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.**

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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.

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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

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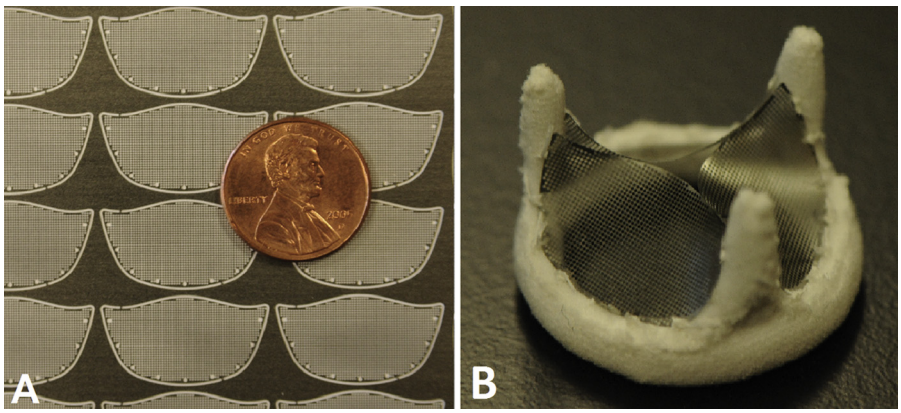

Fig 1. Nitinol scaffold fabricated for growing trileaflet hybrid heart valves. (A) Flat Nitinol mesh cut to the desired shape of a leaflet. (B) Trileaflet hybrid valve scaffold made of Nitinol mesh leaflets.

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

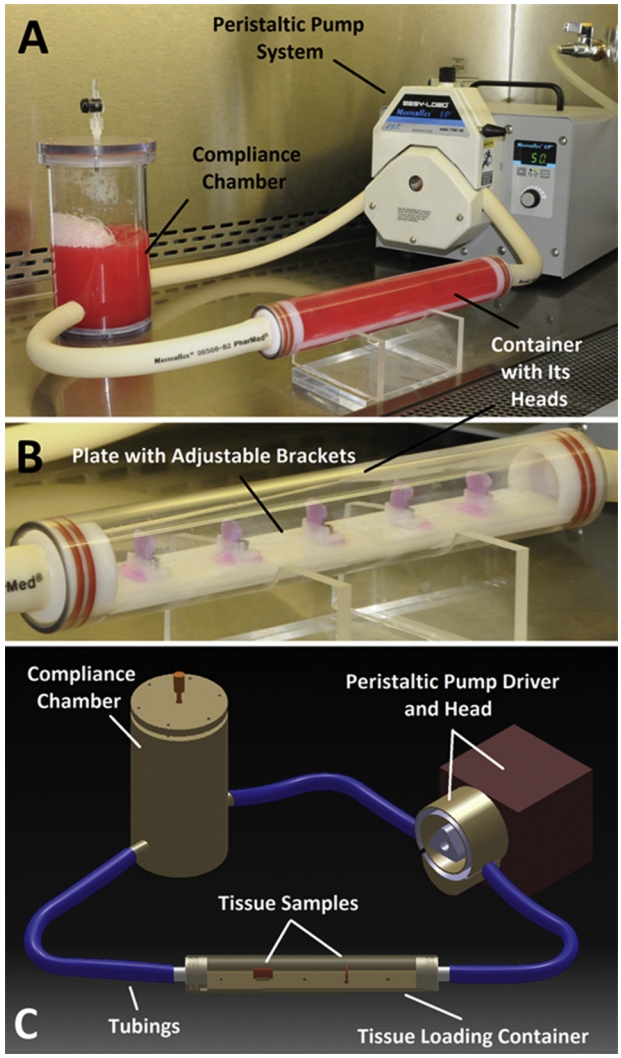

Fig 2. Custom-made bioreactor developed to dynamically condition the leaflet tissues. (A) Experimental setup consists of a peristaltic pump system, tubular container, compliance chamber, and tubing system. (B) Hybrid leaflet samples assembled in the container system and exposed to the experimental flow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

