). The animals remained hemodynamically stable during the entire procedure. Device removal was uneventful and no major complications, such as bleeding or cardiac arrhythmia occurred. As on fluoroscopy (Figs. 3A to 3G, Online Video 1) and as confirmed on computed tomography (Online Figs. 3A to 3G), TEHV could be successfully placed into the aortic valve position, thereby fully excluding the native leaflets while not compromising the coronary perfusion (Fig. 3D to 3G, Online Video 1). In 1 animal of Group B the TEHV appeared to be placed too proximal into the left ventricular outflow tract. Consecutively, after a few hours, it further migrated into the left ventricle and the animal was sacrificed due to beginning acute left heart failure. Another animal of Group B had to be terminated after successful delivery due to severe valvular leaflet dysfunction displaying severe central regurgitation immediately after implantation.

The mean duration from cell harvest until TEHV loading was 64 ± 8 min and the mean crimping time until transapical delivery was 12 ± 6 min. The mean duration of the entire procedure, starting from cell harvest until TEHV implantation was 109 ± 14 min (Table 2).

Performance of TEHV and acute echocardiography findings. Acute TEHV functionality and mobility was controlled via fluoroscopy (Fig. 3G) and epicardial echocardiography. Except for the 2 terminated animals, in all other study animals (n = 10), a sufficient opening and closing pattern of the TEHV was observed and the loading pressure of the systemic circulation was well tolerated (Figs. 4A and 4B,

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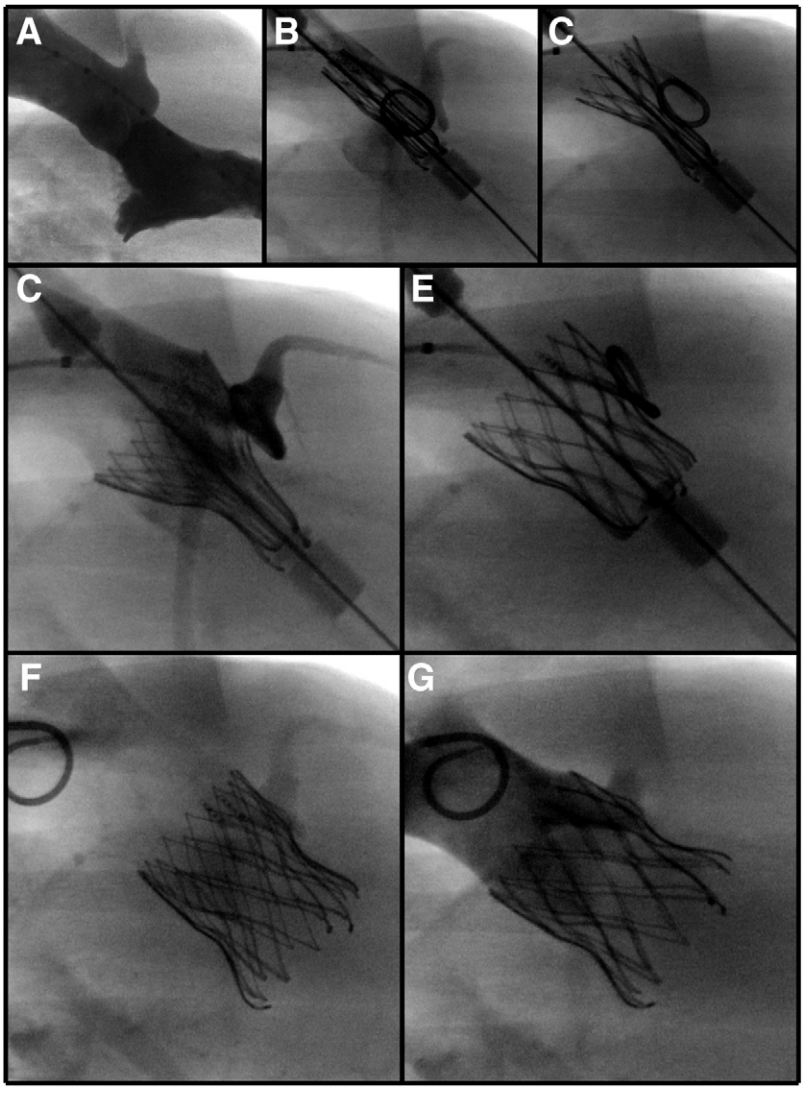

