In vivo monitoring of function of autologous engineered pulmonary valve

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Objectives: Clinical translation of tissue-engineered heart valves requires valve competency and lack of stenosis in the short and long term. Early studies of engineered valves showed promise, although lacked complete definition of valve function. Building on prior experiments, we sought to define the in vivo changes in structure and function of autologous engineered pulmonary valved conduits.

Methods: Mesenchymal stem cells were isolated from neonatal sheep bone marrow and seeded onto a bioresorbable scaffold. After 4 weeks of culture, valved conduits were implanted. Valve function, cusp, and conduit dimensions were evaluated at implantation (echocardiography), at the experimental midpoint (magnetic resonance imaging), and at explant, at 1 day, and 1, 6, 12, or 20 weeks postoperatively (direct measurement, echocardiography). Histologic evaluation was performed.

Results: Nineteen animals underwent autologous tissue-engineered valved conduit replacement. At implantation, valved conduit function was excellent; maximum transvalvular pressure gradient by Doppler echocardiography was 17 mm Hg; most valved conduits showed trivial pulmonary regurgitation. At 6 postoperative weeks, valve cusps appeared less mobile; pulmonary regurgitation was mild to moderate. At 12 weeks or more, valved conduit cusps were increasingly attenuated and regurgitant. Valved conduit diameter remained unchanged over 20 weeks. Dimensional measurements by magnetic resonance imaging correlated with direct measurement at explant.

Conclusions: We demonstrate autologous engineered tissue valved conduits that function well at implantation, with subsequent monitoring of dimensions and function in real time by magnetic resonance imaging. In vivo valves undergo structural and functional remodeling without stenosis, but with worsening pulmonary regurgitation after 6 weeks. Insights into mechanisms of in vivo remodeling are valuable for future iterations of engineered heart valves. (J Thorac Cardiovasc Surg 2010;139:723-31)

Although much progress has been made in the treatment of congenital heart disease, valve replacement technologies for children continue to present distinct clinical challenges. For children, currently available bioprosthetic and mechanical valve replacement devices are limited by lack of growth; pediatric patients outgrow valve replacement devices and subsequently require multiple reoperations to accommodate an increasing body size. In addition, whereas valve and patient lifespan are generally well matched in adult populations, the increasing life expectancy of children with congenital heart disease intensifies the requirement for valve durability. Although more durable, mechanical valves are thrombogenic; implantation of a mechanical valve commits a patient to long-term postoperative anticoagulation and its attendant morbidities. In addition, pediatric patients commonly require reconstruction of the pulmonary valve and right ventricular outflow tract, thereby necessitating a valved conduit, a different device from that which is designed for stented or stentless valve replacement applications in adults.

These distinct design inputs for pediatric patients have led to investigation of tissue engineering approaches for pulmonary valve and right ventricular outflow tract replacement.[1-3] Tissue-engineered heart valves are living tissues offering growth potential and present a possible solution to this challenge. Previous experiments in which autologous cells were seeded onto bioresorbable scaffolds and implanted in an in vivo model have demonstrated the feasibility and potential strengths of this approach.[1-3]

However, insufficient long-term data have been collected to confidently evaluate the long-term function of engineered valves, a requirement for clinical translation. Building on

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Abbreviations and Acronyms

ANOVA = analysis of variance
DMEM = Dulbecco's modified Eagle's medium
FBS = fetal bovine serum
MRI = magnetic resonance imaging
MSC = mesenchymal stem cell

prior experiments, we hypothesized that autologous, stem cell–based engineered valved conduits could be successfully implanted into the circulation, with acceptable initial valve function. We sought to understand the process of tissue-engineered valved conduit remodeling and to evaluate valve function over time in a large in vivo series. To our knowledge, this series of experiments provides a sufficiently large number of observations to allow the meaningful evaluation of intermediate-term performance of implanted tissue-engineered heart valves. We further sought to establish reproducible methods of monitoring in vivo valve function using cardiac magnetic resonance imaging (MRI).

MATERIALS AND METHODS

Autologous valved conduits were created and implanted into sheep, which were then monitored by cardiac MRI at the experimental midpoint. Animals were grouped and monitored according to the schematic representation shown in Figure 1. Valved conduits were explanted at the experimental end point, within the first 24 hours (n = 7), and at 1 (n = 3), 6 (n = 3), 12 (n = 3), or 20 weeks (n = 3) after implantation.

Animals

Female sheep (*Ovis aries,* Dorset subspecies) were obtained at 4 weeks of age, weighing approximately 10 kg. Bone marrow aspiration was performed twice on each animal; at 5 and 6 weeks of age, 10 mL/kg of bone marrow was harvested from the iliac crest under general anesthesia. Use of experimental sheep was approved by the Institutional Animal Care and Use Committee of Children's Hospital Boston. Animals were cared for by a veterinarian in accordance with the ''Guide for the Care and Use of Laboratory Animals.''

