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Gelatin-Coated Resorbable Polymer Mesh as a Novel Scaffold for Aortic Heart Valve Tissue Engineering

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***Abstract*— A gelatin coated resorbable polymer scaffold was used to create tissue engineered heart valves (TEHV) in order to address the issues associated with the longevity of current artificial heart valves. It was hypothesized that the combination of specific elements is essential to development of a functional TEHV: biomimetic geometry, flexible and durable scaffolds, proper cell seeding, and mechanical stimulation. Testing of this hypothesis included iterations of aortic valve scaffold designs to mimic native heart valve architecture using stainless steel and bioabsorbable polymer meshes. The functionality of the valve was tested with a pulsatile flow bioreactor. Preliminary scaffolds were too rigid and displayed only a minimal geometric orifice area (GOA) for blood flow through the valve. The bioabsorbable polymer mesh provided adequate structural support and greater flexibility for the gelatin heart valve when compared to the stainless steel mesh. A new suturing technique was then incorporated to increase the GOA. Initial studies demonstrated that the new suturing technique allowed for natural movement of the root and closure of the cusps. However, improvements are still needed to further increase the GOA during valve opening. Future research plans include modification of suturing techniques as well as the acquisition of more compliant polymer meshes. The gelatin-coated scaffolds were also tested for cytotoxicity by seeding with cells. Preliminary results displayed cell attachment, but ingrowth was limited. Long-term goals include endothelial cell seeding on the most effective polymer scaffold, which will then be cultured in a bioreactor where it will be subjected to mechanostimulation.**

***Keywords* — Degradable, Gelatin, Valve Tester, Polymer**

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I. Introduction

The human heart performs approximately forty million cycles a year – an estimated three billion cycles for the lifespan of the average human [1]. However, increased applied working loads as a result of patient-dependent factors such as stress, high blood pressure, and stenosis can lead to degeneration and premature failure of the native valve [2]. Malfunction of heart valves can also be caused by non-patient-dependent factors such as congenital deformation, inadequate leaflet strength, calcification of leaflets causing stenosis, or inflammation of the valve [1]. The aforementioned pathologies can cause inadequate blood flow through the valve when the leaflets do not fully open, as well as regurgitation when the leaflets do not properly seal the pathway upon closure [3,4]. In modern times, an increasing lifespan has proportionally increased the occurrence of degradation in heart valves. This failure has led to an increased demand for heart valve replacement surgeries [5]. The aortic and mitral valves are more commonly replaced than the tricuspid and pulmonary, with more than 100,000 patients requiring replacement of a diseased or dysfunctional valve every year in the United States [6].

There are currently two categories of replacement heart valves: mechanical heart valves constructed from pyrolitic carbon and bioprosthetic heart valves composed of biological tissue [7]. These heart valves ensure an improved quality of life, but are hindered by a decrease in their viability over time. Mechanical heart valves are more durable, but patients who receive them are administered anticoagulants as long as they have the implant. Bioprosthetic valves are made from animal tissues and materials that are compatible with the body and therefore do not require the administration of anticoagulants post implantation. However, the valves are more likely to degenerate leading to patients requiring revision surgeries [8]. To overcome these issues, researchers have sought to create an entirely tissue-engineered valve capable of integration into the body and remodeling to become living tissue. As a deviation from the ideology of mere disease mitigation, tissue-engineered heart valves promise to be the ultimate fix, serving as a living replacement for a diseased component of the human anatomy

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[9]. Although several groups have demonstrated the feasibility of this new technology using animal studies, there are a number of unmet design problems [10,11]. Therefore, it is pertinent for a new aortic valve design to be fabricated.

The focus of the present study was to create living, tissue engineered heart valves which can surpass the longevity of current artificial heart valves and optimize structural integrity. It was hypothesized that the combination of specific elements are essential in development of a functional tissue engineered heart valve: biomimetic geometry, flexible and durable scaffolds, proper cell seeding, and mechanical stimulation. In this study, gelatin-coated polymer mesh sutured to mimic valve geometry was used as a flexible scaffold and mechanically stimulated in a pulsatile flow bioreactor. Geometric orifice area and movement of the root and cusps without cells seeded were assessed. Fibroblasts were seeded onto the valve materials and were assesed for reaction and ingrowth into the materials. This paper describes the design process in an effort to report both successful and unsuccessful methodologies to assist future tissue engineering heart valve researchers. The final version of the valve, presented herein, is a result of an iterative design process, whereby many fabrication techniques were tested to develop the best method for mimicking the functionality of a native heart valve while also providing optimal durability and biocompatibility.

