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Design of a Composite Scaffold for Myocardial Regeneration Following Infarction

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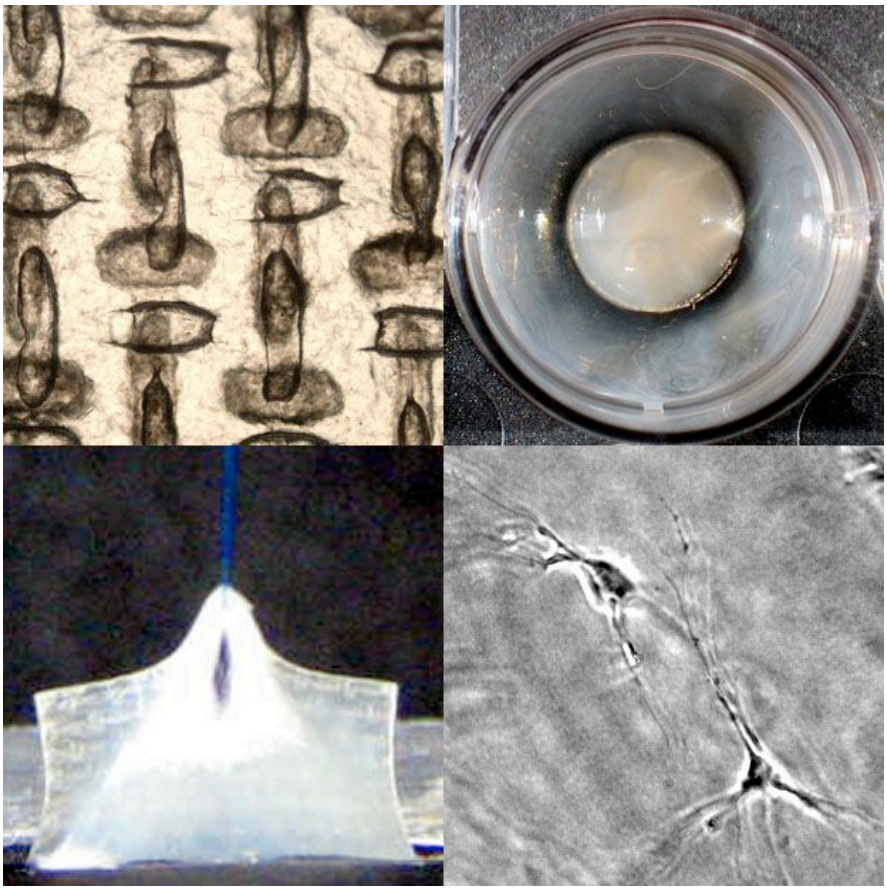

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A Major Qualifying Project to be submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Degree of Bachelor of Science

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Abstract

The heart does not regenerate new tissue when myocardium dies following acute myocardial infarction. We have undertaken an iterative process to develop a prototype patch that will replace infarcted tissue and induce myocardial regeneration to improve heart function. The result is a composite scaffold design composed of an endocardial patch to provide mechanical stability and an injectable filler material to provide a regenerative scaffold environment. This project provides a vital first step towards a final solution for myocardial regeneration.

Executive Summary

Myocardial infarction is one of the leading killers of men and women in the United States. 565,000 new cases and 300,000 recurrent cases occur annually, and complications following myocardial infarction can lead to progressive heart failure and ventricular aneurysm [1]. The treatments currently used for secondary care of myocardial infarction are aimed solely at preventing reinfarction and improving survival. The goal of this project was to progress toward a treatment for myocardial infarction that will not only decrease recurrence and mortality, but will restore heart function by regenerating new myocardium to replace the dead tissue.

Ventricular restoration is an approach for secondary treatment of myocardial infarction that involves excising the infarct and replacing it with a cardiac patch to restore the heart to a more efficient shape. Current commercially available patches are passive; they do not contribute to the work of the heart or assist in regeneration. We propose the design of a new myocardial patch for ventricular restoration that will induce regeneration and improve long-term heart function. To this end, we have undertaken an iterative design process to develop the required characteristics of such a patch. A basic design was developed that is composed of an endocardial patch to provide mechanical stability, and an injectable filler material that will induce myocardial regeneration. The material chosen for the patch must withstand the forces associated with ventricular contraction and suturing during surgery. The filler material is designed to incorporate growth factors and exhibits material properties conductive to the migration and differentiation of bone marrow cells into new myocardium.

