A COMPARATIVE STUDY BETWEEN FETAL AND CALF BOVINE PERICARDIUM

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ABSTRACT

There is a need of a lower profile device of transcatheter heart valves. One way to simply achieve this task is to decrease the size of the device by using a thinner bovine pericardium. The devices currently use calf bovine pericardium which is reported to have a higher tissue thickness compared to that of fetal bovine pericardium. This provided the opportunity to test fetal bovine pericardium as a viable alternative. Uniaxial testing and histology were performed to determine the mechanical and structural properties of both groups (n=10 for each group). We report a statistical difference in ultimate tensile strength and strain at failure between both groups but no statistical difference was found for collagen composition between the two groups. With these results, we are unable to prove fetal bovine pericardium to be a viable alternative. However, there were a couple of outliers from the fetal bovine pericardium had smaller thickness that showed higher ultimate tensile strength and strain at failure compared to those of calf bovine pericardium. In the future, it would provide an opportunity in developing lower profile device by further investigating the cause of this incident.

CHAPTER 1

INTRODUCTION

Cardiovascular disease remains the number one cause of death[1], and among industrialized nations, calcific aortic valve disease (CAVD) is the most prevalent form of cardiovascular disease. Traditionally, surgical aortic valve replacement (SAVR) has been the gold standard for treatment of CAVD, however, over the past decade, transcatheter aortic valve replacement (TAVR) has emerged as a viable alternative for high-risk patients who have been excluded from surgical candidacy due to comorbidities[2]. TAVR uses a transfemoral or transapical catheter approach to deploy a balloon-expandable or self-expanding stent attached to artificial heart valve leaflets constructed from calf bovine pericardium (CBP) or porcine valve tissue.

The use of smaller low-profile valves and catheter systems allows for smaller incisions and easier advancement of the catheter and valves through small and/or tortuous arteries. However, the size of the valve and the delivery catheter systems are limited by the thickness of the tissue used in the valve leaflets and the crimping profile of the stent. The Edwards SAPIEN transcatheter heart valve and the Medtronic CoreValve represent two of the most commonly used TAVR devices. The SAPIEN valve is available in two sizes, 23 mm and 26 mm, and uses transfemoral delivery with sheaths of 22 Fr and 24 Fr internal diameters, respectively[2]. A newer SAPIEN XT valve utilizes a new leaflet geometry allowing for 16 Fr and 19 Fr delivery sheaths. The next-generation SAPIEN 3 valves further reduces the device profile allowing for delivery sheaths as small as 14 Fr. The

Medtronic CoreValve and CoreValve Evolut both utilize an 18 Fr delivery sheath for transfemoral access for all valve sizes.

In the rapidly growing field of TAVR devices it is clear that the industry is trending towards lower-profile devices that minimize incisions to access vessels, facilitate easier advancement of TAVR devices to the aortic annulus and minimize major vascular complications (vascular dissection, perforation and access-site hematoma). The objective of this study is to investigate an alternative material to use in transcatheter heart valves manufacture that exhibits a similar mechanical response to CBP, despite having a decreased thickness. It is hypothesized that fetal bovine pericardium (FBP) can offer similar mechanical properties and morphology with much lower thickness compared to CBP.

Literature Review

Among cardiovascular diseases, calcification of aortic valve is the most common cause of aortic stenosis in adults more than 75 years old in industrialized countries. However, calcification of aortic valve rarely occurs in people less than 50 years of age[3]. Traditionally, the gold standard for treating aortic stenosis is surgical aortic valve replacement[2]. Unfortunately, SAVR is, in many cases, not a viable option in the old patient, which is the group where calcific aortic valve disease is most prevalent. These patients are considered unsuitable for SAVR because of the high risk involved in open-heart surgery. Physicians often do not offer this method or patients themselves refuse the surgery[4]. As a viable alternative to SAVR, TAVR is offered for those high-risk patients who have been excluded from surgical candidacy[3,4]. TAVR is an evolving technology and medical procedure that has been developed with high-risk and inoperable patients suffering

from calcific aortic valve disease or calcific aortic stenosis. The Edwards SAPIEN transcatheter heart valve is one of the most commonly used TAVR devices. The SAPIEN valve is available in two sizes, 23 mm and 26mm[2]. The use of smaller low-profile valves and catheter systems allows for smaller incisions and easier advancement of the catheter and valves through small and/or tortuous arteries. However, the size of the valve and the delivery catheter systems are limited by the thickness of the tissue used in the valve leaflets and the crimping profile of the stent. Up to this point, calf bovine pericardium is the most commonly used bioprosthetic heart valve (BHV) and little research has been done investigating the use of FBP as an alternative[5]. FBP has a lower thickness compared to CBP. By proving FBP as a viable replacement for CBP as a BHV, smaller low-profile valves are possible, thereby improving the quality of life of the patients and introducing the technology to a broader group.