Figure 3. Fluoroscopy-Guided Transapical Delivery of Stem Cell–Based TEHV Into the Aortic Valve Position

The aortic root was visualized **(A)** before the loaded delivery device was inserted and positioned **(B)**. Under instant fluoroscopic control, the tissue-engineered heart valve (TEHV) was stepwise delivered beginning with the deployment of the distal part **(C and D)** followed by the proximal part **(E)**. Immediately after full deployment, coronary perfusion **(F)** and valve functionality **(G)** was confirmed. The animals remained hemodynamically stable during the entire procedure and the TEHV could be successfully placed into the aortic valve position, thereby fully excluding the native leaflets while not compromising the coronary perfusion. See Online Video 1.

Online Video 2A). TEHV functionality was confirmed in the 2-dimensional color mode and the 3-dimensional mode, demonstrating good leaflet mobility and coaptation (Figs. 4C to 4G, Online Videos 2B and 2C). The mean transvalvular gradient was 8.4 ± 2.0 mm Hg and the mean effective orifice area was 1.4 ± 0.1 cm^2 (Table 2). Only 1 animal displayed a mild mitral regurgitation, and none of the animals showed central aortic regurgitation. In contrast, in 40% of the animals, an at least mild paravalvular leakage was present (Table 2), which was related to the stent shape and design, but not to the TEHV itself.

Early post-operative period, complications, and follow-up. Except the 2 terminated animals, all other animals of Group B (3 of 5) and Group C (3 of 3) tolerated the procedure very well without any hemodynamic compromise and could be awakened immediately. Despite of an uneventful TEHV delivery and an excellent TEHV functionality without any signs of regurgitation or paravalvular leakage, 1 animal of Group C died due to acute stent dislocation. All other study animals were monitored regularly and received regular doses of aspirin until the planned harvest.

The 2 remaining animals of Group C (planned to be followed-up for 1 and 2 weeks) displayed a sufficient TEHV in

Table 2. Intraoperative Data and Echocardiography Measurements After Implantation

Duration from cell harvest to TEHV loading, min	64 ± 8
Crimping time until TEHV delivery, min	12 ± 6
Duration of the entire 1-step procedure, min	109 ± 14
TVG mean, mm Hg	8.4 ± 2.0
TVG peak, mm Hg	16.9 ± 5.8
EOA, cm^2	1.4 ± 0.1
Central aortic regurgitation	None
Paravalvular leakage*	2.1 ± 1.7
Regurgitation of mitral valve*	None†
Leaflet motion	Normal
Cardiac output, l/min	5.7 ± 1.2

Values are mean ± SD. *Regurgitation/paravalvular leakage grading: 0 = none; 1 = trivial; 2 = mild; 3 = moderate; 4 = severe. †Except 1 animal displaying mitral regurgitation grade 2.

EOA = effective orifice area; TEHV = tissue-engineered heart valve; TVG = transvalvular gradient.

vivo functionality on TTE and transesophageal echocardiography at 1 week with a mean transvalvular gradient of 8.0 ± 1.7 mm Hg and a mean effective orifice area of 1.5 ± 0.1 cm^2.

Therefore, it was decided to harvest 1 animal at 1 week and to keep the last remaining animal for an additional week to gain further insight into the early remodeling process. The remaining animal was clinically followed-up daily and the pre-final TTE control on day 10 before the planned harvest displayed sufficient TEHV performance. While preparing for the final assessment and harvest, the animal suddenly decompensated hours before the planned sacrifice at day 14 due to a sudden stent dislocation.

