



Reendothelialization of Human Heart Valve Neoscaffolds Using Umbilical Cord-Derived Endothelial Cells

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Background: Heart valve tissue engineering represents a concept for improving the current methods of valvular heart disease therapy. The aim of this study was to develop tissue engineered heart valves combining human umbilical vein endothelial cells (HUVECs) and decellularized human heart valve matrices.

Methods and Results: Pulmonary (n=9) and aortic (n=6) human allografts were harvested from explanted hearts from heart transplant recipients and were decellularized using a detergent-based cell extraction method. Analysis of decellularization success was performed with light microscopy, transmission electron microscopy and quantitative analysis of collagen and elastin content. The decellularization method resulted in full removal of native cells while the mechanical stability and the quantitative composition of the neoscaffolds was maintained. The luminal surface of the human matrix could be successfully recellularized with in vitro expanded HUVECs under dynamic flow conditions. The surface appeared as a confluent cell monolayer of positively labeled cells for von Willebrand factor and CD 31, indicating their endothelial nature.

Conclusions: Human heart valves can be decellularized by the described method. Recellularization of the human matrix resulted in the formation of a confluent HUVEC monolayer. The in vitro construction of tissue-engineered heart valves based on decellularized human matrices followed by endothelialization using HUVECs is a feasible and safe method, leading to the development of future clinical strategies in the treatment of heart valve disease. (*Circ J* 2013; **77**: 207–216)

Key Words: Decellularization; Extracellular matrix; Heart valves; Tissue engineering

Hearth valve disease is a considerable medical problem worldwide. The current treatment methods for heart valve disease include the application of mechanical and tissue valves. Nonetheless, the present substitute heart valves suffer important drawbacks. The practical disadvantages of mechanical heart valves include life-long anticoagulation therapy with the risk of anticoagulant-related hemorrhage, increased risk of infections, and thromboembolism caused by their non-physiological surface and flow abnormalities.^{1,2} Being non-viable constructs, all clinically accessible replacement heart valves, apart from the pulmonary autograft, lack the capability to grow, repair, or remodel.³ Glutaraldehyde-fixed biological

valves, and particularly cryopreserved human allografts, do not need anticoagulation treatment but suffer from progressive tissue deterioration, including early calcification and generally poor longevity because of the preserved viability of the cells with immunogenic rejection potential.⁴⁻⁶ Despite innovative developments to improve the biological valve prostheses durability, long-term results remain unsatisfactory.⁷ To date, none of the currently available heart valve prostheses provide long-term stability, ideal hemodynamic performance, and remodeling capability.

Encouraging alternatives to the current heart valve prostheses are being investigated in the area of cardiac tissue engi-

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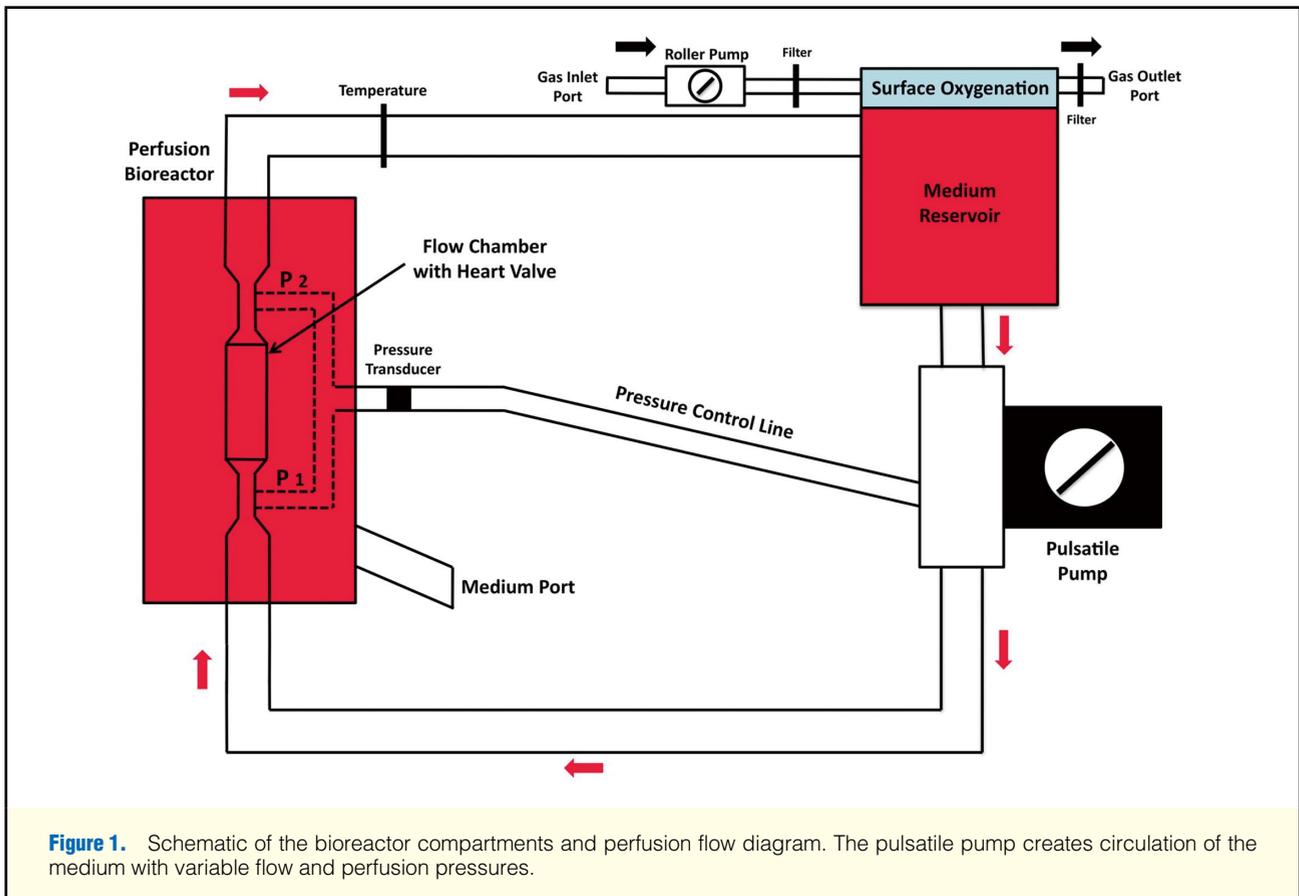
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neering (TE). Despite various scientists reporting promising results, TE of human heart valves is still at the beginning of its evolution.^{3,5,8–10} The best possible scaffold architecture and advanced cell technologies are needed to develop a TE product that will possibly become an applicable valve prosthesis. TE may help to construct viable valve substitutes that feature lifetime durability and the opportunity to use in vitro fabrication of living tissue replacements while holding potential for regeneration.¹¹ Various models featuring polymer biodegradable, biological allogeneic, and xenogeneic decellularized scaffolds have been already developed.^{5,12–15} Our group already reported the successful in vivo application of TE pulmonary heart valves based on decellularized xenograft matrices^{3,8} and generation of 3-dimensional myocardial neoscaffolds by decellularization of whole hearts.⁹