Different materials were evaluated using a quantitative evaluation matrix, and the highest scoring alternatives were chosen for preliminary testing. For the patch component, bovine pericardium-derived collagen matrix (Veritas, Synovis Innovations) and urinary bladder matrix (UBM, ACell Inc.) were assessed for suture retention and uniaxial tension properties. Results revealed that Veritas and UBM were able to hold sutures effectively under clinical conditions, as well as having appropriate strength and elongation properties for this application. UBM was shown to have significantly greater elongation over Veritas and Polyester, making it a closer match to native myocardium. For the filler material, fibrin gel was selected, and experiments were conducted to characterize cell migration and proliferation in the gel. A variety of fibrin formulations were tested at fibrinogen concentrations of 3, 5, and 9 mg/ml. Results showed no significant migration of cells in any of these fibrinogen concentrations after 9 days. The viability

assays revealed the all three formulations of gel demonstrated hMSC viability, with the 9 mg/ml concentration achieving significant population increase after only 7 days, as well as the greatest overall evidence of cell viability.

We conclude that both Veritas and UBM materials are suitable for cardiac patch applications, and that future studies should include more detailed characterization, as well as *in vivo* studies in animal models. Fibrin gel was shown to promote viability of hMSCs, however, no migration was observed. This leads us to recommend that future research focus on increasing the ability of bone marrow cells to migrate from the body into the scaffold through the use of growth factors to recruit native bone marrow cells. The outcome of this project is the final design and characterization of selected materials for a patch for ventricular restoration. This is an important first step toward the ultimate goal of achieving regeneration of myocardium and improved heart function for post-infarct patients in a clinical setting.

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Chapter 1: Introduction

Coronary heart disease is the leading cause of death in the United States [1]. Heart disease can take many forms, the most severe of which is myocardial infarction, also known as heart attack. American men and women experience 565,000 new cases and 300,000 recurrent cases of myocardial infarction annually [1], making treatment for myocardial infarction a significant sector of the healthcare industry.

Myocardial infarction occurs when a coronary artery that supplies blood to the heart becomes blocked, making it unable to adequately perfuse the myocardium. Without the necessary oxygen or nutrients to survive, myocytes enter a state known as ischemia and may soon begin to die [2]. The death of myocytes creates an infarcted region within the myocardium that impedes heart function due to its inability to contract. Over time this infarcted area thins and elongates, causing the heart to take on a more spherical shape to compensate [3]. This leads to a decrease in cardiac output, which in turn leads to an overall decrease in the health and quality of life of the patient [4]. A wide variety of treatments have been developed in an attempt to address myocardial infarction and remodel the heart back to its healthy, elliptical shape. The most direct approach involves cutting out the infarcted region of myocardium and replacing it with a patch. Using this patch, the surgeon is able to restore the original geometry of the heart, thus improving cardiac output [5].

The heart patches currently available for clinical use in the United States are made either of treated bovine pericardium, the synthetic polymer polyethylene terephthalate (PET), or expanded polytetrafluoroethylene (ePTFE). Bovine pericardium is treated to remove cells and crosslinked with a chemical such as glutaraldehyde to alter its mechanical properties [6 , 7]. Minimal cellular infiltration into genepin fixed bovine pericardium along with a thick layer of fibrous tissue was observed four weeks after implantation in rat models by Wei et al. [8]. Although these materials are adequate for restoring ventricular geometry and maintaining ventricular pressure, these studies demonstrate that they result in the development of a region of acellular scar tissue instead of a region of viable muscle cells. The presence of myocardial muscle cells has been found to be related to the work performed by patch implant regions [9]. We therefore hypothesize that if the section of infarcted myocardium were to be replaced with

healthy contractile tissue, the function of the heart could be greatly improved. The new myocardium would contribute to the workload of the heart since the non-contractile region would be replaced with new, beating heart tissue.

The goal of this project is to design and develop a clinically applicable scaffold to replace full-thickness infarctions. The scaffold must be safe for the patient and have structural and mechanical properties similar to healthy myocardium, leading to restoration of an efficient ventricular shape. The scaffold must also be clinically applicable by being easy to implant using existing surgical techniques, customizable in shape, and cost effective to produce. Scaffold implantation should result in immediate heart remodeling followed by regeneration of contractile myocardium to improve heart function. The scaffold will provide temporary biomechanical and biochemical support for cells to grow until they are able to construct new extra cellular matrix. The scaffold will stimulate angiogenesis and as it is replaced by contractile myocardium fed by a neovascular network.

This basic design consists of two components: an endocardial patch sutured to the endocardial region of the ventricular wall, followed by a filler material placed above the endocardial patch filling and sealing off the wall thickness. The endocardial patch provides mechanical support to hold heart pressure and can be used for cardiac remodeling similar to current patches until new regenerated tissue can function mechanically. The filler material provides a provisional matrix for controlled myocardial regeneration and angiogenesis which is an improvement over the formation of uncontrolled scar tissue in the current patches [6].

