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Off-the-shelf human decellularized tissue-engineered heart valves in a non-human primate model

Benedikt Weber [a,b,c,1], Petra E. Dijkman [d,1], Jacques Scherman [c,e,f,1], Bart Sanders [d], Maximilian Y. Emmert [a,b,c], Jürg Grünenfelder [b,c], Renier Verbeek [e,f], Mona Bracher [f], Melanie Black [f], Thomas Franz [e,f,g], Jeroen Kortsmit [e,f], Peter Modregger [h,i], Silvia Peter [h,j], Marco Stampanoni [h,j], Jérôme Robert [a,b], Debora Kehl [a,b], Marina van Doeselaar [d], Martin Schweiger [a,b,c], Chad E. Brokopp [a,b], Thomas Wälchli [a,b,k], Volkmar Falk [b,c], Peter Zilla [e,f], Anita Driessen-Mol [d], Frank P.T. Baaijens [d], Simon P. Hoerstrup [a,b,c,d,*]

[a] Swiss Center of Regenerative Medicine, University of Zürich, Moussonstrasse 13, CH-8091 Zürich, Switzerland
[b] Division of Surgical Research, University Hospital of Zurich, Raemistrasse 100, CH-8091 Zürich, Switzerland
[c] Clinic for Cardiovascular Surgery, University Hospital of Zurich, Moussonstrasse 13, CH-8091 Zürich, Switzerland
[d] Department of Biomedical Engineering, Soft Tissue Biomechanics and Tissue Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands
[e] Chris Barnard Division of Cardiothoracic Surgery, Groote Schuur Hospital, University of Cape Town, South Africa
[f] Cardiovascular Research Unit, Cape Heart Centre, University of Cape Town, South Africa
[g] Programme for the Enhancement of Research Capacity, Research Office, University of Cape Town, Mowbray, South Africa
[h] Swiss Light Source, Paul Scherrer Institute, 5232 Villigen, Switzerland
[i] University of Lausanne, School of Biology and Medicine, Rue du Bugnon 21, CH-1015 Lausanne, Switzerland
[j] Institute for Biomedical Engineering, University and ETH Zurich, ETZ-F-85, Gloriastrasse 35, 8092 Zurich, Switzerland
[k] Department of Health Sciences and Technology, Swiss Federal Institute of Technology Zurich, CH-8057 Zurich, Switzerland

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ABSTRACT

Heart valve tissue engineering based on decellularized xenogenic or allogenic starter matrices has shown promising first clinical results. However, the availability of healthy homologous donor valves is limited and xenogenic materials are associated with infectious and immunologic risks. To address such limitations, biodegradable synthetic materials have been successfully used for the creation of living autologous tissue-engineered heart valves (TEHVs) in vitro. Since these classical tissue engineering technologies necessitate substantial infrastructure and logistics, we recently introduced decellularized TEHVs (dTEHVs), based on biodegradable synthetic materials and vascular-derived cells, and successfully created a potential off-the-shelf starter matrix for guided tissue regeneration. Here, we investigate the host repopulation capacity of such dTEHVs in a non-human primate model with up to 8 weeks follow-up. After minimally invasive delivery into the orthotopic pulmonary position, dTEHVs revealed mobile and thin leaflets after 8 weeks of follow-up. Furthermore, mild-moderate valvular insufficiency and relative leaflet shortening were detected. However, in comparison to the decellularized human native heart valve control – representing currently used homografts – dTEHVs showed remarkable rapid cellular repopulation. Given this substantial in situ remodeling capacity, these results suggest that human cell-derived bioengineered decellularized materials represent a promising and clinically relevant starter matrix for heart valve tissue engineering. These biomaterials may ultimately overcome the limitations of currently used valve replacements by providing homologous, non-immunogenic, off-the-shelf replacement constructs.

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1. Introduction

In spite of the major advances with regard to minimally invasive catheter-based approaches, valvular heart disease remains to be a significant global health problem with increasing morbidity and mortality in the developing world as well as industrialized countries

* Corresponding author. Swiss Center for Regenerative Medicine, University of Zurich, Moussonstrasse 13, 8091 CH-Zürich, Switzerland. Tel.: +41 44 255 3644; fax: +41 44 255 43.
E-mail address: simon_philipp.hoerstrup@usz.ch (S.P. Hoerstrup).
[1] These authors contributed equally to the paper.