As valve replacement surgery outcomes are generally very good, there is almost no contraindication to valve replacement for aortic stenosis[6]. However, older patients have more comorbidity, and they are unlikely to survive open-heart surgery. A study by Iung et al. has shown that surgery is denied in as many as 33% of elderly candidates[7]. Therefore, it is crucial to prove that TAVR is in fact a viable alternative to the traditional golden standard for aortic valve replacement. There have been a number of clinical trials from different countries, including randomized trials comparing TAVR to classical aortic valve replacement[8].

Cribier's research in 2002 provides the results of the first TAVR of aortic valve prosthesis for calcific aortic stenosis[9]. This study was performed in order to identify patients' health after they received TAVR. Although the study had a limited sample size

and is considered to be a single-center study, it was a good study and presented hopeful results. In 2010, the largest TAVR registry in Europe at that time was created in France[2]. A total of 3195 patients were enrolled and Edwards SAPIEN systems were implanted. Gilard et al. reported the results of this prospective multicenter study. The survival rate in this archive resembled the rates in the SOURCE Registry, UK registries, and cohort A of the PARTNER Trial (Placement of AoRtic TraNscathetER Valves)[10]. The PARTNER trial was the world's first randomized and controlled study to test the safety and effectiveness of transcatheter heart valves and the delivery system in people with severe aortic stenosis. This trial had two groups: the high-risk operable patient (cohort A) and the extreme-risk or inoperable patient (cohort B). The high-risk patients were randomized to TAVR or sAVR. The results of these studies come to the conclusion that TAVR is not inferior to classical aortic valve replacement in terms of procedure success and short-term morbidity and mortality.

Unfortunately, TAVR is not a perfect solution for every patient. Like any new procedure, TAVR is associated with predictable and unanticipated complications. One of the most common complications is the vascular complication, which may contribute to procedural mortality[11]. This is particularly true of the transfemoral approach where the valve and catheter are inserted via the femoral artery. The risk is greatest in patients with significant peripheral vascular disease and vascular injury is related to the large-caliber sheaths used for device deployment[12]. These probable vascular injuries may lead to significant bleeding at the insertion site and even lead to mortality. There is an effort in miniaturizing the valves in hopes of decreasing the rate of vascular complications. Bovine pericardium is used for heart valve leaflet replacement where the strength and thinness are critical properties.

Several researches have been conducted focusing on the type of tissue used in such devices. Sizeland et al. focused on pericardium from neonatal animals (4–7 days old.) Their thinner pericardium is considered as an alternative to the valves used in current devices[13]. This study shows that the tissue of neonatal animals shares mechanical properties and collagen structures that are compliant to those of adults or calves. However, this study fails to quantify the different types of collagen present in the tissue. Bovine pericardium is a fibrous collagen extracellular matrix material with structural similarities to skin and other tissues[14]. According to Julkenen's study, methods have been used to study collagen fibril orientation using polarized light microscopy[15]. And it is found that there exists a function-structure relationship between collagen alignment and mechanical strength[16]. Collagen tissue properties change over time and tissue strength varies depending on the collagen fibril diameter[17]. The structural differences between calf bovine pericardium and fetal bovine pericardium tissue that may present desirable differences in their physical properties have not been adequately investigated. This study investigated the structural differences between calf and fetal bovine pericardium.

CHAPTER 2

METHODOLOGY

Sample Preparation

The bovine pericardium tissues were transported to the laboratory in cold isotonic saline (0.9% sodium chloride). Any extraneous fat was removed from the tissues. Then these tissues were mounted in a framed and immersed in a 0.65% glutaraldehyde solution in a phosphate buffer at pH 7.4. The patches were then sterilized in a solution of 2% glutaraldehyde, 4% formaldehyde and 20% ethanol in the same pH 7.4 buffer for 24 hours. The sheets of pericardium were trimmed to dimensions predetermined by the manufacturer and placed in a container with 0.25% glutaraldehyde for storage. Before storing the tissues, the thickness of each tissue fragment was recorded by measuring a series of ten points, using a Mitutoyo micrometer (elecount, series E:A33/8 Digital). All of the trials were carried out at room temperature of 22 to 24°C.