In this paper, we will present the in vitro TE of human heart valve neoscaffolds using human umbilical cord endothelial cells (HUVECs) under simulated physiological conditions. Moreover, the influence of the decellularization protocol on the biochemical composition and ultrastructure of the human scaffolds is characterized to assess the surface properties for consecutive seeding with HUVECs.

Methods

All patients gave written informed consent, allowing the study to be approved by the institutional review board abiding to a protocol for human tissue use (Transplant Registry).

Fresh Human Heart Valves

Human pulmonary (n=9) and aortic valves (n=6) were freshly harvested from explanted hearts of patients suffering from ischemic cardiomyopathy, but having no history of heart valve disease, undergoing heart transplantation (age: 52.3±12.5 years; 6 males/3 females). Additionally, transesophageal ultrasound examination of every heart transplant recipient was done to rule out any pathology of the pulmonary or aortic valve. The heart valves were dissected from the heart under sterile conditions, leaving only a short subvalvular myocardial cuff. After the separation of adhesive tissue, all heart valves were examined macroscopically to exclude any other pathology not detected by echocardiography. Three aortic valves were excluded because of calcification of the commissures. Next, the heart valves were rinsed with phosphate-buffered saline (PBS) at 4°C and embedded in 1% penicillin-streptomycin (PAA Laboratories, Cölbe, Germany) augmented Earle's Medium 199 (PAA Laboratories).

Decellularization Procedure

Human heart valves were decellularized through continuous shaking in 1% sodium dodecyl sulfate (SDS) and 0.05% sodium azide (NaN₃) in PBS (PAA Laboratories) at room temperature for 48 h. The solution was exchanged every 6 h. At the end of the decellularization protocol, the heart valves were washed with PBS for 12 h to remove residual detergents and cell debris. Before seeding, the valves were incubated in culture medium composed of Endothelial Cell Growth Medium (PromoCell, Heidelberg, Germany), 10% fetal bovine serum (FBS, Life Technologies, Darmstadt, Germany) and 1% peni-

cillin-streptomycin, and maintained in a 95% O₂/5% CO₂-incubator at 37°C for 24 h to facilitate cell attachment by deposition of proteins.

Isolation of HUVECs

After obtaining written informed consent, fresh human umbilical cords were obtained from 12 healthy females and stored in PBS at 4°C in the delivery room. The isolation procedure started within 2 h of delivery. The vein was cannulated on both sides with 3-way cocks, then perfused with PBS to wash out blood cells and coagulum. Next, the vein was filled with 20 ml of 0.1% collagenase-dispase (Boehringer Mannheim, Germany) in Hanks' balanced salt solution (Gibco, Grand Island, NY, USA). Following 20 min of incubation at 37°C, the cell effluent was collected by flushing the vessel with 20 ml of Medium 131 (Gibco) supplemented with 10% fetal calf serum (FCS; Gibco) to inhibit further enzymatic activity. After 10 min of centrifugation (12,000 rpm), the pelleted cells were cultured in 25-cm² flasks at 37°C with 5% CO₂. The HUVECs were passaged when 80–90% confluent and were used between passages 3 and 6. The expected endothelial phenotype of the cultured cells was analyzed microscopically (cobblestone appearance typical of endothelial cells) and immunohistochemically for expression of von Willebrand factor (vWF).

DNA Quantification

DNA was isolated from 200-mg freeze-dried, decellularized valve tissue and processed for spectrophotometric quantification to determine the concentration of residual DNA in the decellularized group in comparison with a control group. The total amount of DNA was purified using a silica-membrane-based method, following the manufacturer's instructions, and later quantified by spectrophotometry (QIAamp DNA Mini Kit, Qiagen, Basel, Switzerland).

Perfusion Bioreactor

The cell seeding experiments were performed using a custom-made dynamic bioreactor system, kept in a humidified incubator at 37°C, allowing pulsatile circulation. The bioreactor system consisted of a valve inlet, a pulsatile pump (Stöckert, Freiburg, Germany), and a connected medium reservoir (Figure 1), which was manufactured to be placed in a 37°C conventional incubator (HERAcCell 240, Thermo Scientific, Bremen, Germany). The valve inlet allowed for different sized valves to be used by suturing the valves in the support material (CarboMedics Carbo-Seal Valsalva, Austin, TX, USA). The entire closed loop flow system, which circulates a medium volume of 1.5 L, was constructed from polycarbonate and is completely transparent to allow direct observation. Silicon tubing was used to connect all parts. The decellularized heart valve was perfused continuously with medium from the reservoir using a pulsatile pump (Stöckert) under humidified incubator conditions (37°C, 5% CO₂).

The pressure was controlled constantly using a pressure transducer (Medex Smith Medical) and monitored by a computer system guaranteeing optimal fluid flow and a continuous perfusion pressure report. Fresh gas (average 95% air, 5% CO₂) was transported via a roller pump into the medium reservoir (Duran, Wertheim, Germany). The gas exchange occurred by constant medium surface oxygenation inside the medium reservoir. Sterile filters (Sterifix 0.2 μm; B. Braun Melsungen AG, Germany) were interposed at the top of the medium reservoir to avoid contamination during oxygenation of the circulating medium. The culture medium was replaced daily to provide fresh cell nutrients and to eliminate decomposition products