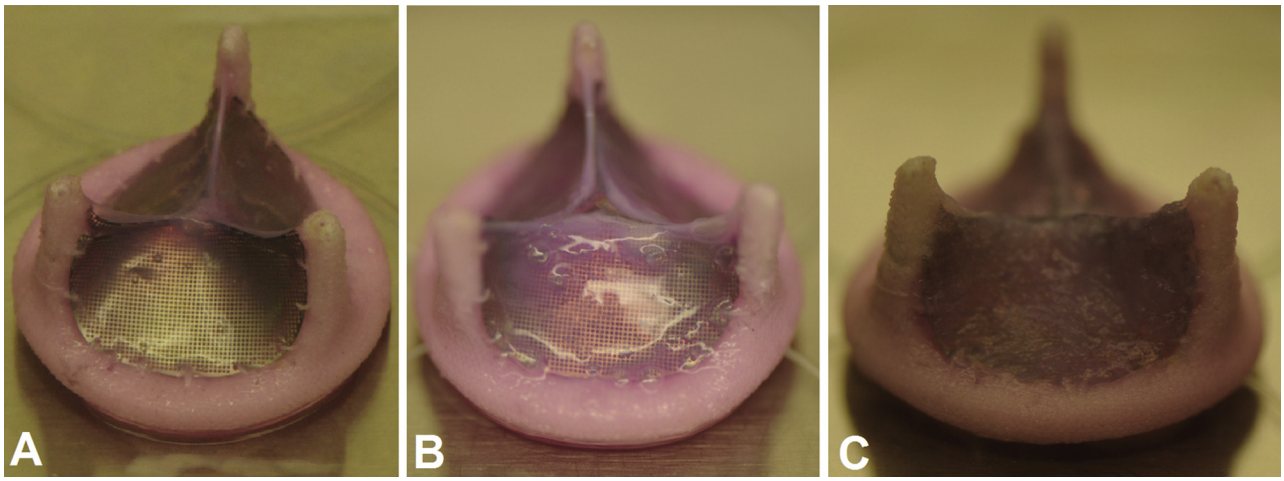

Fig 3. Hybrid heart valve at various stages of development. Nitinol structure along with (A) smooth muscle, (B) fibroblast/myofibroblast, and (C) endothelial cells, all encapsulated in collagen as the first, second, and third layers, respectively.

determined. Total proteoglycan/glycosaminoglycan was measured by a BLYSCAN assay, and FASTIN assay (both from Biocolor, Belfast, Northern Ireland) was used for quantification of elastin content. Hematoxylin and eosin (H&E) staining on the edges of the hybrid tissues was performed for general morphology. Histologic examination on the midparts of the hybrid tissues (ie, the areas containing the mesh) was performed by using a state-of-the-art laser microtome (LLS Rowiak, Hannover, Germany). To do so, the leaflets were stained by stimulated raman scattering for general morphology and Light Green for demonstration of collagen components.

Testing the Hybrid Valves in Heart Flow Simulator

Cultured heart valves and the Nitinol scaffolds were separately tested by use of a heart-pulsed duplicator specifically developed for heart valve studies [4]. The heart flow simulator system is composed of a transparent Silicone chamber, custom shaped according to the molds obtained from patient-specific cardiac magnetic resonance images of the left ventricle. The valves were tested at the aortic position inside the chamber. The system was adjusted for left ventricular pressure waveform with standard diastolic/systolic pressures of 80/120 mm Hg.

Clinical Experience

Hybrid heart valves' leaflets (n = 10) were cultured for 3 weeks. Smooth muscle cells encapsulated in a bovine type I collagen solution constituted the first layer covering the Nitinol mesh (Fig 3A). The rate of collagen contraction and degradation at this step was found very high, owing to the low concentration of collagen (1.1%). After 1 week, fibroblast and myofibroblast cells encapsulated in collagen were cultured over the first layer, with a decrease in degradation rate due to the higher collagen concentration (1.9%) and by the increased cellular population that would increase the rate of ECM formation (Fig 3B). Endothelialization of the valve (Fig 3C) stopped collagen contraction and degradation, which means the cells remodeled the valve based on their own needs.