Uniaxial Testing

Cutting and Marking Samples

The bovine pericardium tissues were cut into a dog-bone shape. This was done so that the when testing, the rupture will occur in the center of the sample where data acquisition is optimal. Then we placed four small markers on the sample to track its elongation/strain using Labview during image analysis. Figure 1 shows a representation of the prepared sample.

Figure 1. Image of dog-bone shaped sample with four markers

Preconditioning

Before testing the samples until rupture, each sample went through a process of preconditioning. Preconditioning went through ten cycles of slightly stretching and relaxing the tissue using a preprogrammed command of the linear actuator (TestResources 200Q Universal Electromechanical Test Machine) at a displacement rate of 50 mm/min. The amount of load of stretching was determined by 10% of maximum load before rupturing the sample. Preconditioning the sample was performed in order to imitate real life conditions.

Uniaxial Testing

Immediately after precondition the samples, the specimens were loaded to failure at a displacement rate of 50 mm/min. The ultimate tensile strength (UTS) and failure strain were defined by the peak stress and maximum deformation withstood by the specimens prior to failure. Stress was calculated by dividing the recorded load by the cross-sectional area of the sample determined prior of the testing. Stress vs. strain curves were plotted and analyzed using a Matlab script.

Histology

<u>Preparation</u>

Ten samples from each group, CBP and FBP, were cut into a 2 to 3 mm wide and 15 mm long rectangle from different locations of the bovine pericardium tissue. The next step was to place these samples in a small plastic box filled with OCT (shown in Figure 2) which is a freezing medium that helps protect the tissue drying and supports the tissue while sectioning. Then plastic boxes were placed in a Styrofoam container and poured liquid nitrogen for at least ten minutes (Figure 3). Once frozen, samples were taken out of the box and sliced the samples for 5μm using the Cryostat (Thermo Scientific CryoStar NX70) and placed onto a glass slide.
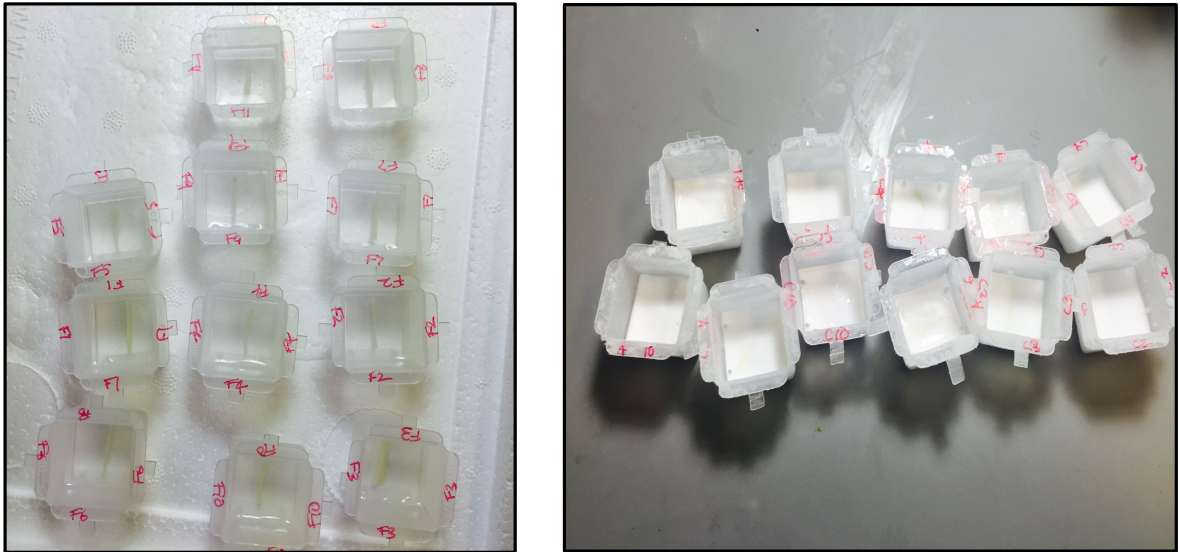
**Figure 2. Samples placed in OCT compound prior to freezing (left).
.Sample blocks after freezing (right).**

<u>Staining</u>

These slides were then stained in Picro-Sirius Red solution for one hour. This gives near-equilibrium staining and shorter times should not be used even if the colors look okay. The next procedure was to rinse the slides in two changes of acetic acid solution and dehydrating in three changes of 100% alcohol. After dehydrating the slides, they were cleared with 2-3 changes of xylene and applied a coverslip over the slide using resinous mounting medium. Picro-Sirius stain is used to stain collagen present in the tissues. Studies show that using Picro-Sirius red stain enhances the detection of collagen when in use of polarized microscopy. Using this stain also shows different colors between collagen Type I and collagen Type III[18].