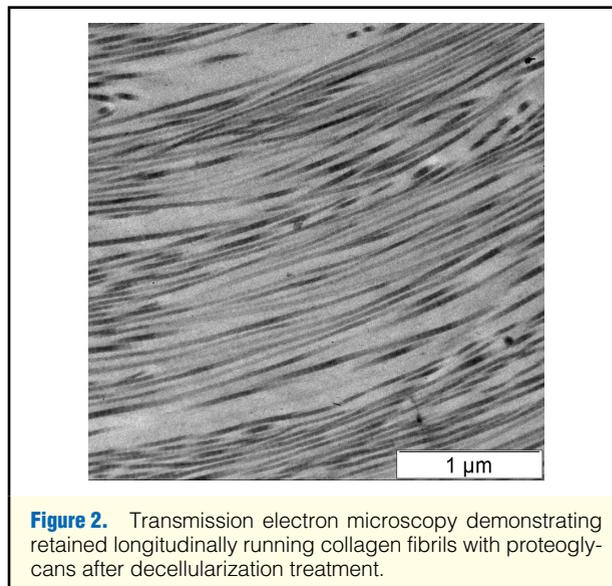


Figure 2. Transmission electron microscopy demonstrating retained longitudinally running collagen fibrils with proteoglycans after decellularization treatment.

from the evolving valve tissue. The culture medium also underwent blood-gas analysis (Rapidlab 860, Siemens, Mannheim, Germany) twice daily. The pH level in the circulating culture medium was adjusted by flexible variation of the CO₂ supply.

Fabrication of Ne endothelium Under Dynamic Tissue Cultivation Conditions

Decellularized human heart valves were placed in cell culture bottles containing a suspension of HUVECs and 50 ml of culture medium supplemented with L-ascorbic acid 2-phosphate (0.25 mg/ml; Sigma-Aldrich, Steinheim, Germany), to promote extracellular matrix (ECM) production. The medium used for cultivation and consecutive tissue fabrication included 1% gentamicin. Subsequently, the leaflets were seeded with a density of 5–6 × 10⁶ cells (HUVEC passage 3–6)/cm². The bottles were gassed with 5% CO₂/air for 20 s and exposed to rotation at 5 rpm for 24 h in an atmosphere of 5% CO₂ at 37°C, exposing the entire valve surface to achieve optimal attachment conditions. Afterwards, the heart valve neoscaffolds were mounted in the bioreactor chamber and perfusion was started at 15 ml/min. The medium circulation through the bioreactor complex was sustained for 4 weeks to a maximum flow of 3.5 L/min.

Analysis of Heart Valve Neoscaffolds

After in vitro endothelialization, characteristic samples of all valve leaflets were investigated by the following methods.

Histology and Immunohistochemistry Heart valve leaflets were dissected in the central cusp area through the adjoining arterial wall and fixed in 10% phosphate-buffered formalin and imbedded in paraffin. Sections (5-μm) were stained with hematoxylin-eosin (HE) to determine if residual nuclear structures could be identified after decellularization. Masson's trichrome stain was used to distinguish the cells from the surrounding connective tissue (Sigma-Aldrich), and the Movat's pentachrome stain (Mastertechs, Lodi, CA, USA) was used to visualize different ECM components such as collagen, elastin, and proteoglycans. All 3 cusps from each heart valve were evaluated.

Frozen tissue specimens were examined for ECM integrity: collagen I (clone Coll-1, Sigma-Aldrich), collagen IV (clone

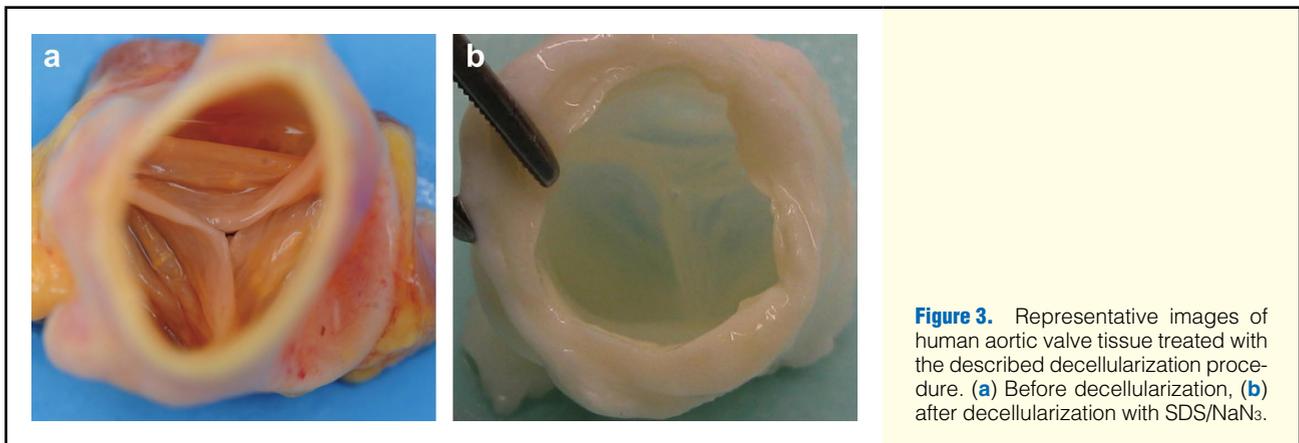


Figure 3. Representative images of human aortic valve tissue treated with the described decellularization procedure. (a) Before decellularization, (b) after decellularization with SDS/NaN₃.

CI22; Dako, Hamburg, Germany); evidence of endothelial cells: vWF (polyclonal rabbit IgG; Dako), and CD 31 (Polyclonal Rabbit IgG, Santa Cruz Biotechnology, Heidelberg, Germany). The fluorescence method of immunohistochemistry used has been described elsewhere.¹³ Based on the intensity and arrangement of labeling, histological evaluations of all stained samples were implemented in a blinded mode. The degree of re-endothelialization and preservation of ECM was evaluated by 2 independent pathologists. Tissue sections were analyzed using routine bright-field microscopy and fluorescence microscopy (Olympus Optical Co, BX 51 and CKX 41 microscopes). Images were acquired with the Cella Soft Imaging System (Olympus Soft Imaging Solutions®, Germany).

Transmission Electron Microscopy (TEM)

Tissue specimens (1 mm³) were fixed in 2.5% glutaraldehyde and embedded in Epon. Ultrathin sections were prepared for microscopy according to standard procedure. Electron microscopy was performed using a Zeiss analytical EM 902 (Zeiss, Oberkochen, Germany) coupled with a Pro-Scan digital camera (Tröndle, Munich, Germany).

