A Hybrid Tissue-Engineered Heart Valve

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Purpose. **This study describes the efforts to develop and test the first hybrid tissue-engineered heart valve whose leaflets are composed of an extra-thin superelastic Nitinol mesh tightly enclosed by uniform tissue layers composed of multiple cell types.**

Description. **The trileaflet Nitinol mesh scaffolds underwent three-dimensional cell culture with smooth muscle and fibroblast/myofibroblast cells enclosing the mesh, which were finally covered by an endothelial cell layer.**

Evaluation. **Quantitative and qualitative assays were performed to analyze the microstructure of the tissues. A tissue composition almost similar to that of natural heart valve leaflets was observed. The function of the valves and their Nitinol scaffolds were tested in a heart flow simulator that confirmed the trileaflet valves open and close robustly under physiologic flow conditions with an effective orifice area of 75%. The tissue–metal attachment of the leaflets once exposed to physiologic flow rates was tested and approved.**

Conclusions. **Our preliminary results indicate that the novel hybrid approach with nondegradable scaffold for engineering heart valves is viable and may address the issues associated with current tissue-engineered valves developed with degradable scaffolds.**

(Ann Thorac Surg 2015;99:2183–7)

Current heart valve replacement options are limited to either mechanical or bioprosthetic heart valves (BHVs). Because of their stronger composition, mechanical heart valves tend to last longer than BHVs. However, they carry a greater long-term risk for thromboembolism, which may lead to stroke and arterial thrombosis [1]. BHVs (including transcatheter valves) that are primarily composed of fixed porcine leaflets or bovine pericardium are not thrombogenic. However, they are prone to calcification and progressive deterioration with restrained durability [2].

Clearly, there is a need for a heart valve that overcomes both the durability and hemocompatibility issues, particularly for younger patients. The leaflets in our hybrid valve are composed of an extra-thin Nitinol mesh core enclosed by multiple layers of the patient's autologous cells. Nitinol was used because of its superelastic nature in addition to its superior strain-controlled fatigue performance. As a result, this novel hybrid tissue-engineered valve retains a biological surface with potential repair and remodeling capabilities, and its thin superelastic Nitinol mesh core strengthens the natural extracellular matrix backbone of the leaflet to withstand the variety of loads applied to the valve in the heart.

Accepted for publication Feb 12, 2015.

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Technology and Technique

Development of the Hybrid Valves

The Nitinol mesh-enclosed leaflets were prepared as described in our previous publication [3]. Briefly, a thin acid-etched, superelastic Nitinol mesh of 25 μm thickness was surface modified and shape-set into the desired leaflet size according to a 21-mm trileaflet valve. The oxidized film was removed at multiple stages by polishing the surface, using hydrochloric acid wash, ultrasonic cleaning wash in ethanol for 15 minutes, and glow discharging for 40 seconds. Then the meshes were irradiated by He^{+} ion beam at 150 keV energy with fluences of 1×10^{14} ions/cm^2 to improve surface biocompatibility. Aortic smooth muscle, aortic adventitial fibroblast/myofibroblast, and umbilical vascular endothelial cells were used to culture hybrid leaflets. The cells (all from Lonza Group, Allendale, NJ) with a total density of 9×10^6 cells per leaflet, were seeded in sequence in an amount inversely proportional to their proliferation rate on the Nitinol mesh leaflets by use of bovine type I collagen gels.

The collagen concentration was set to 1.1% (11 mg/mL) for the first layer, 1.9% (19 mg/mL) for the second layer, and finally 2.7% (27 mg/mL) for the covering endothelial layer. The final collagen solution was sterilized and then polymerized by adding 0.2N HEPES, pH9 and 10× MEM (both from Gibco, Carlsbad, CA) in a ratio of 8:1:1, and 10 μg/mL Laminin (Sigma-Aldrich Inc., St. Louis, MO) was added to increase cell adhesion and proliferation. The solution was UV cross-linked thoroughly with 6.3 * 10^5 mJ/cm^2. The

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2015.02.058

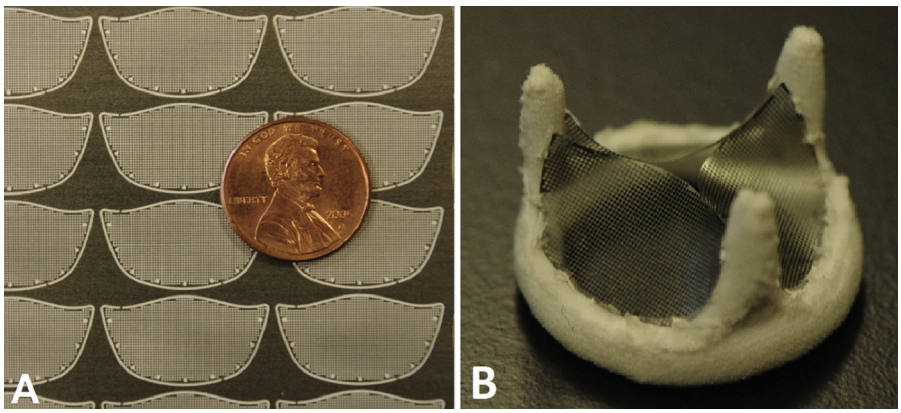

Fig 1. Nitinol scaffold fabricated for growing trileaflet hybrid heart valves. (A) Flat Nitinol mesh cut to the desired shape of a leaflet. (B) Trileaflet hybrid valve scaffold made of Nitinol mesh leaflets.