<u>Microscopic observation</u>

Once the mounting medium was completely dried, the specimens were observed under polarized light using bright field microscope (Zeiss Scope. A1 Axio). Larger collagen fibers are bright yellow or orange, and the thinner ones, including reticular fibers, are green[19].

<u>Color Quantification</u>

Images were processed using ImageJ for a clearer background. The color composition of red, orange, yellow and green was calculated by using Matlab in order to determine the samples' different types collagen composition. We only recorded the hue component that contained 256 possible colors. First we defined the hue values for the specific colors. Red: hue values 2-9 & 230-256; orange: hue values 10-38; yellow: hue values 39-51; and green: hue values 52-128[20]. Any hue values that were not included was considered to be interstitial space confirmed by inspection. We determined the number of

pixels for each hue range and added the them all up to express the total collagen content. Then we calculated the composition for each color by dividing the number of pixels for each color by the total collagen content.

CHAPTER 3

RESULTS

Tissue Thickness and Maximum Load

The following table shows data of the tissue thickness and maximum load (Table 1). The average thickness as well as the average of maximum load were greater with the calf bovine pericardium. Statistical test comparing the thickness between the CBP and FBP using a simple t-test ($P \leq 0.001$) and showed a significant difference between the groups (mean: 0.3214, 0.2312; standard deviation: 0.0279, 0.0158, respectively).

CBP			FBP		
Sample	Thickness (mm)	Load (g)	Sample	Thickness (mm)	Load (g)
CBP 1	0.3	1319	FBP 1	0.235	598
CBP 2	0.367	1940	FBP 2	0.23	356
CBP 3	0.332	1353	FBP 3	0.245	600
CBP 4	0.347	1285	FBP 4	0.22	784
CBP 5	0.302	1344	FBP 5	0.26	838
CBP 6	0.29	2111	FBP 6	0.235	363
CBP 7	0.277	1425	FBP 7	0.195	325
CBP 8	0.312	2075	FBP 8	0.23	711
CBP 9	0.337	1031	FBP 9	0.225	859
CBP 10	0.35	2035	FBP 10	0.235	431

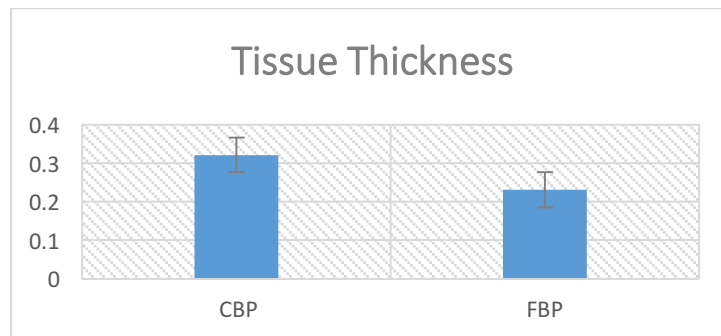**Table 1. Tissue Thickness and Maximum Load of CBP and FBP.**

Figure 3. Tissue Thickness of CBP and FBP.