Cell Isolation and Expansion

Bone marrow was centrifuged on a Ficoll gradient (Sigma Chemical Co, St Louis, Mo) and the mononuclear fraction was plated on uncoated plates in medium containing Dulbecco's modified Eagle's medium (DMEM) with high glucose, 10% fetal bovine serum (FBS), 10% autologous serum, 10 mmol/L HEPES buffer, and 1× antibiotic/antimycotic. Mesenchymal stem cells (MSCs) were isolated by their avid adherence to tissue culture plates as previously reported,[1,4,5] passaged, and expanded in basal medium containing DMEM with high glucose, 10% FBS, 1× antibiotic/antimycotic, and 10 mmol/L HEPES buffer. Cells were expanded by plating at a density of 10^4 cells/cm^2 and passaged every 3 days when 70% to 80% confluence was achieved. Cell passaging continued until approximately 1 billion cells were expanded for each valved conduit. As previously reported, 1 month was required from the harvest of bone marrow to the time of cell seeding on the scaffold.[1]

Scaffold Properties and Assembly

Sheets of nonwoven scaffold containing 50% fibers of polyglycolic acid and 50% fibers of poly-L-lactic acid (Concordia Medical, Warwick, RI) were assembled into a valved conduit by manual and machine needle punching, according to methods established in our laboratory,[1] based on normal dimensions of the ovine right ventricular outflow tract (data not shown).

Custom Tension Device and Scaffold Preparation for Seeding

To immobilize the valved conduit, to prevent geometric changes during culture, and to provide a homogeneous surface for cell seeding, we fabricated a custom device for valved conduits in 3-dimensional culture.[6] The valved conduit scaffold was mounted onto gaskets and tied into position with silk suture material, then sewn circumferentially to the tension device at approximately the level of the cusp base and cusp free edge (Figure 2). The valved conduit was then placed inside a 150-mm glass hybridization bottle and sterilized with ethylene oxide gas. Once sterile, scaffolds were pre-wet with 70% ethanol, washed 3 times with phosphate-buffered saline, and immersed in a solution of 90% FBS and 10% antibiotic/antimycotic (1–2 hours) while cells were prepared for seeding.

Valve Culture

MSCs were seeded onto the 80-cm^2 scaffold at a density of 0.5 to 1.7 × 10^7 cells/cm^2 by adding a dense cell suspension to the bottle housing the scaffold. Valves were seeded in 60 mL of medium containing DMEM high glucose, 20% FBS, 10% autologous sheep serum, 2× antibiotic/antimycotic, 10-mmol/L HEPES buffer, 82-μg/mL ascorbic acid-2-phosphate, and 2-ng/mL basic fibroblast growth factor. During seeding, the scaffold was rotated at 1 rpm, and medium was changed every 12 hours. After 72 hours, the valve was removed from the glass bottle and placed in an 850-cm^2 roller bottle containing 500 mL of medium as above without sheep serum. The medium was changed every 3 days for 1 month (Figure 2).

Preoperative Blood Preparation for Cardiopulmonary Bypass

Blood donation bags were prepared with acid–citrate–dextrose buffer for blood banking. An adult sheep was exsanguinated for blood donation; blood was stored at 4°C overnight and used for blood prime for cardiopulmonary bypass.

Operation

Twenty-two autologous valves were prepared according to these methods, 19 of which were implanted in vivo. The remaining 3 valves served as in vitro controls and were analyzed in parallel to characterize tissues at the time of implantation. At operation, a thoracotomy was performed in the fourth intercostal space, and under cardiopulmonary bypass with a beating heart, the native pulmonary valve cusps and a 1- to 2-cm main pulmonary artery segment were excised. The engineered valved conduit was removed from the tension device and interposed between the cut pulmonary artery segments (Figure 2). Epicardial echocardiography was performed to evaluate valve function at implantation (Figure 3). Maximum transvalvular pressure gradient was obtained by continuous-wave spectral Doppler methods. In addition, a semiquantitative evaluation of proximal pulmonary regurgitation was performed, on the basis of vena contracta color jet width and the presence of retrograde diastolic flow in the main pulmonary artery: trivial = long-axis color jet width ≤ 1 mm; mild = color jet width between 1 and 2 mm; moderate = color jet width > 2 mm but < 50% of the conduit width; severe = color jet width > 50% of conduit width and presence of retrograde diastolic flow in the main pulmonary artery. After chest closure, the animal was transferred from the operating table to a recovery sling, allowed to recover until in hemodynamically stable condition, allowed to wake from anesthesia, and extubated. All tubes and drains were removed before return to the cage on the evening of the operation.

MRI

At each experimental midpoint, animals underwent cardiac MRI (1.5 T Achieva; Philips, Best, The Netherlands) while under general anesthesia.