II. Methods and Materials

A. Valve Design Process

The valve was designed by selecting materials, patterns, and techniques based on effectiveness and ease of assembly. In general, the desired cusp and wall shapes were cut from a mesh, assembled in the form of a tri-leaflet valve, coated with gelatin, and tested to assess valve functionality compared to native valves. The first generation design began with the use of a stainless steel mesh (Small Parts, Inc., Miramar, Florida, USA) that was cut using the contiguous scalloped leaflet template (Figure 1a) and sutured (Ethicon, Inc., Somerville, New Jersey, USA) to the valve wall (Figure 1b). The sutured mesh was then fit onto the silicon mold and coated using a gelatin injection technique (described below). Initial testing in a heart valve bioreactor showed very little cusp movement and an opening in the center of the valve where the cusp edges were not large enough to touch each other upon closing.

The second generation valve was constructed using a high-density biodegradable polymer mesh sutured together to create the valve scaffold. We used the contiguous peaked leaflet template (Figure 1c) to allow the cusps to touch in the center, and finalized it with the gelatin injection technique. This valve was tested in the heart valve bioreactor (see description below) and showed adequate closure. However, cusp movement was not ideal due to an accumulation of gelatin at the base of each leaflet. For this reason, a gelatin submersion technique (see description below) was developed and replaced the injection technique to provide a thinner layer of gelatin that would better facilitate cusp movement.

This new gelatin submersion technique was used in combination with a more pliable mesh of the same material that had a lower density (AM6-A) and a new design: the individual