Quantification of Collagen and Elastin Content

To quantify collagen and elastin, Biocolor assays (Biocolor, Carrickfergus, UK) were performed in decellularized heart valve samples and compared with native human heart valve tissue. Collagen was extracted with 0.5 mol/L acetic acid (Sigma-Aldrich) and 1:50,000 protease inhibitor cocktail at 4°C. Elastin was extracted with 100°C 0.25 mol/L oxalic acid (Sigma-Aldrich). Samples and calibrators were treated with the respective dyes and later quantified by spectrophotometry.

Measurement of Mechanical Stability

Mechanical stability of heart valve tissue was analyzed using a static material testing instrument (Zwick Roell, Ulm, Germany). Decellularized heart valve samples were stretched until complete tearing in the longitudinal and circumferential directions. Passive tensile strength was constantly registered during the displacement. As a control, samples from untreated native heart valves were used.

Statistical Analysis

Quantitative analysis and mechanical stability data are expressed as mean ± standard error of the mean (SEM). Student's *t*-test was used to determine significant differences between the untreated native heart valve and decellularized neoscaffold groups

in terms of mechanical stability. A value of $P < 0.05$ was considered statistically significant.

Results

Human heart valves were decellularized by the detergent treatment for 48 h, and consecutively seeded with HUVECs. Under bioreactor conditions, a confluent monolayer of endothelial cells formed on the decellularized human heart valves.

Analysis of Valve Morphology, Histology and Decellularization Treatment

Decellularization of human heart valves according to the protocol described here resulted in complete removal of cellular components, as shown by standard histology, immunohistochemistry, and TEM. Histological examination of aortic and pulmonary heart valves did not show any significant differences after decellularization. However, during the decellularization process, the heart valves became translucent, consistently losing their natural color. TEM examination demonstrated large amounts of collagen-fiber bundle networks lacking intracellular components (Figure 2). The initial structural configuration with intact leaflet and vascular wall morphology was maintained. The endothelial cell layer was completely removed. The decellularized cusps appeared smooth-walled, comparable to native heart valve leaflets (Figure 3).

Residual total DNA content of the neoscaffolds was notably decreased in comparison with the control group. After decellularization, the DNA content was reduced to 21.8 ng DNA/mg tissue vs. 450.7 ng DNA/mg tissue in the control group, indicating a 95.2% reduction in DNA content.

Morphological and histological analyses revealed an optically preserved 3-dimensional neoscaffold composition (HE stain) with complete maintenance of the basement membrane as depicted through collagen IV staining. Further examination using Masson's trichrome, Movat's pentachrome and collagen I stain visualized different ECM components such as mesh-like collagen formations, elastin and proteoglycans (Figures 4,5).

In conclusion, cell nuclei and other intracellular components were successfully extracted from native heart valve tissue, while preserving the typical ECM composition on both the histological and ultrastructural level.

Isolation and Characterization of HUVECs

HUVECs were successfully isolated from human umbilical cord tissue. As indicated through positive immunostaining with anti-