Ultimate Tensile Strength and Strain of Failure

Ultimate tensile strength (UTS) and strain at failure were determined of the maximum stress and deformation prior to failure, respectively, by examining the stress vs strain curve of each sample. Figure 4 represents a stress vs strain curve of CBP and FBP. A simple t-test was performed (P≤ 0.001) between the CBP and FBP of UTS (mean: 22.438, 12.324; standard deviation: 5.619, 4.352, respectively.) and strain of failure (mean: 0.460, 0.608; standard deviation: 0.0901, 0.0704, respectively) determined a statistically significant difference between the two groups. (shown in Table 2, Figure 5)
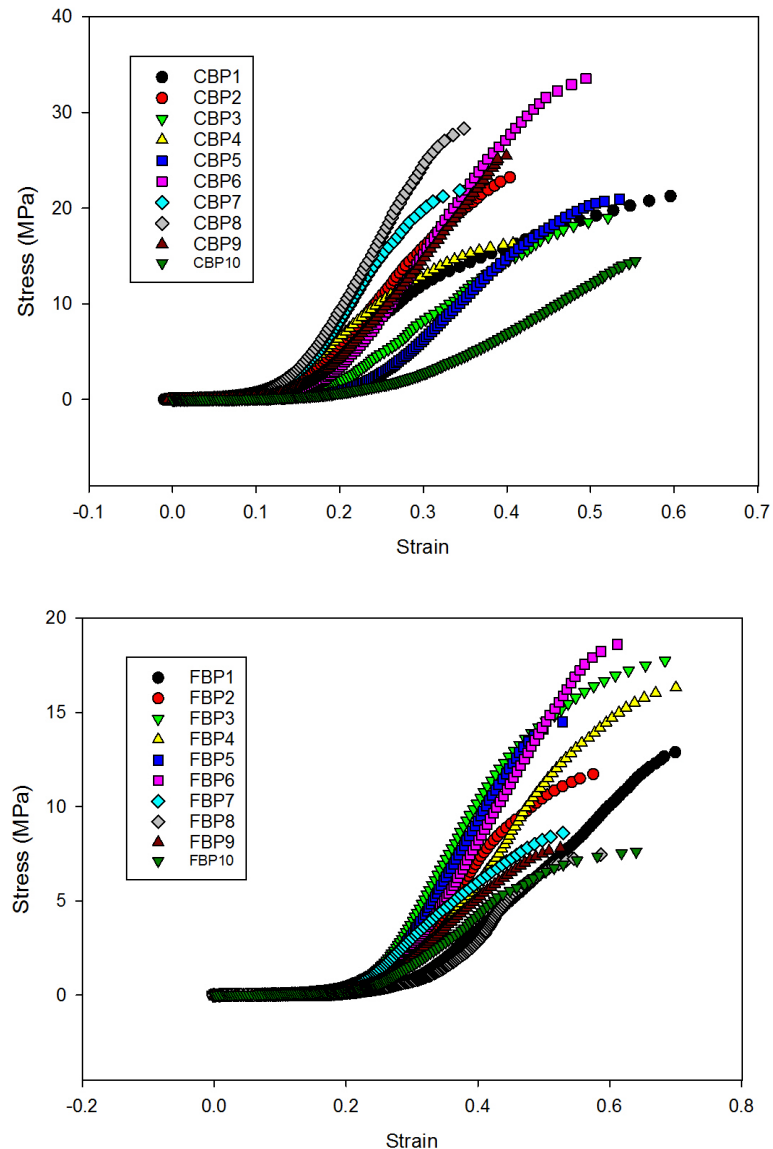

Figure 4. Stress vs Strain curve of CBP (top) and FBP (bottom).

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CBP			FBP		
Sample	UTS (MPa)	Strain	Sample	UTS (MPa)	Strain
CBP 1	21.26	0.5956	FBP 1	12.88	0.6993
CBP 2	23.22	0.4036	FBP 2	11.72	0.5749
CBP 3	19.03	0.5208	FBP 3	17.75	0.6837
CBP 4	16.31	0.4074	FBP 4	16.31	0.7002
CBP 5	20.92	0.5346	FBP 5	14.49	0.5286
CBP 6	33.54	0.4945	FBP 6	18.61	0.6114
CBP 7	21.84	0.3437	FBP 7	8.59	0.5292
CBP 8	28.31	0.3486	FBP 8	7.45	0.5862
CBP 9	24.73	0.3994	FBP 9	7.79	0.5252
CBP 10	14.78	0.5535	FBP 10	7.62	0.6401

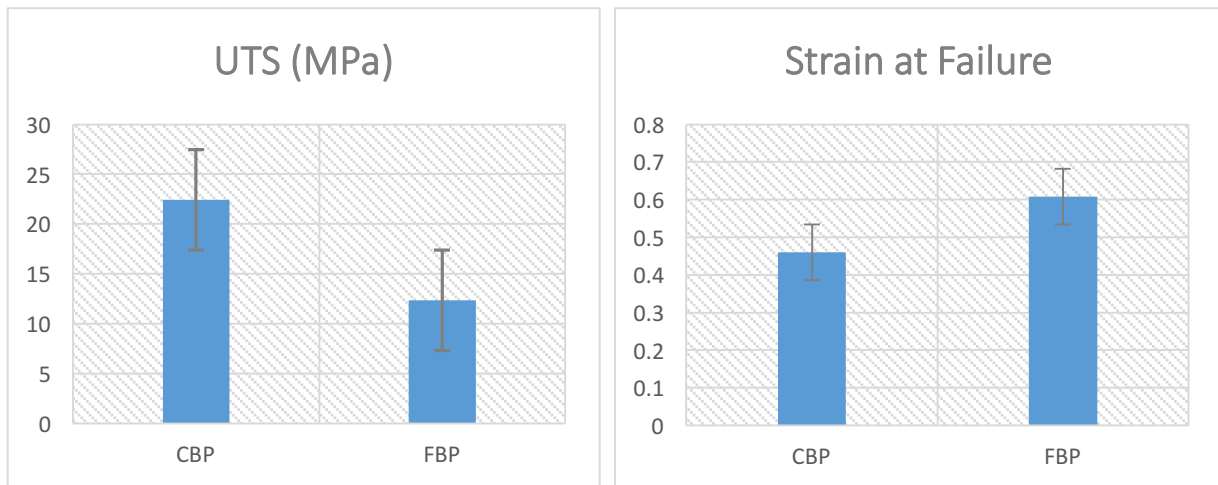**Table 2. UTS and strain at failure for CBP and FBP.**