Explant macroscopy. Except the animal that had to be terminated due to sudden valve dysfunction, all other TEHV (n = 11) displayed intact leaflet structures with well-defined cusps and sufficient coaptation, without signs of thrombus formation, thickening, shrinking, or structural damage (Figs. 5A and 5B). The TEHV harvested at 1 and 2 weeks after implantation appeared to be well integrated into the surrounding tissue by complete tissue covering of the stent frame (Fig. 5C). The TEHV explanted at 2 weeks showed tissue formation and coaptation in 2 fully intact leaflets. The third leaflet (noncoronary) displayed a thin fissure that we assume was possibly related to the harmful explantation procedure as the stent was completely entrapped in the mitral valve and adequate trileaflet TEHV functionality was confirmed on TTE 3 days before.

Explant microscopy. After explantation, harvested tissues were analyzed using scanning electron microscopy, histology, and extracellular matrix analysis. In scanning electron microscopy, acute TEHV explants revealed a surface with

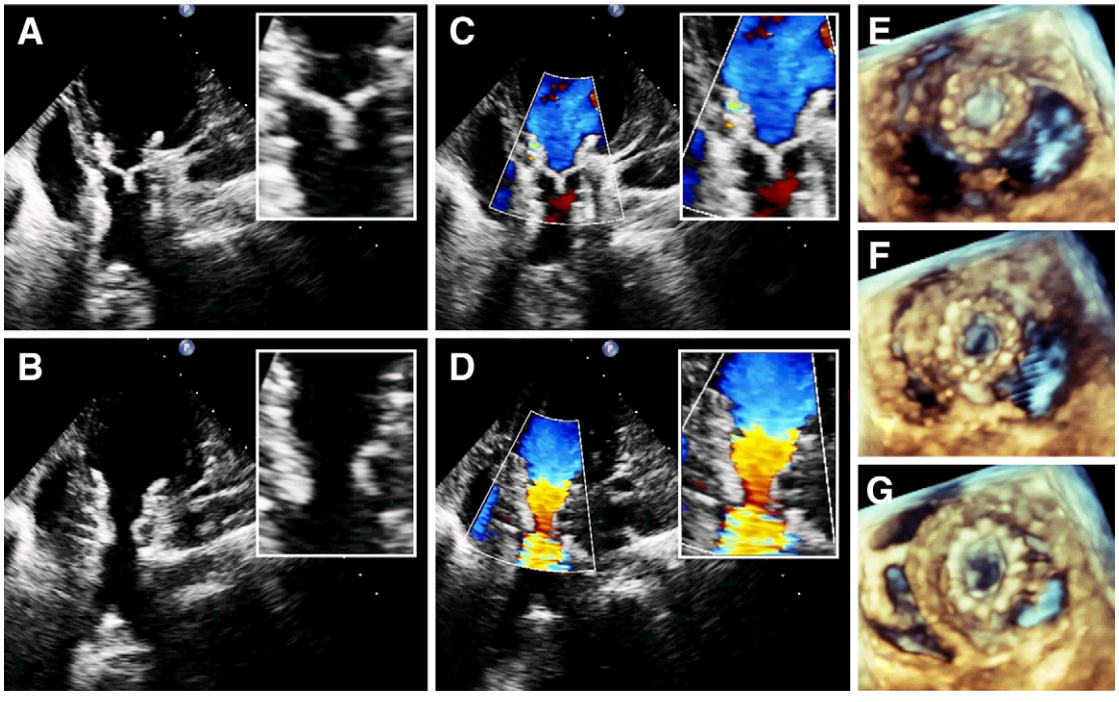

Figure 4. Performance of TEHV and Echocardiography Findings

Tissue-engineered heart valve (TEHV) functionality and mobility was controlled via epicardial and transesophageal echocardiography. TEHV tolerated the loading pressure of the systemic circulation adequately and demonstrated a sufficient coaptation **(A, B, and insets).** TEHV functionality and absence of regurgitation was confirmed in the 2-dimensional color mode **(C, D, and insets)** and in the 3-dimensional mode demonstrating adequate leaflet mobility **(E to G, and insets).** See Online Videos 2A, 2B, and 2C.