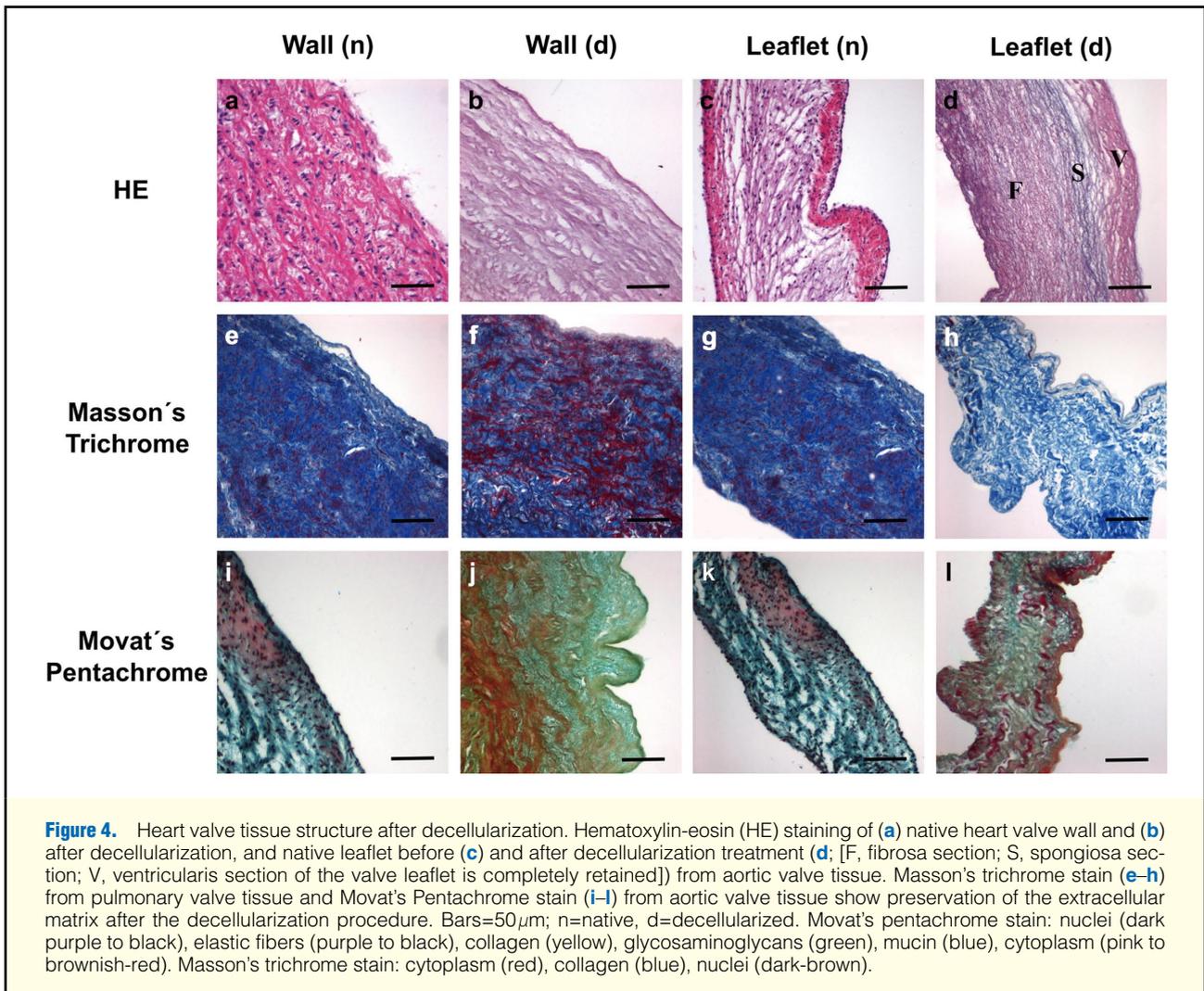


Figure 4. Heart valve tissue structure after decellularization. Hematoxylin-eosin (HE) staining of (a) native heart valve wall and (b) after decellularization, and native leaflet before (c) and after decellularization treatment (d; [F, fibrosa section; S, spongiosa section; V, ventricularis section of the valve leaflet is completely retained]) from aortic valve tissue. Masson's trichrome stain (e–h) from pulmonary valve tissue and Movat's Pentachrome stain (i–l) from aortic valve tissue show preservation of the extracellular matrix after the decellularization procedure. Bars=50 μ m; n=ative, d=decellularized. Movat's pentachrome stain: nuclei (dark purple to black), elastic fibers (purple to black), collagen (yellow), glycosaminoglycans (green), mucin (blue), cytoplasm (pink to brownish-red). Masson's trichrome stain: cytoplasm (red), collagen (blue), nuclei (dark-brown).

vWF factor, they retained their endothelial character in successive passages. At passages 3–6, chosen for all of our experiments based on cell numbers and appropriate cell characteristics, the cells were characterized. They showed good in-vitro proliferation and formed confluent, cobblestone-like monolayers after 3 weeks (Figure 6).

Perfusion Bioreactor System

During our experiments, the perfusion bioreactor worked exceptionally well with constant flow. No leakages from the various bioreactor compartments were observed. All experiments were performed in the bioreactor system without any infection and under normal aerobic cell metabolic conditions. Gas concentration analysis of the circulating medium showed physiological conditions of pO₂, pCO₂, and pH.

Analysis of Tissue-Engineered Heart Valve Neoscaffolds

Both aortic and pulmonary heart valve neoscaffolds demonstrated cell attachment on both sides of the leaflet surface following 3 weeks in dynamic culture conditions. Moreover, after reendothelialization the luminal surfaces of the aortic and pulmonary heart valve neoscaffolds were similarly covered. Immunohistological analysis revealed that the cells on the scaffold expressed vWF and were CD31-positive, indicating a de

novo endothelial layer (Figure 7). Under dynamic, pulsatile flow at a rate of 3.5 L/min, the endothelial cells converged and formed a complete monolayer. The original endothelial cells of the heart valve leaflets were replaced by HUVECs and they had spread and divided on the neoscaffold surface. The HUVECs fully coated the leaflet surface and were oriented toward the flow direction. The leaflet coating consisted of typical cobblestone-like endothelial cells covering the basal lamina of both sides of the cusp, including the pulmonary and aortic wall. The leaflets appeared intact and flexible without fenestrations or adhesions and presented a pale, shiny surface, comparable to native tissue samples. There was no cusp thickening or reduction of cusp mobility upon gross inspection, and the heart valve neoscaffolds demonstrated acceptable resistance and stability, comparable to that of native tissue, posing no hindrance to suture application.

Analysis of Collagen and Elastin Content

Collagen and elastin content of decellularized heart valve samples was compared with that of untreated native heart valve tissue, which was taken as 100% (Figure S1). Quantification of the collagen and elastin content was 87.70 \pm 2.86% and 85.15 \pm 2.81%, respectively, compared with native heart valve tissue.

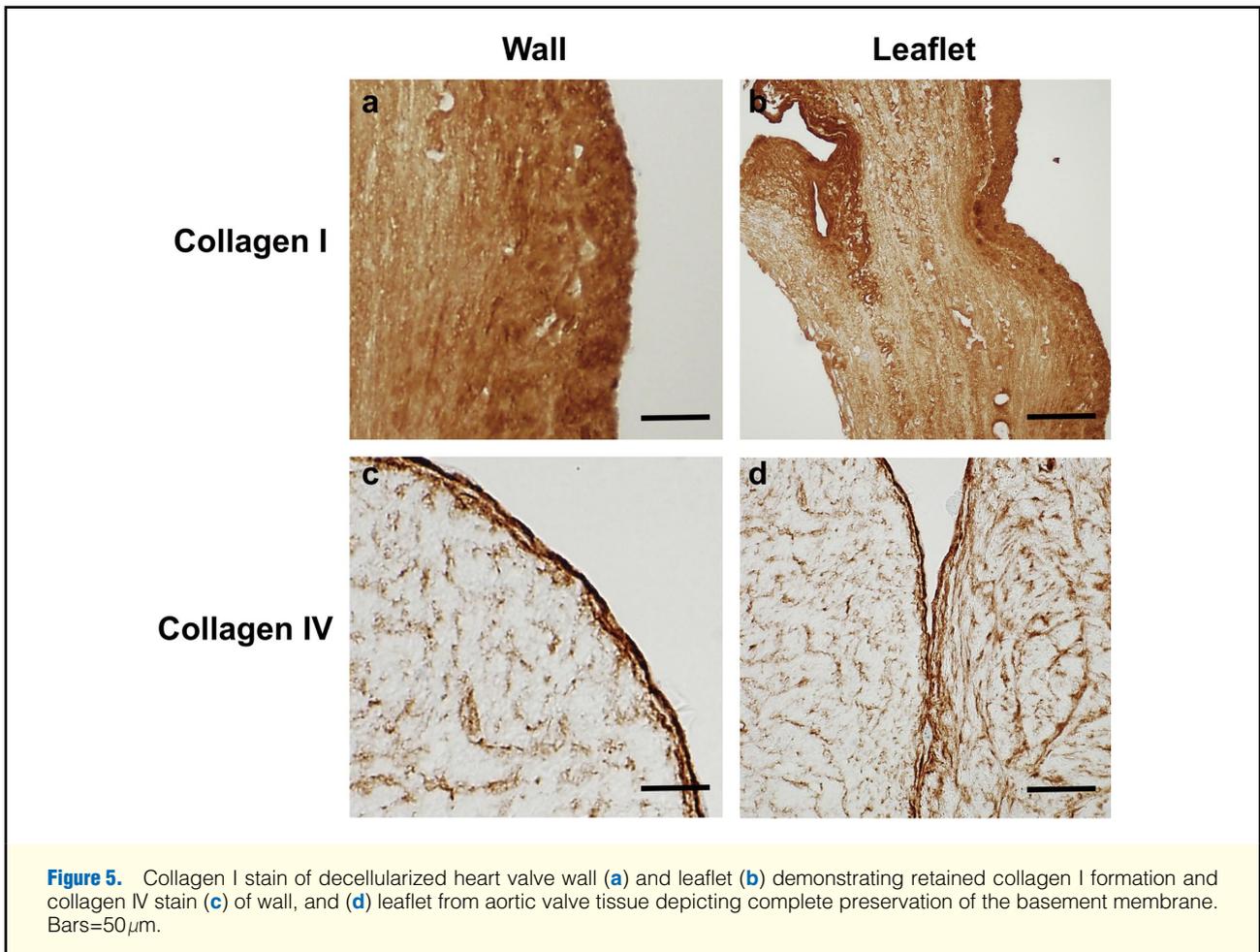


Figure 5. Collagen I stain of decellularized heart valve wall (a) and leaflet (b) demonstrating retained collagen I formation and collagen IV stain (c) of wall, and (d) leaflet from aortic valve tissue depicting complete preservation of the basement membrane. Bars=50 μ m.