Figure 5. Statistically significant difference was found using t-test $(P \leq 0.001)$ for both UTS and strain at failure between the groups.

Collagen Composition

Images were taken using polarized light to identify collagen compositions (Figure 6). It was determined that there were no statistical differences between the two groups of the colors red, orange and yellow. However, there was a statistical difference (P=0.008) between the groups of the color green (Figure 7).
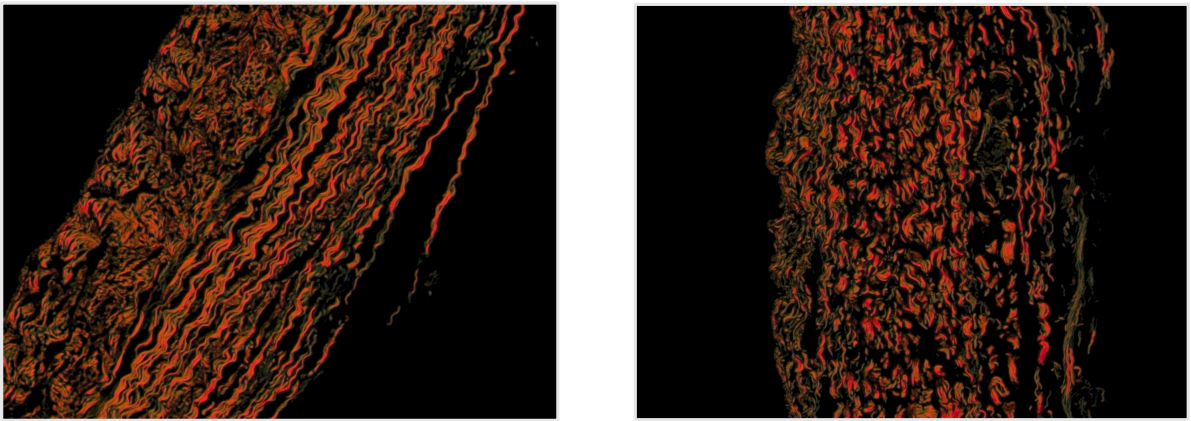

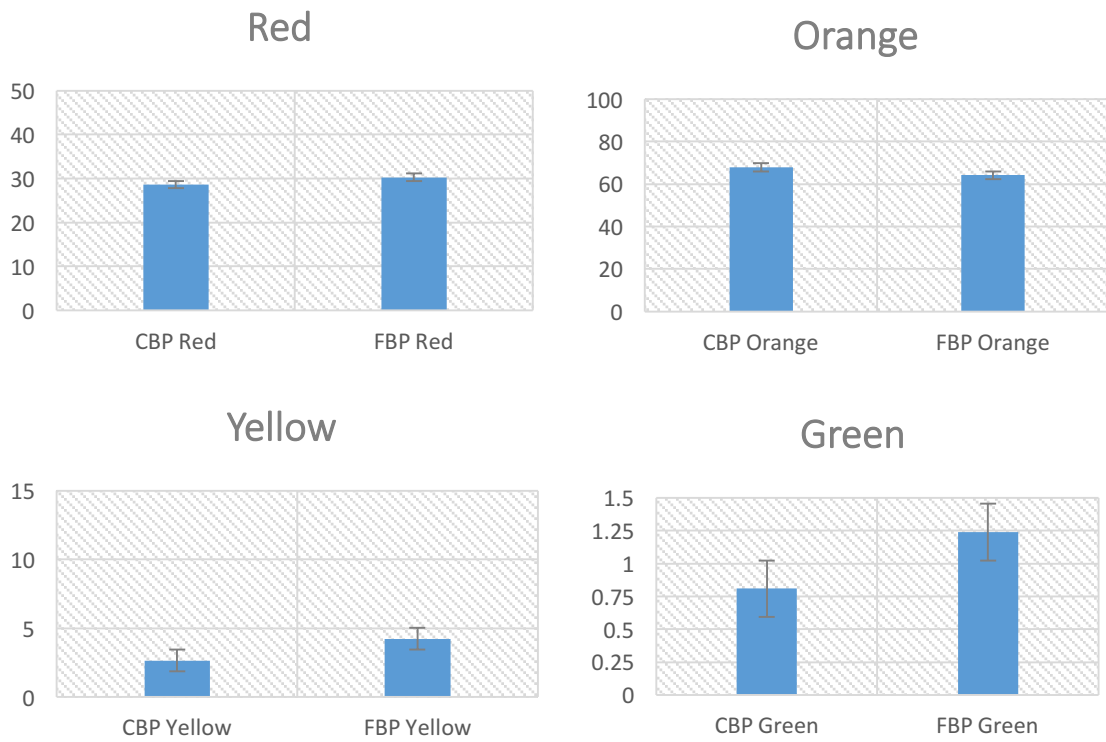**Figure 6. Image of CBP4 (left) and FBP7 (right) taken under polarized light after staining with Picro Sirius Red.**

