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PRE-CLINICAL RESEARCH

Minimally-Invasive Implantation of Living Tissue Engineered Heart Valves

A Comprehensive Approach From Autologous Vascular Cells to Stem Cells

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Objectives The aim of this study was to demonstrate the feasibility of combining the novel heart valve replacement technologies of: 1) tissue engineering; and 2) minimally-invasive implantation based on autologous cells and composite self-expandable biodegradable biomaterials.

Background Minimally-invasive valve replacement procedures are rapidly evolving as alternative treatment option for patients with valvular heart disease. However, currently used valve substitutes are bioprosthetic and as such have limited durability. To overcome this limitation, tissue engineering technologies provide living autologous valve replacements with regeneration and growth potential.

Methods Trileaflet heart valves fabricated from biodegradable synthetic scaffolds, integrated in self-expanding stents and seeded with autologous vascular or stem cells (bone marrow and peripheral blood), were generated in vitro using dynamic bioreactors. Subsequently, the tissue engineered heart valves (TEHV) were minimally-invasively implanted as pulmonary valve replacements in sheep. In vivo functionality was assessed by echocardiography and angiography up to 8 weeks. The tissue composition of explanted TEHV and corresponding control valves was analyzed.

Results The transapical implantations were successful in all animals. The TEHV demonstrated in vivo functionality with mobile but thickened leaflets. Histology revealed layered neotissues with endothelialized surfaces. Quantitative extracellular matrix analysis at 8 weeks showed higher values for deoxyribonucleic acid, collagen, and glycosaminoglycans compared to native valves. Mechanical profiles demonstrated sufficient tissue strength, but less pliability independent of the cell source.

Conclusions This study demonstrates the principal feasibility of merging tissue engineering and minimally-invasive valve replacement technologies. Using adult stem cells is successful, enabling minimally-invasive cell harvest. Thus, this new technology may enable a valid alternative to current bioprosthetic devices. (J Am Coll Cardiol 2010;56:510–20) © 2010 by the American College of Cardiology Foundation

Minimally-invasive valve implantation techniques are rapidly evolving as alternative treatment option for patients with valvular heart disease. These techniques are expected to have a major impact on the management of patients with valvular heart disease over the next several years (1). Various transvascular, catheter-based implantation approaches have been developed and successfully used in both experimental and clinical settings (2). Alternative minimally-invasive surgical

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techniques, such as the transapical approach, have been described as safe and successful procedures particularly for patients with more advanced atherosclerosis and/or complex heart valve pathologies (2,3). However, the currently available valve substitutes for minimally-invasive replacement procedures are bioprosthetic and as such will be associated with calcification and progressive dysfunctional degeneration similar to that of conventional nonviable tissue valves (glutaraldehyde fixed, and so forth). That suggests their primary clinical application in elderly patients (2).

Autologous, viable valve substitutes with regeneration potential would overcome the limitations of today's valve prostheses and enable the application of minimally-invasive treatment modalities also in younger patients (4,5). In recent years, tissue engineering techniques have been developed on the basis of decellularized allografts or rapidly degrading synthetic scaffold materials and autologous cells, aiming at living heart valve replacements with regeneration potential. Feasibility and functionality of such tissue engineered heart valves (TEHVs) implanted by conventional surgical procedures have been demonstrated by in vitro as well as in vivo experiments and initial clinical trials (6–12). Therefore, TEHV implanted by a minimally-invasive technique could become a viable autologous alternative to bioprosthetic valve replacements. A clinically relevant heart valve tissue engineering concept would ideally comprise both minimally-invasive techniques for cell harvest and valve implantation. Here, the first in vitro and in vivo experiences in an animal model toward a "complete" minimally-invasive heart valve tissue engineering approach based on rapidly degrading composite scaffolds and autologous vascular and adult stem cells are presented.

Abbreviations and Acronyms

DNA = deoxyribonucleic acid
eNOS = endothelial nitric oxide synthase
GAG = glycosaminoglycans
OD = outer diameter
SMA = smooth muscle actin
TEHV = tissue engineered heart valve
UTS = ultimate tensile strength

Methods

This comprehensive study comprises 2 sequential experimental setups. In a first set of experiments (study A), the principal feasibility of creating in vitro TEHV, which can be implanted by transapical, minimally-invasive delivery with adequate in vivo functionality, was investigated. A second set of experiments (study B) was focused on the feasibility to utilize autologous stem cells (bone marrow, peripheral blood) as a less invasive, multipotent alternative cell source. Both concepts were tested in sheep, representing the most used animal model for heart valve prostheses.

Experimental study A: merging TEHV and minimally-invasive implantation technologies, in vitro and in vivo feasibility. Valve replacements (n = 16) for minimally-invasive implantation technologies were manufactured by integrating TEHV into self-expandable stents (Figs. 1A and 1B). The TEHV were cultured in vitro using dynamic in vitro culture protocols. In vivo performance up to 8 weeks of TEHV was evaluated after transapical implantation in sheep (n = 6) in pulmonary position. Next to each im-

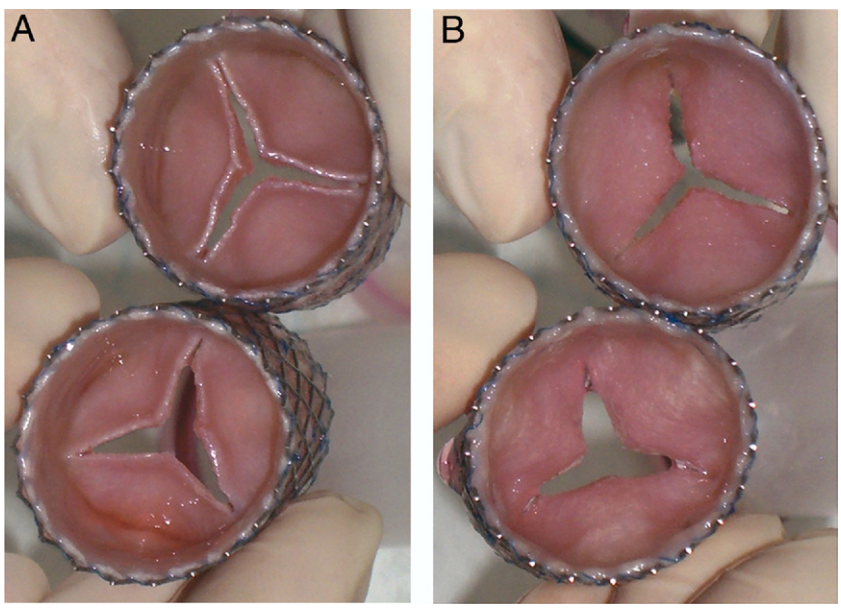

Figure 1 Macroscopic Appearance of TEHV Before Implantation

Macroscopic picture of autologous tissue engineered heart valve (TEHV) based on vascular-derived cells integrated into a self-expanding nitinol stent, **(A)** distal view and **(B)** proximal view.