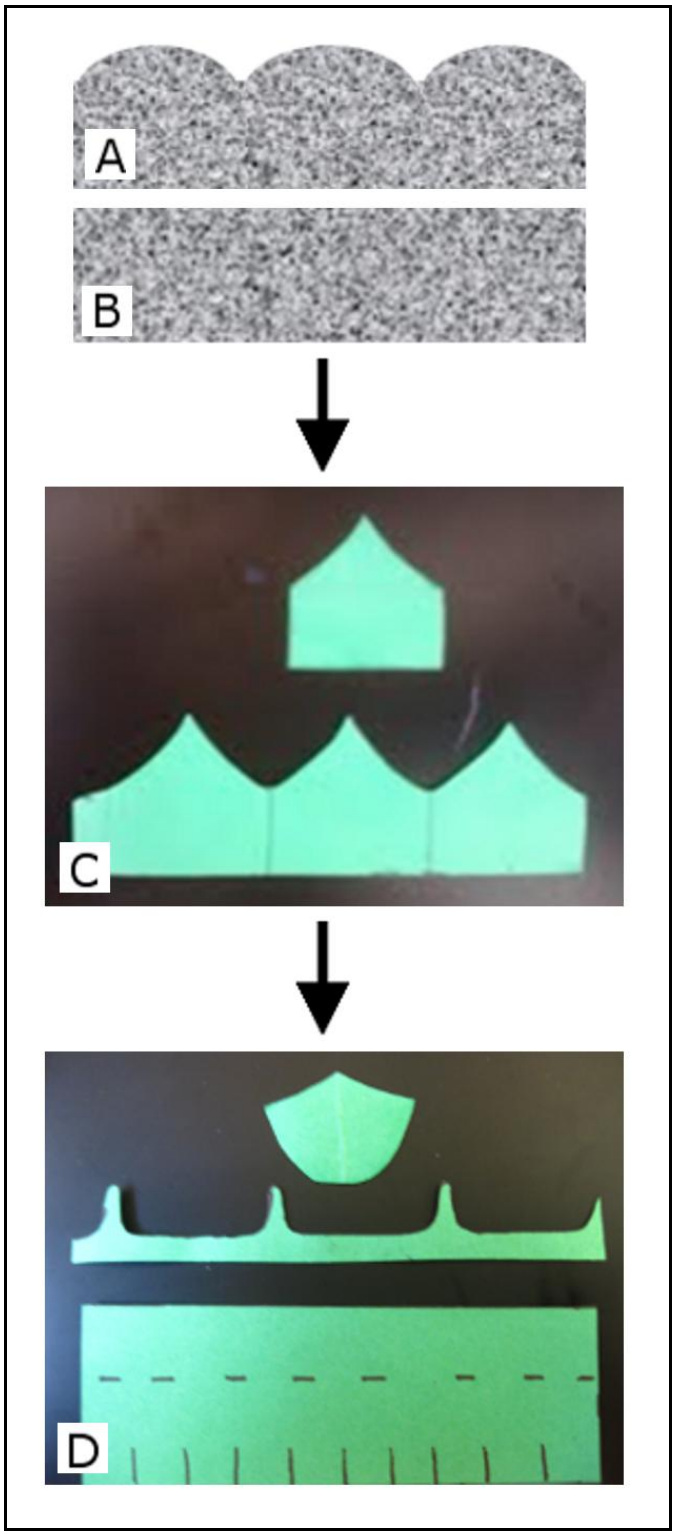

Fig. 1. Leaflet Templates
(2a, b) Contiguous scalloped leaflet design. (2c) Valve root design as used with original continuous scalloped leaflet design. (2d) Individual peaked leaflet design with valve root and valve wall.

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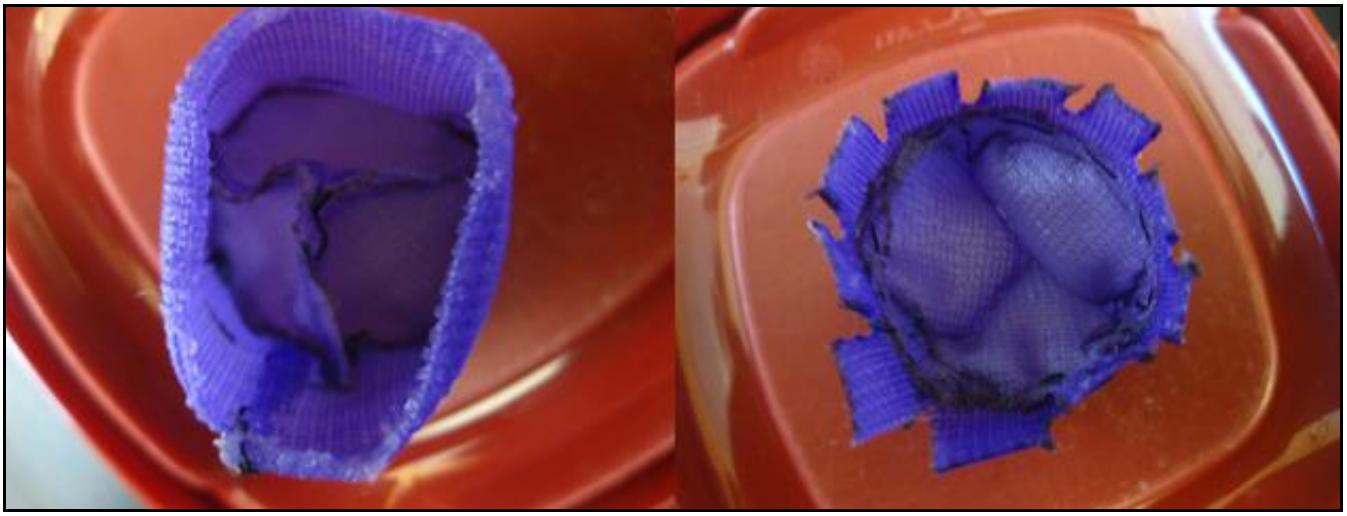

Fig. 2. Dipped Mesh Scaffold
Apical view (left) and basal view (right) of valve scaffold after dipping showing feet in basal view.

peaked leaflet template (Figure 1d). This new leaflet template was designed to create more freely flowing movement of the valve with the use of individual leaflets. After testing, these selections were seen to provide optimal valve function and served as the third generation valve.