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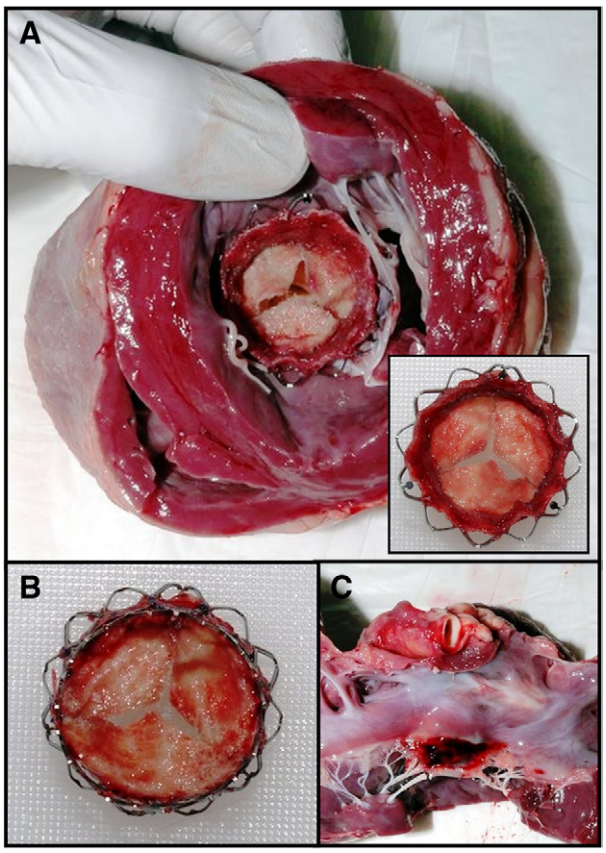

Figure 5. Explant Macroscopy of TEHV

Explanted tissue-engineered heart valves (TEHV) displayed intact leaflet structures with well-defined cusps and sufficient coaptation, without signs of thrombus formation, thickening, shrinking, or structural damage from the lower view **(A and inset)** and the upper view **(B).** The TEHV harvested at 1 and 2 weeks after implantation appeared to be well integrated into the surrounding tissue by complete tissue covering of the stent-frame **(C).**

fibrin reaction characterized by thrombocyte aggregation as well as leukocyte attachment (Online Fig. 4). This was also confirmed in histology, where acute explants showed clear cellular infiltrates and fibrin formation in hematoxylin and eosin staining (Figs. 6A to 6D). Interestingly, this cellularity only slightly increased in the tissues of the 24-h explants, whereas all later explant stages—including the 1-week and the 2-week explant tissues—showed a clearly increased cellularity (Figs. 6E to 6L). This observation was also supported by the extracellular matrix analysis, which showed increasing deoxyribonucleic acid and glycosaminoglycan values in the TEHV with increasing time in vivo (Online Figs. 5A and 5B).

Discussion

TAVI, which has been recently implemented into clinical routine as an attractive alternative for conventional aortic valve replacement, is expected to have a major impact on the management of valvular heart disease (1–4). Although these techniques are primarily applied to elderly, high-risk patients (1,3), the extension of indication for younger patients is awaited for the near future. However, despite the tremendous potential of transcatheter techniques, the currently used valvular prostheses for these approaches are bioprosthetic and all major disadvantages apply, including progressive calcification and functional degeneration (9,20–22).

The concept of TEHV has been repeatedly suggested as a potential solution to overcome the limitations of currently used bioprostheses (21,23). The use of living, autologous cell–based valvular constructs with the capacity of growth and regeneration may be significantly beneficial for both the congenital and adult settings. Numerous tissue-engineering concepts as well as cell sources are under evaluation for their clinical relevance, and we have recently demonstrated the principal feasibility of combining the concept of HVTE and transcatheter approaches in a pre-clinical large-animal model (8). However, so far, most of these concepts require extensive technical, logistical, and financial efforts, limiting their broad implementation into the clinical arena. Therefore, more simplicity is mandatory for an efficient translation into the clinical arena. A clinically relevant HVTE concept comprises minimally invasive techniques for both cell harvest and valve delivery.

We have recently introduced a new concept of combining autologous, TEHV, and transcatheter approaches in a 1-step procedure (9,17). Based on various previous animal studies and clinical pilot trials (12,14,15) to generate BMMC-derived tissue-engineered vascular grafts, in recent proof-of-concept studies, we were able to show the successful implantation of autologous BMMC-derived TEHV in a 1-step intervention into the pulmonary position in a primate model (9), as well as in the descending aorta using a modified Hufnagel procedure in an acute ovine model (17).

