Biomaterials 125 (2017) 101–117

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

In situ heart valve tissue engineering using a bioresorbable elastomeric implant – From material design to 12 months follow-up in sheep

Jolanda Kluin [a, b], Hanna Talacua [a, b], Anthal I.P.M. Smits [c, d], Maximilian Y. Emmert [e, f, g], Marieke C.P. Brugmans [h], Emanuela S. Fioretta [e], Petra E. Dijkman [e], Serge H.M. Söntjens [i], Renée Duijvelshoff [c, d], Sylvia Dekker [c], Marloes W.J.T. Janssen-van den Broek [c], Valentina Lintas [e], Aryan Vink [j], Simon P. Hoerstrup [c, e, g], Henk M. Janssen [i], Patricia Y.W. Dankers [c, d], Frank P.T. Baaijens [c, d], Carlijn V.C. Bouten [c, d, *]

[a] Department of Cardiothoracic Surgery, Academic Medical Center, Amsterdam, The Netherlands
[b] Department of Cardiothoracic Surgery, University Medical Center, Utrecht, The Netherlands
[c] Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands
[d] Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, The Netherlands
[e] Institute for Regenerative Medicine (IREM), University of Zürich, Switzerland
[f] Heart Center Zürich, University Hospital Zürich, Switzerland
[g] Wyss Translational Center Zürich, ETH and University of Zürich, Switzerland
[h] Xeltis BV, Eindhoven, The Netherlands
[i] SyMO-Chem BV, Eindhoven, The Netherlands
[j] Department of Pathology, University Medical Center, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form
21 December 2016
Accepted 6 February 2017
Available online 8 February 2017

Keywords:
Cardiovascular tissue engineering
Endogenous regeneration
Supramolecular chemistry
Biodegradable polymers
Pulmonary valve replacement
Regenerative biomaterials

ABSTRACT

The creation of a living heart valve is a much-wanted alternative for current valve prostheses that suffer from limited durability and thromboembolic complications. Current strategies to create such valves, however, require the use of cells for *in vitro* culture, or decellularized human- or animal-derived donor tissue for *in situ* engineering. Here, we propose and demonstrate proof-of-concept of *in situ* heart valve tissue engineering using a synthetic approach, in which a cell-free, slow degrading elastomeric valvular implant is populated by endogenous cells to form new valvular tissue inside the heart. We designed a fibrous valvular scaffold, fabricated from a novel supramolecular elastomer, that enables endogenous cells to enter and produce matrix. Orthotopic implantations as pulmonary valve in sheep demonstrated sustained functionality up to 12 months, while the implant was gradually replaced by a layered collagen and elastic matrix in pace with cell-driven polymer resorption. Our results offer new perspectives for endogenous heart valve replacement starting from a readily-available synthetic graft that is compatible with surgical and transcatheter implantation procedures.

1. Introduction

Current heart valve prostheses have serious drawbacks, such as thromboembolic complications or calcification-induced limited durability [1–3]. Most importantly, current prosthetic valves, including cryopreserved donor valves, are non-living structures that do not adapt to functional demand changes, which inherently limits their durability in comparison to a viable valve replacement (i.e. a pulmonary homograft) [4]. As a result, pediatric patients in particular are faced with a lifelong risk of valve-related morbidity and up to 50% reduction in life expectancy [5]. The creation of living, tissue engineered heart valves that can last a lifetime is believed to overcome these limitations [6,7]. Classical heart valve tissue engineering (TE) in which cells are harvested, expanded *in vitro*, seeded on a rapidly-degrading scaffold and conditioned in a

* Corresponding author. Eindhoven University of Technology, Dept. of Biomedical Engineering, Groene Loper 15 (GEM-Z 4.117), P.O. Box 513, 5600 MB, Eindhoven, The Netherlands.
E-mail address: c.v.c.bouten@tue.nl (C.V.C. Bouten).

http://dx.doi.org/10.1016/j.biomaterials.2017.02.007

bioreactor for several weeks to ensure fast matrix production that can withstand hemodynamic forces, has been explored for over 20 years [8–13]. Yet, translation to the clinic has proven difficult. This is mainly due to the complexity of the procedure and suboptimal long-term *in vivo* performance – the prevalent issue being valve leaflet retraction by the seeded cells [11–13]. To reduce these drawbacks, *in situ* heart valve TE has emerged to create living valves at the site of destination inside the heart. In this approach, a malfunctioning valve is replaced by a cell-free scaffold that gradually transforms into a living valve by recruiting endogenous cells and using the body as a "bioreactor".