To further improve function and ease of assembly, a number of different meshes and attachment methods were tested. These included a 19 courses per inch (cpi) low tension reverse locknit, a 28 cpi low tension reverse locknit, and a 40 cpi low tension tricot (generously donated by Poly-Med, Inc., Anderson, SC). Mesh materials and architecture were analyzed for valve functionality as well as cellular compatibility. In addition, the suturing method of assembly was compared with a cauterizing method and a combined suturing/cauterizing method.

Gelation Injection Technique

For non-sterile studies, Knox gelatin (Kraft Foods, Tarrytown, New York, USA) was dissolved in boiling water on a hot plate at a concentration of 0.1 g/mL and 2% antibiotic-antimycotic solution (Mediatech, Inc., Manassas, Virginia, USA) was added after cooling. Once the mixture thickened, it was injected into a silicone mold containing the mesh framework and wrapped in plastic piping to prevent gelatin from seeping out. The mold was then stored at 4°C in a refrigerator for 24 hours to allow thermal gelation to occur.

Gelation Submersion Technique

Gelatin was prepared in the same method as the gelation injection technique. Using tweezers to hold the feet of the valve wall, the valve was submerged in the gelatin. After each submersion, a paintbrush was used to evenly distribute gelatin on the valve and avoid undesirable gelatin accumulation at the base of the leaflets. Submersion and painting was repeated four times and the valve was allowed to set for three minutes. After setting, the valve was submerged and painted three more times to allow for sufficient coverage. The valve was stored in a humidifier box at 4°C for 24 hours in an inverted position to encourage the leaflets to remain in the closed position post thermal gelation.

B. Bioreactor Studies

To effectively reproduce and measure the effects of the human body on the proposed tissue-engineered heart valve, a pulsatile flow bioreactor designed for heart valves [12] was used. The entire assembly has been tested thoroughly and has performed consistently in more than 30 experiments with various valve designs [13]. It allowed for testing the opening and closing of leaflets in order to qualitatively assess similarities to native leaflet movement.

The bioreactor was designed to allow for mounting valves of various sizes and shapes, as well as allow proper exposure to mechanical stimuli for preconditioning of valves. To mount a valve in this bioreactor, the valve base must be clamped between two o-rings. For these experiments, the valve design consists of a valve wall with attachment feet (Figure 2) that allow for easy installation into this bioreactor.

C. Manual Heart Valve Tester

This novel tester is intended for quick assembly and testing of valves to assess integrity and function. By requiring fewer steps during its assembly than the bioreactor (described above), it provides a faster alternative when evaluating new valve designs. As shown in Figure 3, the primary components in the design are: a ventricular chamber, bracing rings, an aortic chamber, and a cap. The aortic chamber is attached to the ventricular chamber via three adjustable draw latches that can quickly secure the two chambers while allowing for adjustments in height for different valve designs. For convenience, the tester was designed to utilize the same set of bracing rings as used in the bioreactor described by Sierad, et al., which are placed between the two chambers. Three screws are used to ensure the cap stays in the correct position and a seal is maintained. To manipulate the valve, the user operates two bellows so that when the ventricular bellow is squeezed (collapsed), fluid moves through the valve into the aortic chamber and the valve opens. The user then collapses the aortic

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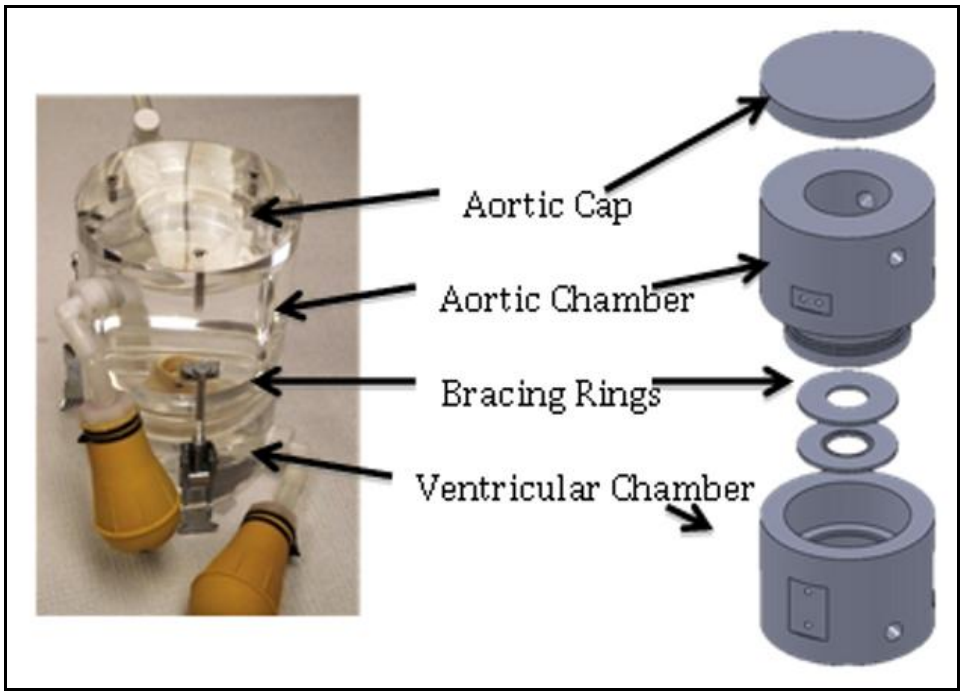