Tissue Analysis

Figure 4 shows the results of the quantitative biochemical assays. The amount of DNA in hybrid leaflets was found to be comparable with the natural valve tissue in static group ($p \sim 0.15$), with an increase to ~140% for the dynamic group. The ECM production increased in all dynamic groups versus the static groups, with a significant increase ($p < 0.05$) for collagen. H&E staining showed a uniform organized structure in a spongy form for the static groups (Fig 5A) and a more dense and layered fashion for the dynamic groups (Fig 5B). Stimulated raman scattering staining at the tip of the leaflet showed the mesh tightly enclosed within the tissue (Fig 5C). Light Green staining (Fig 5D) at the midpart of the leaflet

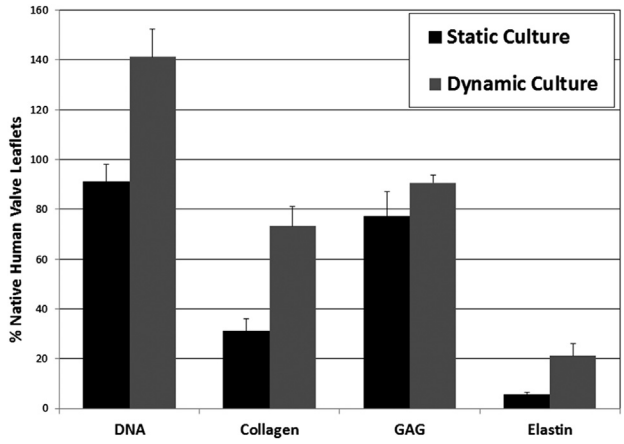

Fig 4. Quantitative biochemical assays performed on the statically and dynamically conditioned hybrid valves, showing increase in DNA content and matrix protein values for dynamic groups versus static groups. (GAG = glycosaminoglycan.)

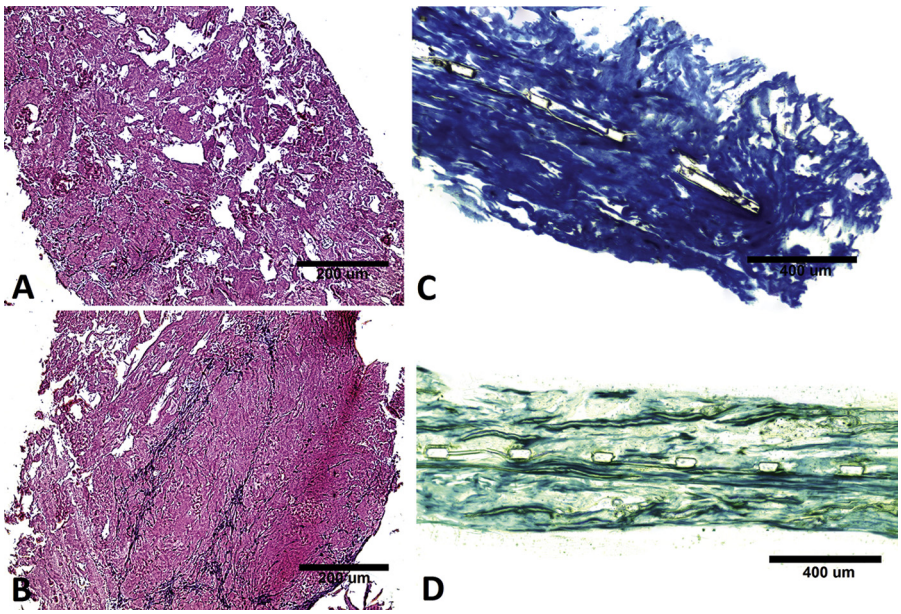

Fig 5. Qualitative histologic assays performed on the statically and dynamically conditioned groups. H&E stain representing a spongy structure for (A) static group versus (B) dense composition for dynamically conditioned leaflets. (C) Stimulated raman scattering staining shows that the mesh is completely enclosed within the tissue. (D) Light Green staining demonstrates a fibrous structure with a uniform cellularity throughout the leaflets.

showed a fibrous collagenous structure with smooth inflow and outflow sides. Cell's nuclei distributed evenly throughout the tissue confirmed the migration of cells since the initial seeding density was not uniform.