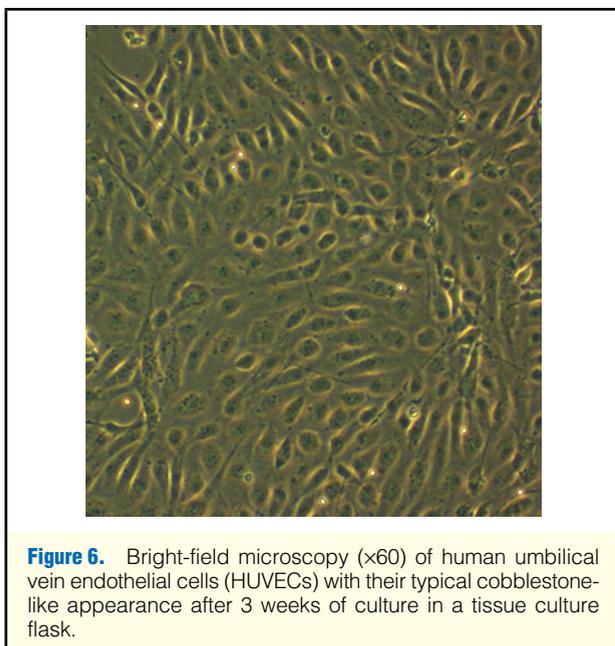


Figure 6. Bright-field microscopy ($\times 60$) of human umbilical vein endothelial cells (HUVECs) with their typical cobblestone-like appearance after 3 weeks of culture in a tissue culture flask.

Analysis of Mechanical Stability

Figure 8 demonstrates the stress-strain curves of the decellularized heart valve neoscaffolds. Using the decellularization protocol described here, the decellularized heart valve neoscaffolds showed similar mechanical stability as untreated native heart valve tissue for longitudinal (a) and circumferential (b) stretch ($P > 0.05$).

Discussion

TE through endothelial cell transfer onto specially designed neoscaffolds is a promising experimental approach to diminishing the drawbacks of allografts, non-living xenografts, and prosthetic heart valves. Our group has already reported on clinical experience with in-vivo-developed TE pulmonary heart valves, based on decellularized xenograft matrices^{3,8} and the creation of decellularized hearts as potential neoscaffolds for whole heart TE.⁹

The present investigation was based on total decellularization of human heart valve scaffolds, dissected from explanted hearts of heart transplant recipients. Repopulation under bio-reactor conditions was accomplished through in-vitro reseeding with HUVECs, which in turn formed a functional endothelial cell layer on the surface of the decellularized heart valve neoscaffolds.

It has been shown that decellularized heart valve neoscaffolds implanted in a sheep model were subject to calcification