In this study, and for the first time, we demonstrate the feasibility of this 1-step procedure comprising minimally invasive cell harvest and transapical delivery for the aortic valve position successfully replacing aortic valves in an ovine model. With the help of an improved catheter system allowing for orthotopic, aortic valve implantation, we were able to further develop and adapt this novel, clinically, highly relevant 1-step procedure for the high-pressure circulation. Our data indicate that BMMC-based TEHV can be successfully implanted into the aortic position exhibiting competent valve functionality and leaflet coaptation sufficiently withstanding the systemic pressure load, whereas any signs of valvular rupture, tear, or structural damage were absent.

Importantly, this study reveals that the TEHV fully accepted the systemic pressure responding with a high degree of early cellularization indicative for an active and

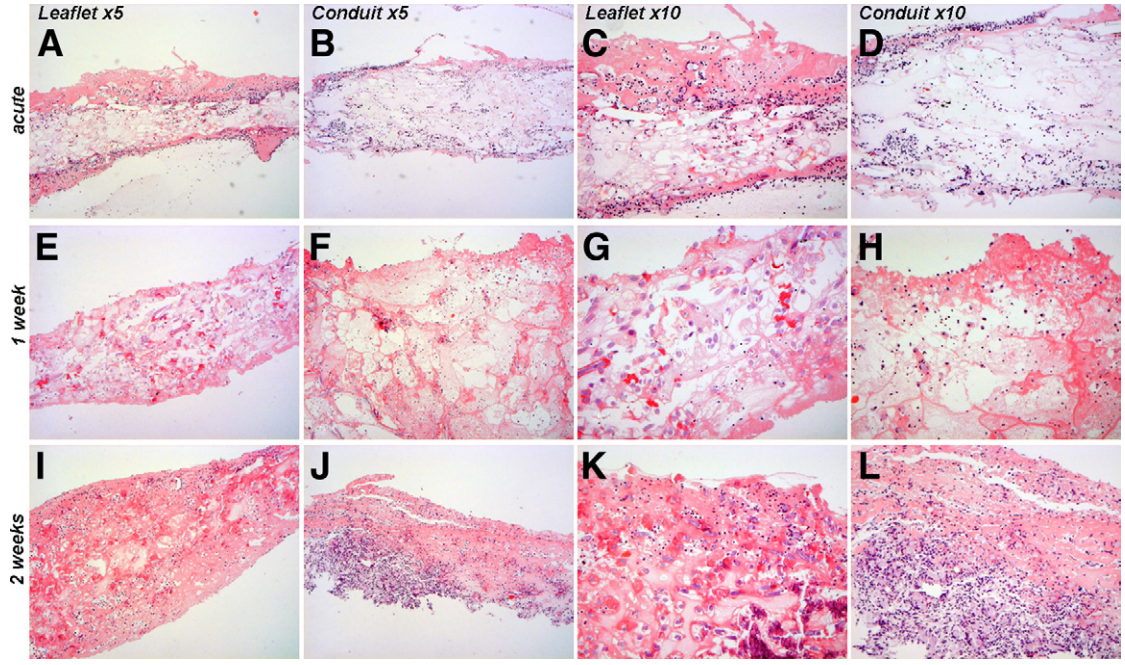

Figure 6. Histological Analysis of TEHV Explants

On histology, acute explants showed clear cellular infiltrates and fibrin formation in hematoxylin and eosin staining **(A to D)** (magnification 5× and 10×). Interestingly, this cellularity only slightly increased in the tissues of the 24-h explants (images not shown), whereas all later explant stages at 1 week and 2 weeks showed a clearly increased cellularity **(E to L)** (magnification 5× and 10×). TEHV = tissue-engineered heart valve(s).

extensive tissue formation and remodeling process. As previously described, sufficient hemodynamic loading appears to play a key role in tissue remodeling and formation and is a well-established mechanism (24). The histological findings of this study are supported by the interesting observation in our recent study that elucidated the crucial impact of sufficient hemodynamic loading on functional tissue remodeling in a nonhuman primate model. In contrast to orthotopically delivered TEHV fully excluding the native leaflets, TEHV that were implanted into the supravalvular position—not excluding the native leaflets and thereby being fully unloaded—lacked significant leaflet structure, being indicative for complete absence of tissue formation as well as BMMC-mediated remodeling processes (9).