leaflets were cultured in basal media in the presence of growth factors such as human epidermal growth factor 0.01%, insulin 0.01%, human fibroblast growth factor-B 0.02%, vascular endothelial growth factor 0.01%, and ascorbic acid 0.01% (all from Lonza, Allendale, NJ). To increase the rate of extracellular matrix (ECM) production, 10 ng/mL TGF-β1 (R&D Systems Inc., Minneapolis, MN) was added to the collagen gels at each layer.

To develop the hybrid valves, each three mesh leaflets (Fig 1A) were initially sewn to a biocompatible Steralloy structure (Hapco, Hanover, MA) along with a surgical ring, as shown in Figure 1B. The 25-mm hybrid valves (n = 10) were prepared by casting collagen solution mixed with cells of each layer around the trileaflet Nitinol mesh scaffold by use of a two-piece shell made of biocompatible polyether ether ketone. The two components of the shell secured and separated the mixture of scaffold and cells. To test the effect of pulsatile flow on the valve composition, half of the valves were exposed to pulsatile flow during the cell culture (dynamically conditioned), and the rest were cultured without exposure to flow (statically conditioned).

Bioreactor Design for Testing the Hybrid Leaflets

A bioreactor was developed to dynamically condition the leaflets and to test the tissue–metal attachment under the pulsatile flow. Additionally, this bioreactor was used to test whether presence of a metallic nondegradable mesh scaffold inside the tissue may lead to any adverse effects on the cellular activities and the process of extracellular matrix formation under dynamic loading conditions. The hybrid tissue leaflets were placed in the bioreactor and exposed to physiologic flow rates. The bioreactor is described in detail in Figure 2. The entire system was placed inside an incubator with basal media as the circulating media.

Leaflet Tissue Analysis

To analyze the cellular and extracellular elements, biochemical assays were performed for both statically and dynamically conditioned groups. Total DNA as an indicator of cell numbers was quantified. Hydroxyproline as an indicator of total collagen content was quantitatively

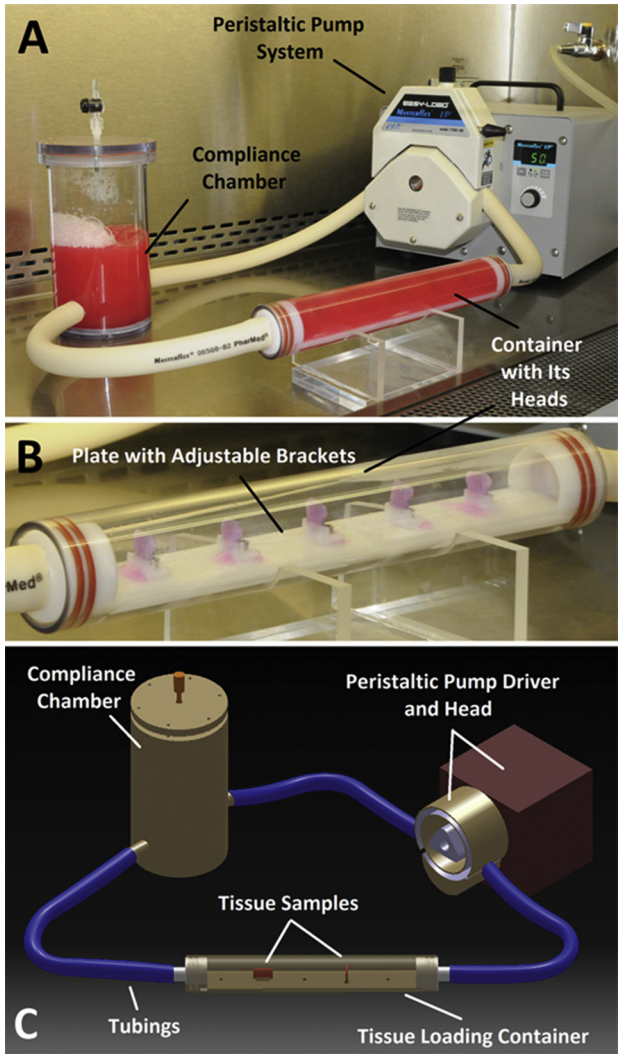

Fig 2. Custom-made bioreactor developed to dynamically condition the leaflet tissues. (A) Experimental setup consists of a peristaltic pump system, tubular container, compliance chamber, and tubing system. (B) Hybrid leaflet samples assembled in the container system and exposed to the experimental flow rates. (C) Schematic representation of bioreactor setup.