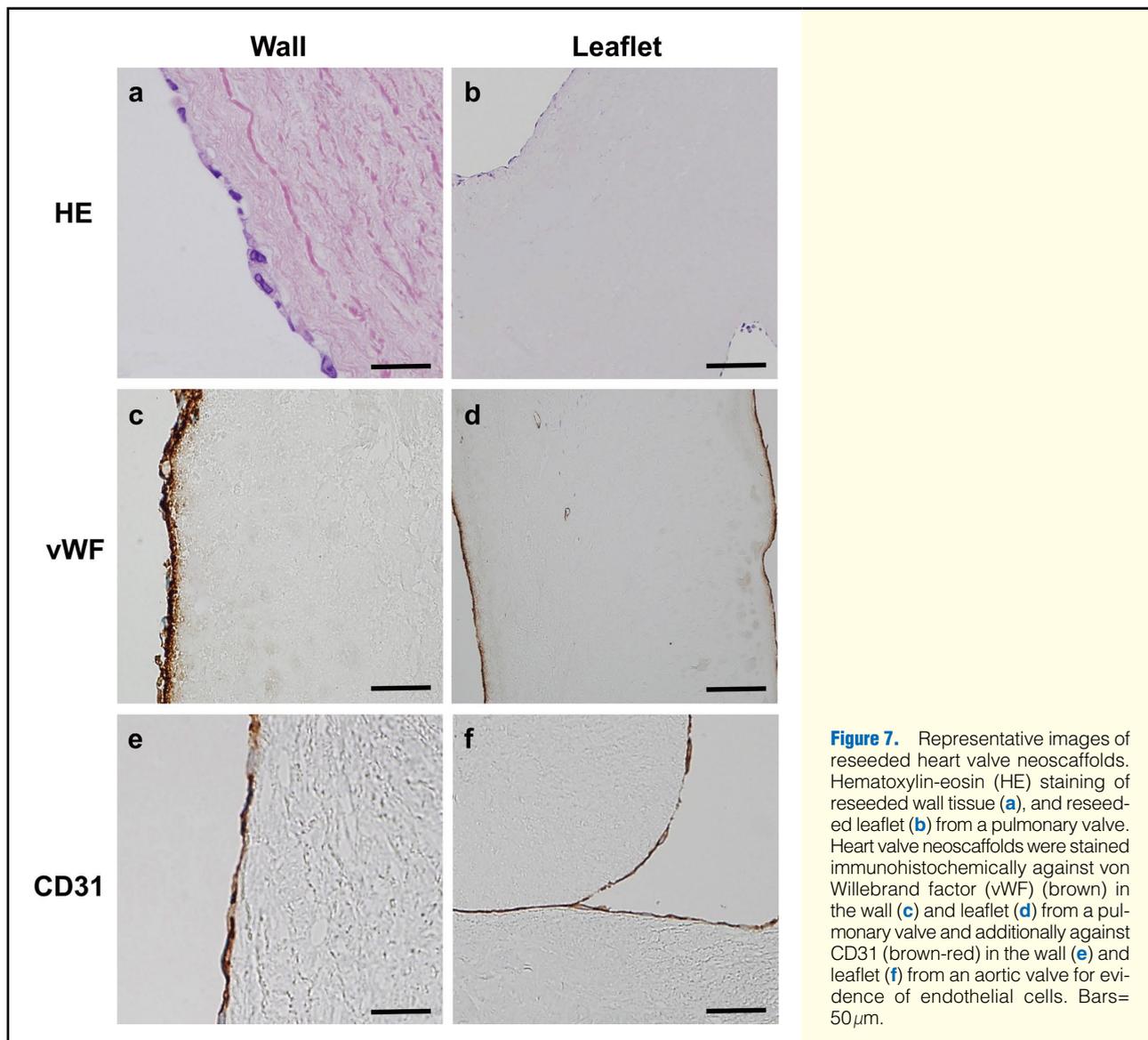


Figure 7. Representative images of reseeded heart valve neoscaffolds. Hematoxylin-eosin (HE) staining of reseeded wall tissue (a), and reseeded leaflet (b) from a pulmonary valve. Heart valve neoscaffolds were stained immunohistochemically against von Willebrand factor (vWF) (brown) in the wall (c) and leaflet (d) from a pulmonary valve and additionally against CD31 (brown-red) in the wall (e) and leaflet (f) from an aortic valve for evidence of endothelial cells. Bars= 50 μ m.

and endocarditis without prior reendothelialization.¹⁵ Hence, decellularized heart valve neoscaffolds should be endothelialized before further manipulation to prevent the risks of calcification and infection. We exclusively used an allograft ECM because it would not introduce cross-species immunological conflict. For example, repopulation with recipient cells could convert the allograft tissue to autologous tissue having a syngeneic origin, and the potential ability to remodel.

Scaffold Source

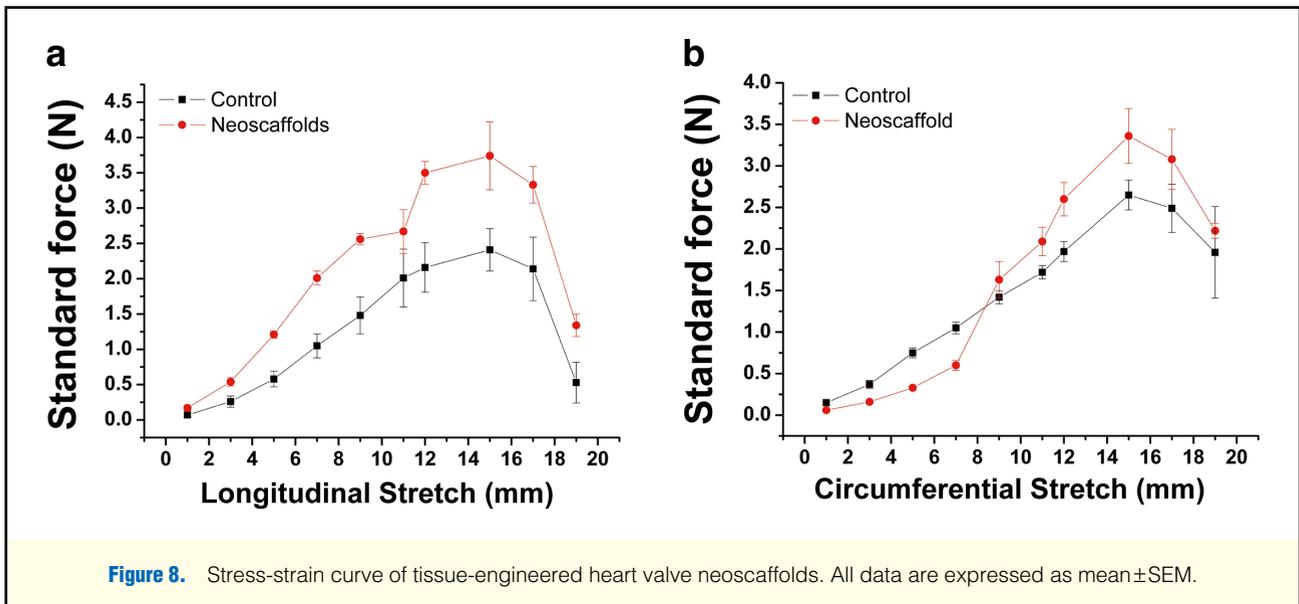
In this study, we used decellularized human heart valve neoscaffolds as biologically active structures because they not only provide a 3-dimensional (3D) supporting matrix, but also important ECM proteins such as collagen, elastin, and proteoglycans, parts missing in synthetic polymer materials.⁹ We are convinced that the key to successful TE lies in the preservation of the anatomical structure. Our human scaffold provides such a structure, as well as a higher immune histocompatibility compared with synthetic materials.

In contrast is the application of biodegradable polymers with

limited mechanical properties and variable degradation times for heart valve TE. The advantages of these synthetic-based scaffolds are mainly their biocompatibility, availability and ability to be reproduced with predictable chemistry,^{16,17} but they do not approximate the composition of a natural matrix. On the other hand, synthetic-based scaffolds are expensive and potentially immunogenic, and additionally, suffer from toxic degradation and inflammatory reaction.⁹ Usually, there are 2 major drawbacks: (1) synthetic scaffold sources cannot express the important bioactive molecules and ligands that are necessary for neoscaffold maturation and (2) effective diffusion of vital cell nutrients is aggravated.¹⁷

Furthermore, synthetic polymer scaffolds are rigid, thrombogenic, and induce an overshooting fibrosis that can induce leaflet retraction with insufficiency following implantation.^{18–20} Preliminary studies have proven that biodegradable polyglactin-PGA copolymer matrices used to produce vascular scaffolds are unstable and cause aneurysm formation.²¹

There have also been reported efforts from other researchers of the use of synthetic scaffold components in heart valve



TE,^{22–24} but none of these concepts has achieved the criterion of safe human implantation.