Figure 7. No statistical difference found for red, orange and yellow. Significant difference found for green (P=0.008).

CHAPTER 4

DISCUSSION

Mechanical Properties

We conducted uniaxial testing in order to find the mechanical properties of bovine pericardium. We looked at the ultimate tensile strength and strain at failure to identify mechanical properties of each group. Given the significant differences in ultimate tensile strength and strain at failure between CBP and FBP ($P \leq 0.001$), and the fact that they had significant differences in thickness as well, it was determined that the two groups had different mechanical properties. It was observed that the ultimate tensile strength differed almost twice (CBP: 22.438 MPa and FBP: 12.324MPa) but only differed less than 30% in tissue thickness (CBP: 0.3214 mm and FBP: 0.2312 mm). Looking at the results we can see that with higher thickness associates a higher ultimate tensile strength ranging from 14 MPa to over 33 MPa. However, there is also a trend of decreasing strain at failure with increasing ultimate tensile strength (Figure 8). We can interpret this data as for the tissues that withstand a higher stress will not elongate as much before they rupture.
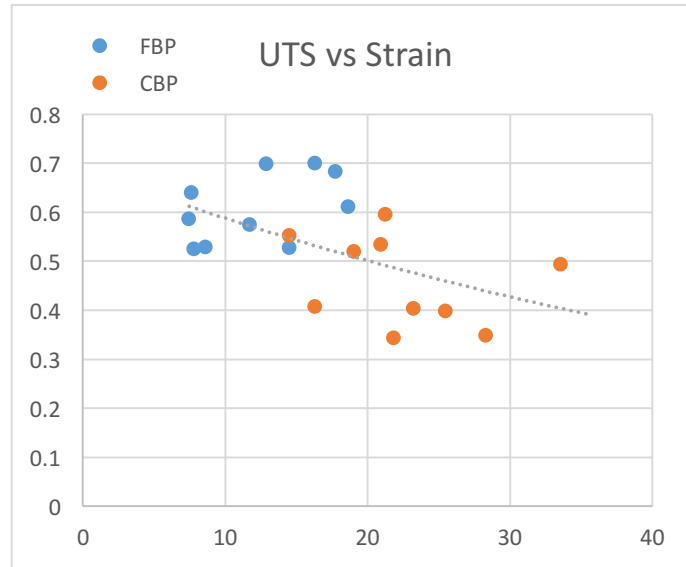
Figure 8. UTS vs Strain

Structural Properties

Histology was performed to identify structural properties of bovine pericardium. We used Picro Sirius to stain the tissue samples to look at their collagen compositions under polarized light. Studies show that using Picro-Sirius stain enhances the detection of collagen when in use of polarized microscopy. We calculated the percent composition of the specific colors as follows: CBP (Red: 28.09%, Orange: 59.53%, Yellow: 2.67%, Green: 0.81%) FBP (Red: 27.87%, Orange: 65.90%, Yellow: 4.36%, Green: 1.36%). The color orange had the most collagen composition having nearly or over 60% for both bovine groups. There was no statistically significant difference found between them. The least composition was found in the color green which composed less than 2% of the whole sample and had a statistical difference between the groups through a t-test statistical test ($P < 0.05$).

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CHAPTER 5

CONCLUSION

In this study, two types of bovine pericardium were tested through uniaxial testing and and histology to identify its viability of an alternative material for prosthetic heart valves. We hypothesized that calf and fetal bovine pericardium will have similar mechanical and structural properties. However, though the experiment it was shown that there was a significant difference in mechanical properties between the two groups. Our hypothesis was rejected and we did not have sufficient data to show that FBP can be a viable alternative for CBP used in prosthetic heart valves although they had similar collagen compositions.