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[1,2]. Although the currently available heart valve replacement procedures change the universal fatal outcomes of valvular heart disease, prosthesis-related complications are reported in up to 50% of prosthetic heart valve recipients within 12 years after the operation [3]. While mechanical, metal-based prostheses require lifelong anticoagulation therapy as they are inherently prone to thromboembolic complications, bioprosthetic substitutes undergo progressive calcification and structural deterioration [4,5]. In addition, none of the currently used non-viable valve substitutes hold the potential for regeneration and growth [6].

Striving for improvement of these suboptimal materials in clinical use, decellularized xenogenic or allogenic heart valves have been used as starter matrices for tissue-engineering of valve replacements with promising (pre-)clinical results [6–8]. However, xenogenic grafts are associated with the risk of immunogenic reactions as well as zoonotic disease transmission [9,10] and the availability of homografts is limited [11]. Therefore, biodegradable synthetic materials have been successfully used for the fabrication of fully autologous tissue-engineered heart valves (TEHVs) [12,13]. These living autologous heart valve substitutes with regeneration and growth potential could potentially overcome the limitations of the available heart valve prostheses in clinical use. In recent studies we demonstrated the principal feasibility of merging autologous cell-based heart valve tissue engineering procedures and minimally invasive delivery methods in the ovine [14–16] as well as the senescent non-human primate model [17], which extended the spectrum of tissue engineering to transcatheter heart valve replacement. However, although these initial experiences were promising and demonstrated first in vivo functionality of minimally invasive bioengineered heart valves, autologous cell-based living TEHVs are associated with substantial and almost prohibitive technological and logistical complexity, also involving regulatory approval of ex-vivo cell processing procedures [18].

Therefore, decellularized TEHVs based on biodegradable synthetic materials and homologous cells have been recently developed as potential 'off-the-shelf' alternatives [19]. These constructs combine major advantages of apparent technologies: i) they represent human cell-derived starter matrices for guided tissue regeneration and host cell repopulation and may harbor the advantages of (living) bioengineered constructs – such as regeneration and growth; ii) they would provide homologous (non-xenogenic) replacement structures lacking the risk of zoonotic disease transmission; and iii) they could be produced as 'off-the-shelf' constructs and may principally offer an unlimited supply of valvular replacement structures.

The present study investigates the implantation of human cell-derived decellularized tissue-engineered heart valves (dTEHVs) using minimally invasive implantation techniques in a senescent non-human primate model. Although most previous tissue engineering in vivo investigations have focused on the sheep preclinical animal model (as also requested by some regulatory authorities), recent studies revealed that the significance of the results obtained in the ovine model may be limited given the species-specific remodeling response different to a human-like cardiovascular environment. In contrast, recent experiments suggest that the non-human primate model displays a more appropriate and predictive preclinical model, in particular with regard to endogenous cell repopulation and (neo-)tissue formation, thereby representing an important step prior to the clinical translation of the tissue engineering technologies [17].

2. Materials and methods

2.1. TEHV scaffold fabrication

Trileaflet heart valve scaffolds ($n = 8$) were fabricated as previously described [16,19]. Briefly, scaffolds made from non-woven polyglycolic-acid meshes (PGA; thickness 1.0 mm; specific gravity 70 mg/cm^3; Cellon, Bereldange, Luxembourg) were integrated into radially self-expandable nitinol stents (length = 30.0 mm; OD = 20.0 mm; pfm-AG, Köln, Germany) by using single interrupted sutures (Polypropylene, Ethicon, USA) and coated with 1% poly-4-hydroxybutyrate (MW 1×10^6; P4HB; TEPHA Inc., Lexington, MA, USA) in tetrahydrofuran solution (THF; Sigma Aldrich, St. Louis, MO, USA). After solvent evaporation and vacuum drying overnight, the scaffolds were placed into a 70% EtOH (70% ethanol absolute (VWR international S.A.S. Fontenay-Sous-Bois, France) and 30% autoclaved ultrapure water) twice for 15 min to obtain sterility. Thereafter, scaffolds were incubated for 30 min at 37 °C in PBS (Sigma Aldrich Inc., USA) supplemented with 10% Penicillin-streptomycin (Lonza, Verviers Belgium) and 50 μg/ml Fungin (Invitrogen, San Diego, USA), followed by two washing cycles with PBS.

2.2. Isolation of human fibroblasts

Human vascular-derived cells were harvested by plating from the human vena saphena magna of a 77-year-old patient, according to the Dutch guidelines for secondary used materials. Cells were expanded up to the sixth passage using standard cell culture methods in a humidified atmosphere containing 5% CO_2 at 37 °C. Culture medium consisted of DMEM advanced (Gibco, Invitrogen, Carlsbad, USA), supplemented with 10% Fetal Bovine Serum (FBS; Gibco, Invitrogen), 1% GlutaMax (Gibco, Invitrogen), and 1% penicillin-streptomycin (Lonza, Verviers Belgium).