Fig. 3. Manual Heart Valve Tester
This tester can be very quickly assembled and is used for quick valve testing. Valves are placed between the bracing rings and the metal latched hold all other components together with a quick latch. The two rubber bellows are used to pump fluid through the valve to evaluate opening and closing.

bellow to shut the valve and the fluid travels through the external tubing to return to the ventricular chamber. The components of the tester in contact with fluid are made entirely from clear acrylic and are easy to sterilize. This allows the valve being tested to be clearly viewed from all angles.

D. Analysis of Geometric Orifice Area

One important measure of valve functionality is the geometric orifice area (GOA). GOA is the area between the leaflets when a valve is in the open position. GOA relates to the amount of work it takes for the heart to push blood through the valve. The larger the orifice, the more blood can go through with one pump of the heart and the less work required when compared to a smaller opening.

Geometric orifice area was calculated using ImageJ software (National Institutes of Health) for valves consisting of high-density bioabsorbable polymer mesh sutured using the contiguous scalloped leaflet template (second generation valves) and the thin mesh sutured using the individual peaked leaflet template (third generation valves). The GOA for an aortic porcine valve was calculated as a control comparison. Images were taken of the valves during testing in the pulsatile flow bioreactor. From a series of images taken, images of the valve when open were collected and analyzed using ImageJ. The images were made binary and inverted so that the valve opening was black. A freehand selection drawing was made around the opening and the 'analyze particles' command was used to calculate the amount of black space in the freehand selection. The particle size was set to 50-infinity pixels^2 and the circularity was 0-1.00. The area reading was recorded. The oval selection command was used to evaluate the maximum geometric orifice area possible if the leaflets were fully open (the inner diameter of the valve). This evaluation was performed using the 'measure' command. The geometric orifice area was divided by the total area possible and multiplied by 100. In this study, GOA is represented as a percentage of the total possible opening of the valve.

E. Cell Study

To test cytotoxicity and cell integration of the low-density bioabsorbable polymer mesh coated with gelatin (used in the third generation valves), a 3-week cell study was performed. 12 samples of the mesh were cut into 1 cm^2 pieces, sterilized with ethylene oxide, and coated with gelatin using the gelatin submersion technique. Once coated, the samples were placed in a 12-well plate, one sample per well. After thermal gelation, the gelatin coated mesh samples were crosslinked using 0.075% glutaraldehyde in 0.1 M Hepes buffered saline at pH 7.4 for 24 hours. The glutaraldehyde was then removed and replaced with a 1:1 ratio of Fetal Bovine Serum (FBS) to Dulbecco's Modified Eagle's Medium (DMEM) for 24 hours for neutralization of glutaraldehyde residues. After the allotted time, the FBS/DMEM was removed and the mesh was coated in glycine for 2 hours. 3T3 fibroblasts were seeded at a seeding density of 100,000 cells/well in DMEM/10%FBS media. After 3, 7, 14, and 21 days, samples were examined using the Live/Dead assay (Invitrogen) and Hematoxylin and Eosin staining. Samples without cells served as a control.