Resilience and Compliance of the Valve's Leaflets

The experiment with the heart flow simulator was aimed to test whether the ventricle's contractile force could robustly open and close the leaflets. The series of images acquired from a recorded movie clip in Figure 6A show that the Nitinol leaflets properly behaved under physiologic flow rates (eg, 5 L/min). These images were imported into ImageJ software to measure the open area of the valve during systole. The effective orifice area measured 2.6 cm^2 (75% for a 21-mm valve); this number is 42% and 44% for a 21-mm commercial BHV made of bovine pericardium and porcine tissue, respectively [5]. Nevertheless, mesh porosity did not allow proper in vitro hemodynamic testing due to the valve leakage through the mesh holes. To test the hybrid valves, the chambers and the sac were filled with basal media (Fig 6B). The valves were found to operate properly even under the low flow rates (2.5 L/min) at 60 beats/min, a compliance comparable with that of the BHVs (Fig 6C). No tissue separation or decomposition of the valves was observed—even under the higher flow rates—until the occurrence of cellular necrosis resulting from a lack of nutrition and oxygen, given that the heart flow simulator is not designed as a bioreactor for long-term testing of live tissues. To measure the hemodynamics determinations, high-speed particle image velocimetry is required, which uses a high-energy laser sheet to obtain the velocity field. However, use of the high-energy laser would produce heat shock and immediate necrosis of the cells. This was a limitation of our study.

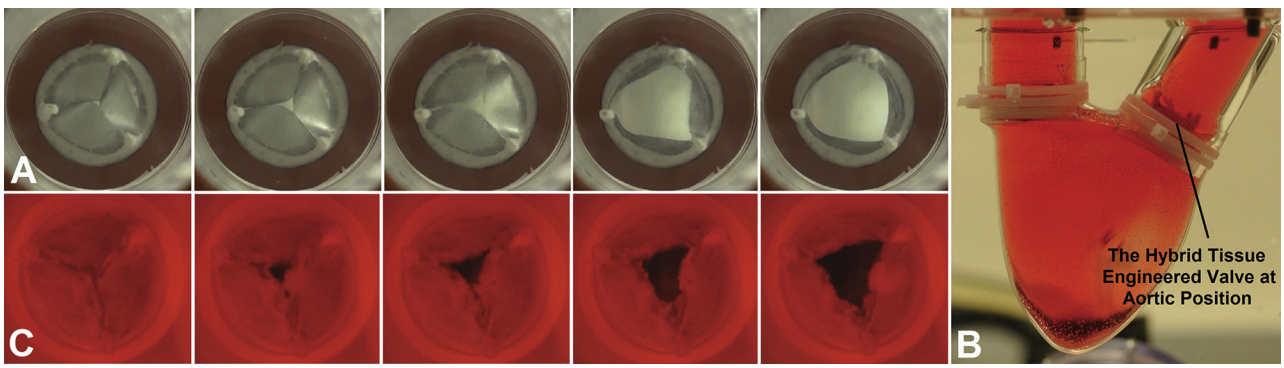

Fig 6. Testing the valve performance inside a heart flow simulator. (A) Function of the porous Nitinol scaffold under the flow rate of 5 L/min. (B) Left ventricle sac of the heart pulsed flow simulator filled with basal media. (C) Function of hybrid valves under 2.5 L/min showing comparable performance with native valves.

Comment

Besides the meaningful and early encouraging results of tissue-engineered heart valves [6], these valves are mostly unable to adjust their composition to withstand the various types of dynamic loads to which they are exposed in the heart, principally the left ventricle [6, 7]. These valves were mainly developed using degradable scaffolds (eg, synthetic polymers and purely biological material). Their slow or rapid rate of degradation may either lead to deficiency in composition or result in tissues that are thicker or stiffer than the native valves [8]. Additionally, the tissue-engineered leaflets shrink as a result of rapid degradation or contraction of their scaffolds [9].

To overcome these limitations, we approached the problem differently by creating a nondegradable superelastic scaffold that permanently remains and supports the leaflets in contrast to the currently used degradable ones [3]. Through this approach, multilayered hybrid heart valves were developed that are composed of three different cell types arranged in a fashion similar to that of a native valve.

The resilience and compliance of the developed valves were tested by use of a heart-pulsed flow simulator. Our results indicate that a very thin superelastic Nitinol sheet as the scaffold can serve as the primary load-bearing component of the hybrid leaflet, and it should preserve the structural integrity of the engineered valve when subjected to the high pressure in the heart, particularly in the left ventricle. Hybrid tissue was also previously shown to be biocompatible and to evoke only a minimal immune response in vitro [10]. This approach to engineering heart valves holds promise for combining the long-term durability of mechanical valves with the improved biocompatibility and hemodynamics of bioprosthetic heart valves.

Disclosures and Freedom of Investigation

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The tested technology has been solely developed at Kheradvar laboratory at University of California, Irvine, and the authors had full control of the design of the study, methods used, outcome parameters, analysis of data and production of the written report.

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