2.3. Phenotyping of human fibroblasts

Isolated cells were characterized using immunofluorescence staining. Primary antibodies used for characterization of cells were against α-smooth muscle actin (mM-anti-human, clone 1A4, DakoCytomation, Copenhagen, Denmark), desmin (mM-anti-human desmin, clone D33, DakoCytomation), vimentin (mM-anti-human vimentin, clone Vim 3B4, DakoCytomation), and CD45 (mM-anti-human CD45, clone HI30, Biolegend). Alexa 546 phalloidin (A22283, Invitrogen Corp., USA) and DAPI (AD8417, Sigma Co., USA) were used as control staining. Primary antibodies were detected with Cyanine-2 goat-anti-mouse (Jackson Immunoresearch Laboratories Inc., West Grove, PA).

For immunostainings cells were first washed with PBS and then fixed with 4% paraformaldehyde in PBS for 10 min. Following a second washing phase (PBS), they were blocked and permeabilized in 0.2% Triton X-100 (Sigma) in PBS for 10 min. After blocking with 5% goat serum and 1% bovine serum albumin in PBS for 30 min, primary antibodies were added and incubated for 1 h at room temperature. After three washing steps with PBS for 5 min, secondary antibodies were added and incubated for 45 min. The specimens were washed again three times in PBS for 5 min and mounted in Aqua-Poly/Mount™. Analysis of the stained sections and cells was carried out using an inverted fluorescence microscope equipped with a CCD camera (ZEISS Axiovert 40 CFL and ZEISS Axioplan II; Carl Zeiss AG, Oberkochen, Germany). Image processing was performed using the ZEISS AxioVision™ software (Carl Zeiss AG) and the Office Picture Manager (Microsoft Inc., USA).

2.4. Heart valve tissue engineering

Isolated human fibroblasts were expanded up to passage six and were seeded (1.5×10^6 cells/cm^2) onto stented heart valve scaffolds ($n = 8$) using fibrin as a cell carrier [20]. After seeding and static pre-incubation, the constructs were placed into a diastolic pulse duplicator system for culture in closed configuration [21]. The TE-medium (DMEM Advanced, supplemented with 0.1% lamb serum, 1% GlutaMax, 1% Penicillin-streptomycin, and Lascorbic acid 2-phosphate (0.25 mg/ml; Sigma–Aldrich)) was replaced every 2–3 days. The leaflets were exposed to dynamic strains by applying increasing transvalvular pressure differences that started from 3 mm Hg after 5 days and build up to 15 mm Hg in the 4 following days and kept as such until the fourth week.

2.5. Decellularization

After 4 weeks of dynamic conditioning the in vitro grown TEHVs ($n = 8$) were decellularized using the previously described decellularization protocol [19]. Briefly, the TEHVs were washed three times in PBS (Sigma Aldrich) and incubated overnight in PBS supplemented with 0.25% Triton X-100, 0.25% sodium deoxycholate (SD, Sigma–Aldrich), and 0.02% EDTA (Sigma Aldrich) at 37 °C followed by two washing cycles in PBS. Next, the TEHVs were treated with a nuclease digestion solution of 50-mM TRIS-HCI buffer (Tris (hydroxymethyl)-aminomethane (Merck) pH 8.0, supplemented with 100 U/mL Benzonase® (25 units/μl, Novagen, Madison WI USA) and 1 mmol/l of $MgCl_2$ (Merck)) at 37 °C to remove remaining nucleic remnants. After 5–8 h the nuclease digestion solution was replaced with a nuclease digestion solution supplemented with 80 U/mL Benzonase® and incubated overnight. Thereafter, the solution was replaced again by a 20 U/mL Benzonase® solution and incubated for 5–8 h. The valves were washed twice in PBS followed by a washing cycle with M-199 medium (Gibco, Invitrogen Inc.) for more than 24 h at 4 °C to remove cellular remnants. Subsequently, the valves were incubated for 30 min at 37 °C in PBS supplemented with 10% Penicillin-streptomycin and 50 μg/ml Fungin, sterilized in 70% EtOH for 15 min, and again washed twice in PBS. All steps were conducted under continuous shaking. Decellularized TEHVs (dTEHVs) were stored in fresh M-199

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