The underlying molecular mechanisms of autologous BMMC-based tissue remodeling appear to be complex and have not been completely understood so far. Recent studies suggest a multifactorial, BMMC-mediated chemoattractive process (9,13,22,25,26). This extensive, paracrine process appears to involve numerous cells, including monocytes as well as mediator cytokines controlling the different steps of remodeling and tissue formation (9,13,22,25,26). In the setting of tissue-engineered vascular grafts, Roh et al. (13) recently demonstrated that seeded BMMC secrete significant amounts of monocyte chemoattractant protein-1 and lead to an increased early monocyte recruitment. Importantly, they also showed that the seeded BMMC were no longer detectable within a few days after implantation, suggesting that the scaffolds were initially repopulated by host monocytes by a monocyte chemoattractant protein-1–mediated process (13). In line with that, the recent study of Hibino et al. (25,26) confirmed the early absence of seeded BMMC declining to <1% after 2 weeks indicating that the bone marrow does not represent the significant source of endothelial or smooth muscle cells that comprise the newly formed vessel. In contrast, the adjacent vessel wall appeared to be the primary source of these cells forming the major part of the neotissue. The investigators concluded that tissue-engineered constructs function by mobilizing the body's innate healing capabilities to "regenerate" neotissue from pre-existing committed tissue cells (25). The mechanism of a BMMC-mediated chemoattractive process appears to be confirmed in the present study, indicating an early and extensive remodeling process primarily characterized by an early monocytic infiltration that may even be accelerated due to the systemic pressure loading.

Another interesting observation in this study was that macroscopically the TEHV did not display any sign of thickening or shrinking when compared with the previous reports in sheep (8,18) or primates (9) that presented with functional, but shortened leaflets limiting an optimal coap-

tation area. A potential reason may be reflected in the fact that this study was the first trial performed in the high-pressure circulation, whereas all other studies have been performed in the low-pressure pulmonary circulation (8,9,18). Although highly speculative, the high-pressure environment may play a key role in this regard and may prevent the TEHV from shrinking and thickening. Consecutive studies will be mandatory to further elucidate the important key issue of shrinking and to determine the role of pressure in this regard.

Study limitations. Based on this proof-of-concept study demonstrating the principal feasibility of transcatheter aortic valve replacement using BMMC-based valves in a 1-step procedure, long-term studies are mandatory to further assess the fate and the underlying remodeling mechanisms of such TEHV. Furthermore, the role of the seeded BMMC and the associated multifactorial, chemoattractive remodeling process needs to be systematically evaluated to define quality criteria of tissue formation representing a key prerequisite for a safe translation into the clinical setting. Finally, additional effort is needed to improve the stent design to further minimize the principle problem of paravalvular leakage and stent dislocation that is associated with current transcatheter approaches, as it was also encountered in the present study.

Conclusions

Considering the tremendous clinical potential of a BMMC-based tissue-engineering approach that has already received U.S. Food and Drug Administration approval for clinical application in the setting of tissue-engineered vascular grafts and is expected to have an even vaster impact with regards to TEHV (10), our study provides the first evidence to facilitate this concept for the successful replacement of aortic valves using a minimally invasive transcatheter approach. Its long-term durability proven, and taking into account heart valve pioneer Dr. D. E. Harken's "ten commandments" (27) that define an optimal heart valve prosthesis and thereby characterize a prosthesis with native valve attributes, such TEHV may represent the next generation of heart valve substitutes potentially overcoming some of the limitations of currently used bioprosthetic valves.

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Key Words: aortic valve ■ stem cells ■ tissue engineered heart valves ■ transcatheter ■ transcatheter aortic valve implantation.

APPENDIX

For supplemental methods, figures, and videos, please see the online version of this paper.