Future Work

Although we tried to emulate real life conditions during our testing, the tissue sample was constantly exposed in air during uniaxial testing. In the future, we wish to perform this test similar to the real life conditions. When cutting the tissues into dog-bone shapes, we did not consider the orientation of the fibers. We think that the orientation of fiber, whether cut perpendicularly or in parallel, will affect the results. We have identified incidents where samples from FBP having lower thicknesses compared to that of CBP actually had higher ultimate tensile strength and strain at failure. Further investigating this might lead to finding the solution of having a lower profile device.

REFERENCES

1. World Health Organization. *Cardiovascular diseases.*
   http://www.who.int/mediacentre/factsheets/fs317/en/. Accessed January 2015.

2. Lachell, A., Henry, L. *Transcatheter aortic valve replacement options for severe aortic stenosis in high-risk patients*. <u>J Cardiovasc Nurs</u>, 30(3), 242-247. 2015.

3. O'Brien, K. D. *Epidemiology and genetics of calcific aortic valve disease*. <u>Journal Of Investigative Medicine: The Official Publication Of The American Federation For Clinical Research</u>, 55(6), 284-291. 2007

4. Ozkan, A. et al. *Assessment of left ventricular function in aortic stenosis*. <u>Nature Reviews</u>. 8(9), 494-501. 2011.

5. Singhal, P. et al. *Bioprosthetic Heart Valves: Impact of Implantation on Biomaterials*. <u>ISRN Biomaterials</u>, 2013, 1-14. 2013.

6. Kurtz, C. E., & Otto, C. M. (2010). Aortic stenosis: clinical aspects of diagnosis and management, with 10 illustrative case reports from a 25-year experience. Medicine, 89(6), 349-379

7. Iung, B., Cachier, A., Baron, G., Messika-Zeitoun, D., Delahaye, F., Tornos, P., . . . Vahanian, A. (2005). Decision-making in elderly patients with severe aortic stenosis: why are so many denied surgery? Eur Heart J, 26(24), 2714-2720

8. Pasic, M., Unbehaun, A., Dreysse, S., Drews, T., Buz, S., Kukucka, M., . . . Hetzer, R. (2010). Transapical aortic valve implantation in 175 consecutive patients: excellent outcome in very high-risk patients. J Am Coll Cardiol, 56(10), 813-820

9. Pasic, M., Unbehaun, A., Dreysse, S., Drews, T., Buz, S., Kukucka, M., . . . Hetzer, R. (2010). Transapical aortic valve implantation in 175 consecutive patients: excellent outcome in very high-risk patients. J Am Coll Cardiol, 56(10), 813-820

10. Gilard, M. et al. *Registry of transcatheter aortic valve implantation in high-risk patients.* The New England Journal of Medicine. 2012.

11. Windecker, S. *Clinical outcome and predictors for adverse events after transcatheter aortic valve implantation with the use of different devices and access routes.* Am Heart J, 161(6), 1114-1124. 2011.

12. Fishbein, G. A. et al. *Transcatheter aortic valve implantation: status and challenges.* Cardiovasc Pathol, 23(2), 65-70. 2014.

13. Sizeland, K. H. et al. *Age dependent differences in collagen alignment of glutaraldehyde fixed bovine pericardium.* Biomed Res Int. 2014.

14. Sizeland, Katie et al. *Age dependent differences in collagen alignment of glutaraldehyde fixed bovine pericardium.* BioMed Research International. 2014

15. Julkunen et al. *Maturation of collagen fibril network structure in tibial and femoral cartilage of rabbits.* Osteoarthritis Cartilage. 18(3):406-15. 2010

16. Fratzl et al. *Nature's hierarchical materials.* Progress in Materials Science. 52(8):1263–1334. 2007

17. Willet, TL. *Changes in collagen with aging maintain molecular stability after overload: evidence from an in vitro tendon model.* J Bimech Eng. 2010

18. Junqueria, L.C.U. et al. *Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections.* Histochemcial Journal. (11) 447-455. 1979.

19. Kiernan, John A. *Sirius Red for collagen staining protocol.* Department of Anatomy and Cell Biology.

20. Aikawa,E. et al. *Human semilunar cardiac valve remodeling by activated cells from fetus to adult: Implications for postnatal adaption, pathology, and tissue engineering.* Circulation: Journal of the American Heart Association. 2006.

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