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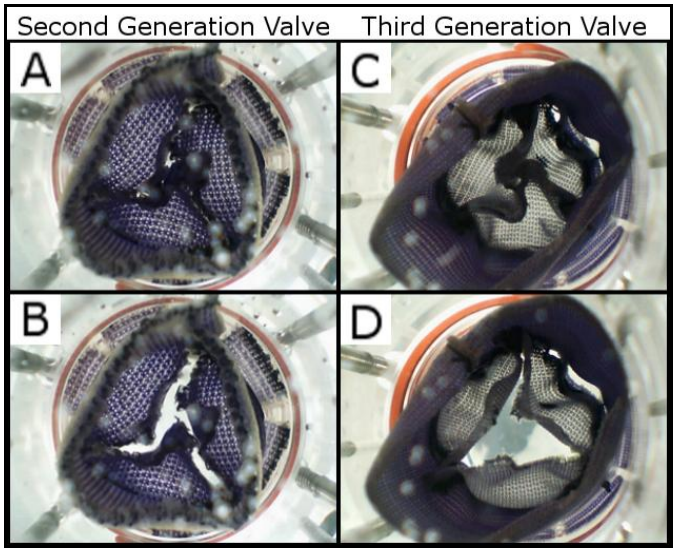

Fig. 4. Biroeactor Testing of Mesh Valves
Apical view of the second and third generation valves in the bioreactor, showing the inability of the initial valves to fully close and open.

III. Results

A. Geometric Orifice Area

Figure 4 shows one of the initial valves mounted in the bioreactor. Looking closely, you can see spaces between the cusps, showing the inability of the initial valves to completely coapt. For the low-density bioabsorbable polymer mesh valve made using the individual peaked leaflet template (third generation valve), the average percent opening was 16.6% for the images from the first day and 15.7% for the second day. A student's T test determined that there was no significant difference in GOA between the first and second day. This shows consistency in the valve over the course of the time spent in the bioreactor. The average percent opening for the high-density bioabsorbable polymer mesh valve made using the contiguous scalloped leaflet template (second generation valve) was 6.6%. The average percent opening for the decellularized porcine valve was 15.4%. There was no significant difference in average percent opening between the low-density bioabsorbable polymer mesh valve (third generation valve) and the porcine valve. These results reveal that the GOA of the low-density bioabsorbable polymer mesh valve (third generation valve) is comparable to that of the porcine valve and there is a significant difference between the high-density bioabsorbable polymer mesh valve (second generation valve) and the porcine valve showing a large increase in valve functionality in the transition to a thinner mesh and a different scaffold shape.

B. Cell Study

The results of the LIVE/DEAD Viability study are shown in Figure 5. The images are overlays of the green and red fluorescent images created using ImageJ software. The results of Day 3 and Day 7 showed only a few dead cells and a large amount of live cells surrounding the low-density bioabsorbable polymer mesh. Day 14 and Day 21 had an increased amount of dead cells, but still showed an abundance of live cells surrounding and incorporating within the mesh fibers.

The results of the hematoxylin and eosin stains are shown in Figure 6. The controls and samples were stained and observed under bright field microscopy. The results of cellular integration with the mesh were inconclusive, but the images depict the gelatin in purple integrating throughout the circular polymer mesh fibers.

IV. Discussion

A. Analysis of Valve Efficacy

The first iteration of the valve, constructed with a stainless steel scaffold, was tested in a pulsatile flow bioreactor for 3 days. This valve exhibited a small geometric orifice area. Bioreactor testing showed that motion was hindered by an accumulation of gelatin at the base of each leaflet. Further, complete coaptation was not evident and the leaflets did not display biomimetic motion. These problems can be attributed to the choice of material and the template used to make the valve. Understanding that this valve exhibited poor compliance to flow lead to the development of a new valve design that more accurately mimics the native valve.

In the second iteration, the high-density bioabsorbable polymer scaffold used a contiguous peaked architecture that attempted to better represent native leaflets. This valve, however, showed poor movement due to an accumulation of gelatin at the base of each leaflet, preventing proper motion in vitro. It was determined that the gelatin injection technique applied more gelatin than desirable for geometric leaflet motion.

