Trilayered Design in Aortic Valve Tissue Engineering: A Polymeric Approach

Jun Liao[1] and Jianjun Guan[2]

[1]Department of Agricultural and Biological Engineering, Mississippi State University, Mississippi State, MS
[2]Department of Material Science and Engineering, Ohio State University, Columbus, OH

Statement of Purpose:
The most common surgical therapy for heart valve disease is heart valve replacement via mechanical or bioprosthetic valves. These valves succeed in restoring blood hemodynamics but fail in the repairing/remodeling aspect that is essential for the pediatric population. Tissue engineered heart valves (TEHV) aim to solve this issue by implanting a functional living tissue with the ability to grow and remodel with the heart. Current efforts in the fabrication of synthetic scaffolds for TE heart valves have been lacking in structural consideration, treating it as a uniform mass. In this study, we addressed the mechanical integrity and structural characteristics of aortic valve leaflet (AVL). By utilizing electrospinning, we have generated a three-layered polymer scaffold mimicking the trilayered structure in the AVL: fibrosa, spongiosa, and ventricularis.

Methods:
Biodegradable polyurethaneurea (PUU) was synthesized as previously described.[1,2] Thermosensitive copolymer (TGel) based on N-isopropylacrylamide and N-acryloxysuccinimide was synthesized via free radical polymerization. PUU/TGel blends were dissolved in *hexafluoroisopropanol (*HFIP), and a diamine was added to crosslink the TGel. The crosslinked blend solution was fed by a syringe pump into a positively charged stainless steel tube. The electrospun nanofibers were collected on a negatively charged rotation mandrel (Fig. 1). The tissue engineered AVL consisted of three layers. The 1st and 3rd layers were designed to have fiber-preferred directions that were orthogonally oriented, and the 2nd layer was designed to have a random structure.

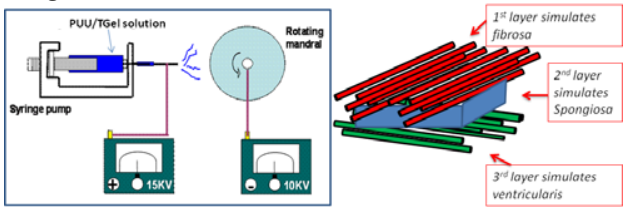

Figure 1. Experimental setup and multilayer electrospinning procedure.

TEHV scaffolds were subjected to histological and SEM studies to reveal the trilayered structure. Biaxial mechanical characterizations were performed to assess TEHV behavior under biaxial loading condition in the physiological range. Uniaxial mechanical testing was carried out to assess material properties of the trilayered polymer scaffold.

Results:
The thickness of TEHV scaffolds ranged from 400 µm - 500 µm, a typical thickness of AVL. H&E staining showed a highly integrated three-layered structure (Fig. 2), with a morphology similar to native AVL. SEM study showed that there were distinct fiber orientations in the 1st and 3rd layers, which simulated the collagen and elastin fiber orientations in the fibrosa and ventricularis, respectively; moreover, 2nd layer was more like a cushion layer and lack of fiber orientation, similar to that of the spongiosa.

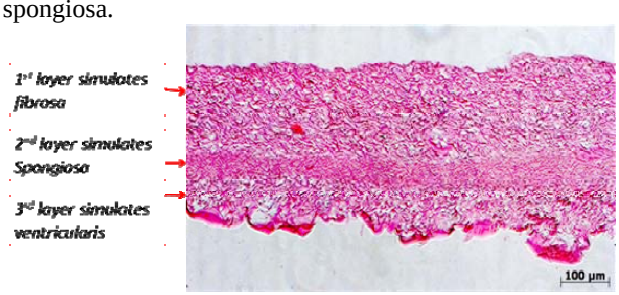

Figure 2. Trilayered polymeric scaffold mimicking AVL

Biaxial test of TEHV showed a comparable response to that of a native AVL in terms of tissue anisotropy and overall extensibility. Uniaxial test showed that TEHV material strength (UTS) reached ~55% of the native AVL. Although UTS provides a good safety factor (UTS/physiological loading) for TEHV, future improvement is still needed.

Conclusions:
We have generated a novel trilayered polymeric scaffold that mimics the structural properties of AVL. By utilizing electrospinning, we were able to manipulate the fiber orientation and composition of each layer of TEHV. The highly integrated trilayered structure of TEHV has been verified by both histology and SEM. The biaxial mechanical properties of the scaffold are very comparable to that of the native AVL. The material strength reaches ~55% of the native AVL. In conclusion, the biocompatibility of PUU and TGel, the feasibility of subtle microstructure manipulation, and mechanical properties mimicking the native AVL makes this scaffold a promising option for tissue engineered heart valves. Future study will include co-spraying of valve interstitial cells and optimization of TEHV construct.

References:
1. Guan J, et al., J Biomed Mater Res, 2002;
2. Feng Wang, et al., Acta Biomaterilia, 2009;