Decellularization Treatment

For the first time, a mono-regime SDS/NaN₃ decellularization protocol has been successfully used for human heart valve scaffold cell elimination. The ionic detergent, SDS, and the ionic solid, NaN₃, have already demonstrated excellent cell removal properties with preservation of major structural ECM molecules.^{9,25,26} In our investigation, decellularization with SDS/NaN₃ resulted in a biological neoscaffold free of any visible cellular material with maintenance of the heart valve architecture and geospatial arrangement of its matrix. The 3D fibrous network of lengthwise folded collagen fibers and bundles inside the cusps, as well as other ECM components, were well-conserved and properly arranged, suggesting that the tissue's matrix structures were not significantly compromised. In both aortic and pulmonary heart valves the normal structure was optimally preserved. Furthermore, the applied decellularization protocol was able to conserve cusp coaptation and macroscopic valve geometry. In the present study, both the collagen and elastin content and the mechanical properties of the decellularized heart valve neoscaffolds demonstrated similar characteristics to untreated controls. Taken together, these findings are favorable for continued recellularization of the neoscaffold once implanted *in vivo*.

Other investigators have used enzymatic decellularization treatments for heart valve TE and demonstrated scaffold injury with basal membrane damage.^{25,27} Other decellularization protocols include the use of glutaraldehyde, which leads to structural deterioration *in vivo*. It is imperative to avoid a multimodal aggressive decellularization treatment.²⁸

In our study, we did not achieve complete reduction of nuclear material. However, we observed the removal of virtually all DNA material from the decellularized heart valves, based on histological staining and TEM results. The impossibility that any decellularization method will remove 100% of all cell components is well known.⁹ There has yet to be a study proving a direct correlation between these nuclear fragments and immunological rejection.²⁸

The final biologic neoscaffolds had the majority of their cellular content removed, potentially enabling safe implantation.

Cell Source

In this evaluation, endothelial cells were successfully isolated from human umbilical cord tissue through enzymatic digestion. As the cells were transplanted onto the luminal surface of the decellularized human neoscaffolds, they formed a monolayer of neoendothelium on the valve leaflets, as has already been shown in animal models.²⁹ In the present study, we showed that after reendothelialization the luminal surface of human aortic and pulmonary heart valve neoscaffolds was identically covered with a solid layer of HUVECs. The formation of a confluent endothelial cell layer is essential in the development of a TE heart valve, because it provides an antithrombotic surface and physiological continuous remodeling.²¹

Endothelial cells are key players in the regulation of important processes such as inflammation, angiogenesis, immune reaction, and transport of molecules. Moreover, they are able to produce various genes through different signaling pathways and contain receptors, which are important for cell metabolism and homeostasis.¹⁶

Additional research has shown that a population of mesenchymal stem cell-like cells exist in human umbilical cord tissue and cord blood.^{30,31} Umbilical cords are readily available and contain different juvenile cell types for regeneration therapy. The possibility of cryopreserving a patient's own umbilical cord tissue after birth would allow for the preparation of TE heart valve grafts for future autologous use. Available allografts could be incubated with appropriate cells from the cryopreserved umbilical cord until maturity to become living and functional scaffolds and later be implanted as an autologous tissue heart valve. Furthermore, degeneration of these autologous heart valves based on immunological reactions may be reduced.

Conceivable future studies using progenitor cells from umbilical cord tissue could become a new approach to cardiac valve TE. This advance would overcome the lack of living autologous replacements with regeneration potential.

Perfusion Bioreactor

In the present investigation, we developed a cell culture bioreactor that provided homogeneous cell allocation on a heart valve scaffold under physiological flow conditions, which enabled the fabrication of viable and surgically feasible vascular tissue.

The bioreactor system is so compact that it can fit in a standard humidified incubator, which minimizes the contamination threat that comes with larger constructions. Because of the reactor's transparent materials, the vascular scaffold can be continuously observed, allowing for defects and contaminations to be detected. Furthermore, the reactor can be easily modified using a sample-mounting adapter to take other cardiovascular constructs; for example, to colonize vascular grafts with different cells prior to implantation.

It is common knowledge that cardiovascular cells such as endothelial cells are influenced by local fluid dynamics. Mechanical shear stress is induced through the modification of the bioreactor flow, circulating volume, and pressure. This influences the growth, orientation, and phenotypic remodeling of the endothelial cells.^{32–35} It has been shown that a rapid increase in bioreactor flow causes significant damage of the reseeded endothelium and complete cell wash-off from cusps.¹² This is especially common near the valve leaflet, where turbulent flow is present.

To avoid cell detachment, we slowly adjusted the endothelial cells to physiological flow levels using incremental increases in the flow rate. This strategy allowed endothelial cells to attach and fully align to the valve scaffold. Nevertheless, the optimal flow conditions for cell adhesion, differentiation, and proliferation are still unknown and may vary between various bioreactor constructions.

Study Limitations

Whether or not the human heart valve neoscaffolds, produced using the methodology presented here, will provide the microenvironment needed for further cellular differentiation, has to be evaluated in future studies. This implicates the invasion of valvular interstitial cells into the heart valve scaffold, either in vivo after implantation or in vitro by means of cultivated cells.

Conclusion

To our knowledge, this is the first report of the generation of a TE human heart valve neoscaffold with preserved ECM composition containing an in vitro reseeded HUVEC neoendothelium as a peripartial cell source.

The most important results of the presented work are: (1) fabrication of TE human heart valve neoscaffolds reseeded with HUVECs is feasible; (2) there is evidence that the architecture of the ECM is retained after treatment with SDS/NaN₃ and is comparable to native tissue, based on our light- and electron-microscopic, quantitative and mechanical analyses; and (3) HUVECs can be easily extracted and preserved, which allows for individuals to store their own samples for possible future autologous use. Although the results shown here are limited, this investigation demonstrates the ability of our bioreactor system to develop the optimal in-vitro environment for TE heart valve neoscaffolds. The bioreactor enables consistent and uniform delivery of cells, nutrients, gases, and hydrodynamic shear forces.

The presented approach could be used to transform human allografts into autologous tissue heart valves. Our work has the potential for an important clinical contribution, which is a strong argument for evaluating our approach experimentally in addi-

tional studies. A preclinical animal study is currently being conducted to demonstrate the viability of endothelialized valve scaffolds in vivo. Taken together, these studies serve as proof of concept to generate bioengineered, recellularized human heart valve neoscaffolds as a potential matrix for further efforts in heart valve regeneration therapy.

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Disclosures

None.

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Supplementary Files

Supplementary File 1

Figure S1. Quantitative analysis of collagen and elastin content of tissue-engineered heart valve neoscaffolds.

Please find supplementary file(s);
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