In comparison with the stainless steel scaffold, the second iteration using the high-density bioabsorbable polymer scaffold appeared to allow sufficient motion, but valve architecture was still a hindrance to valve movement and coaptation. It was determined that a contiguous design was preventing valve

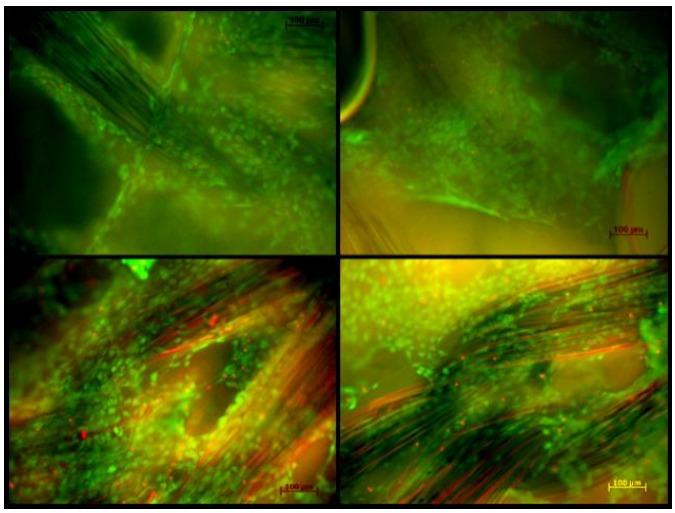

Fig. 5. Cell Coverage on the Scaffold
Live/Dead Images at (clockwise from top left) 3 Days, 7 Days, 14 Days, and 21 Days.

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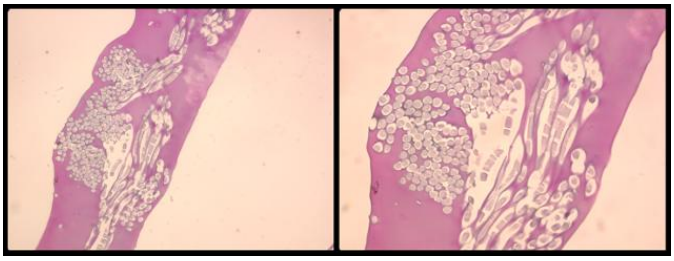

Fig. 6. Gelatin Integration Around Scaffold
Hematoxylin & Eosin sample showing gelatin integration at 100x (left) and 200x (right).

responsiveness to flow and leading to fabrication of a stenotic valve. With these results in mind, alternatives for gelatin coating, as well as polymer mesh density and leaflet architecture were considered. A gelatin submersion technique, as well as a single peaked leaflet design and use of more porous polymers were developed for the third iteration of the valve.

In the third iteration valve using low-density bioabsorbable polymer scaffold, each leaflet was constructed with a mesh of different courses per inch (cpi), allowing for monitoring of individual leaflet motion. Bioreactor analysis suggested that improvements were made by switching from the stainless steel to a polymer mesh, but more improvements are necessary to reach a fully functional tissue-engineered heart valve.

The third valve, a low-density bioabsorbable polymer scaffold, addressed the issues discussed above and the tests were more successful. The results of testing in the bioreactor showed an increase in the GOA of the valve during opening. The low-density bioabsorbable polymer scaffold leaflets presented mechanical properties similar to native heart valve leaflets, as a result of their compliance to flow. Tight closure of the leaflets was observed with little or no backflow.

V. Conclusions

Our data suggests that by using the individual peaked leaflet design, the geometry and movement of our valve more closely mimicked that of a native human aortic valve. Combining a low-density, bioabsorbable mesh with a gelatin submersion technique allowed for an increased GOA as well as more natural and flexible physiological motion of the scaffold. Further advancements should include modification of suturing techniques and improved gelation techniques to produce a more compliant scaffold while maintaining structure integrity, thus increasing GOA.

Growth of fibroblast cells on the gelatin and mesh scaffold demonstrated that the chosen materials could foster the growth of tissue. The growth of new tissue in parallel with scaffold degradation will allow for preservation of the original valve architecture and ensure normal blood flow. Future work includes the growth of endothelial cells, the native blood contacting surface, on the low-density bioabsorbable polymer scaffold and exposing this construct to mechanical stimulation in the bioreactor. Mechanostimulation is known to promote the growth of cells, and would lead to an improved design.

VI. Acknowledgments

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