Recent studies employing the *in situ* heart valve TE principle have shown compelling results using decellularized biological scaffolds, such as small intestine submucosa (SIS) [14,15] or *de novo* engineered extracellular matrices [13,16–18]. Moreover, promising results have been achieved using decellularized allografts, which have led to the large-scale clinical trials of these valves over the last decade [19–21]. However, these approaches do not negate the need for a (engineered) biological starter matrix and offer limited control over scaffold properties. Here we propose and demonstrate proof of concept of *in situ* heart valve TE starting from a cell-free synthetic bioresorbable micro-porous scaffold as a novel concept in heart valve replacement therapy (Fig. 1A). Compared to other (*in situ*) tissue engineering approaches, this fully synthetic approach is advantageous in that it does not require the use of any donor, using either human donor valves (decellularized allografts) or animal-derived tissue (e.g. SIS), or even *in vitro* cell and tissue culture. The use of a synthetic starter matrix offers off-the-shelf availability at substantially reduced costs and logistic complexity by omitting any tissue culture or tissue preparation [22,23]. In addition, synthetic materials offer high control over scaffold design and manufacturing, including the modulation of scaffold properties (e.g. resorption rate, biophysical properties) to induce functional, healthy regeneration [24,25]. Last, but not least, regulatory complexity is drastically reduced because the synthetic scaffolds can be considered as medical device at the time of implantation. While this concept has been demonstrated for tissue engineered vascular grafts [26–28], synthetic material-based *in situ* heart valve TE poses more complex challenges related to the valvular geometry and the complex dynamic opening and closing of the valve. The scaffold should not only withstand hemodynamic loading immediately upon implantation, but also maintain stable valve function with time and during scaffold resorption and neo-tissue formation. To our opinion, safe clinical use requires that scaffold resorption should be mainly cell driven, meaning that the scaffold will only degrade, and thus lose strength and durability, when sufficient extracellular matrix has been synthesized by the cells to take over mechanical functionality.

The goal of the present study was to design a bioresorbable synthetic heart valve that can maintain long-term functionality as a pulmonary valve in sheep, recruit host cells, and support the *in situ* formation of neo-tissue by these cells in pace with scaffold resorption. Valve structural and mechanical properties, opening and closing behavior, and resorption mechanisms were tested *in vitro*, while long-term functionality and *in situ* cell recruitment and neo valve formation were studied during long-term follow-up in an ovine model.

2. Materials & methods

2.1. Valve design and in vitro testing

For the development of the valvular scaffold we considered the relevant design criteria over multiple length-scales. At the molecular level, we employed a custom-developed bioresorbable supramolecular elastomer, based on bis-urea-modified polycarbonate (PC-BU). Using electrospinning, this material was processed into microporous scaffolds with fiber diameters and pore sizes optimized to advocate homogenous cell colonization and regenerative remodeling of the scaffold [28–30]. To characterize the mechanisms of scaffold resorption, scaffolds were subjected to accelerated hydrolysis and oxidative *in vitro* resorption tests. On the macroscopic scale, we developed a crown-shaped polyether ether ketone (PEEK) reinforcement ring to stabilize valve geometry. Prior to implantation, the polymer was seeded with fast-degrading fibrin gel in analogy with our previous *in vitro* heart valve TE approaches [31,32]. *In vitro* function of the resulting valvular device was tested in accordance with ISO 5840 using a pulsatile test system.

2.1.1. PC-BU polymer synthesis and characterization

PC-BU was developed and synthesized in-house in an analogous fashion to the preparation of the polycaprolactone bis-urea biomaterial as reported by Wisse et al. [33,34], by replacing the amine functional polycaprolactone used by Wisse et al. with amine functional polycarbonate in the chain extension polymerization reaction with butylene diisocyanate. The PC-BU material was analyzed by attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy as measured on a Spectrum Two IR spectrometer (Perkin Elmer). The neat PC-BU material was thermally analyzed by differential scanning calorimetry (DSC) using a Q2000 machine (TA Instruments). Melting (T_m) and glass (T_g) transition temperatures were measured from the melt, i.e. after the sample had first been brought to the isotropic state, in the second or ensuing heating runs. Heating scan rates of 10 °C/min and 40 °C/min were used for T_m and T_g assessment, respectively. The T_m was determined by the peak temperature, while the T_g was given by the inflection point in the thermogram. The degradative properties of PC-BU were assessed using accelerated *in vitro* tests, as previously described [35] (Supplementary Dataset 1).

2.1.2. Cytotoxicity test

A PC-BU solvent-cast film was prepared using chloroform/methanol as solvent, and was dried under vacuum to remove traces of solvent. Film samples were incubated in complete culture medium (DMEM from Gibco, supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) Penicillin Streptavidin) at 37 °C and 5% CO_2. Extraction of the PC-BU samples was performed for 24 h at a weight per volume of 20 mg PC-BU per mL complete medium. 3T3 mouse fibroblasts were seeded at a density a 5×10^3 cells per well in a 96-well plate and were maintained for 24 h under standard culturing conditions until cells were grown to 50% confluence. Next, the medium was removed and the 3T3 fibroblasts were cultured for an additional 24 h in the presence of 100 μL of filtered medium extract ($n = 4$). Cells exposed to complete medium supplemented with 1% (v/v) Triton-X 100 served as a control for cytotoxic conditions. The cytotoxicity was determined using an MTT cytotoxicity assay. Briefly, thiazolyl blue tetrazolium bromide (MTT, from Sigma) was dissolved in phosphate buffered saline to a concentration of 5 mg/mL; the solution was filtered and further diluted in complete medium to a final concentration of 1 mg/mL. The extract medium was removed and replaced with 50 μL of the MTT/culture medium. Fibroblasts were incubated for 2 h under standard culturing conditions, before the MTT solution was removed and replaced with 100 μL of isopropanol (acidified with 0.04 M HCl) until all formazan crystals dissolved. Subsequently, the absorbance was measured at 570 nm (650 nm reference wavelength) on a Tecan Safire microplate reader. Cell viability is presented relative to that of 3T3 fibroblasts that were maintained in untreated culture medium during the course of the study, where this reference is set at 100% cell viability.