The ability of the scaffold to meet its functional requirements were analyzed by putting it through a series of tests. Mechanically, it must hold the pressure inside the left ventricle, withstand tensile forces experienced during wall contraction, retain mechanical properties under fatigue conditions, and comply with the stretch ratio of heart muscle. To this end, the materials chosen for the device were subjected to uniaxial tensile testing, measuring tensile strength and stretch under normal loading conditions of the heart. To test the interaction of the scaffold with sutures, the materials also underwent testing to characterize their suture retention capabilities. Suturability is another concern that will be tested by measuring the force required to puncture the scaffold with a suturing needle. To assess the regenerative capability of the filler material, we focused on assessing the cellular migration and viability properties of a variety of different filler

formulations. We used human mesenchymal stem cells (hMSCs) to model a cellular therapy intended to regenerate myocardium. hMSC migration into the filler scaffold material was measured over a period of 9 days. The ability of hMSCs to proliferate in the scaffold was also measured using an MTS viability assay.

The results of this project indicated that that both Veritas and UBM materials are suitable for cardiac patch applications, and that future studies should include more detailed characterization, as well as *in vivo* studies in animal models. Both materials demonstrated appropriate strength properties in order to withstand the stresses in the wall of the heart. Elongation of both materials also fell within the desired range, although UBM had a significantly greater rate of elongation, making it a closer match to native myocardium. Suturability tests of both materials also passed specification. Fibrin gel was shown to promote viability of hMSCs, however, no migration was observed. The strong viability data suggests that fibrin gel will serve as a good myocardial scaffold material of the migration of cells into the material can be increase through further research. The outcome of this project is the final design and characterization of selected materials for a patch for ventricular restoration. This is an important first step toward the ultimate goal of achieving regeneration of myocardium and improved heart function for post-infarct patients in a clinical setting. Future research may include *in vivo* testing of materials and the overall device design in applicable animal models.

Chapter 2: Background

This chapter describes the deterioration of a healthy heart to an infarcted heart and the major effects that this has on overall heart function. Traditional treatments such as medications and surgical interventions are explained with special emphasis on the use of cardiac patches and ventricular restoration. In addition, new approaches involving the regeneration of myocardium using biomaterial scaffolds and growth factor therapies is examined.

2.1 The Healthy Heart

The heart is the center of the circulation system and distributes blood throughout the body. The function of the heart is vital to supply oxygen and nutrients to, and remove waste products from the body via the blood in order to maintain the balance that is necessary to sustain life [3]. It beats an average of 100,000 times and pumps 2,000 gallons of blood throughout the vascular system on a daily basis. A healthy heart is of the utmost importance to support day to day life [10].

Strong muscular contractions in the left ventricle pump blood out of the heart and into the circulatory system. These muscular contractions are produced by the muscle tissue that makes up the walls of the ventricle [11]. Healthy heart muscle wall is composed of three layers as seen in Figure 1: two collagenous membranes, the endocardium and epicardium, on either side of a muscular sheet, the myocardium.

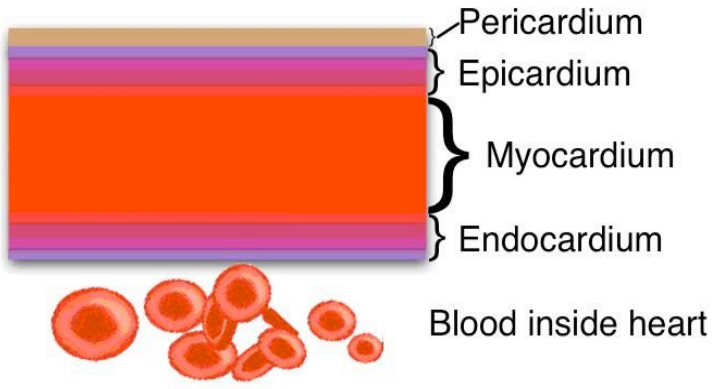

Figure 1: Schematic of Myocardium

The outer and inner layers are networks of type I and type III collagen and elastin, with the endocardium being populated by endothelial cells [12]. The myocardium is the layer of functional beating muscle that consists of fibroblasts and highly oriented myocyte muscle fibers in a matrix of collagen. Myocytes in this functional tissue layer also facilitate conduction of the electrical signals needed to initiate contractile movement in order to pump blood out of the ventricles [11]. Electrical conduction occurs with the help of intercalated discs that join the cells together. Gap junctions in these intercalated discs allow the action potential to travel through the membranes of the myocytes, thus facilitating signal propagation and a synchronized contractile pulse [13].

The coronary circulation system provides blood flow through the myocardium, nourishing the myocytes as they work to pump blood throughout the rest of the body. When blood flow to the myocardium is interrupted, the muscle becomes starved of oxygen and cells enter a serious and potentially fatal pathway, diagramed in Figure 2. This sets the stage for ischemic heart conditions and can lead to myocardial infarction.

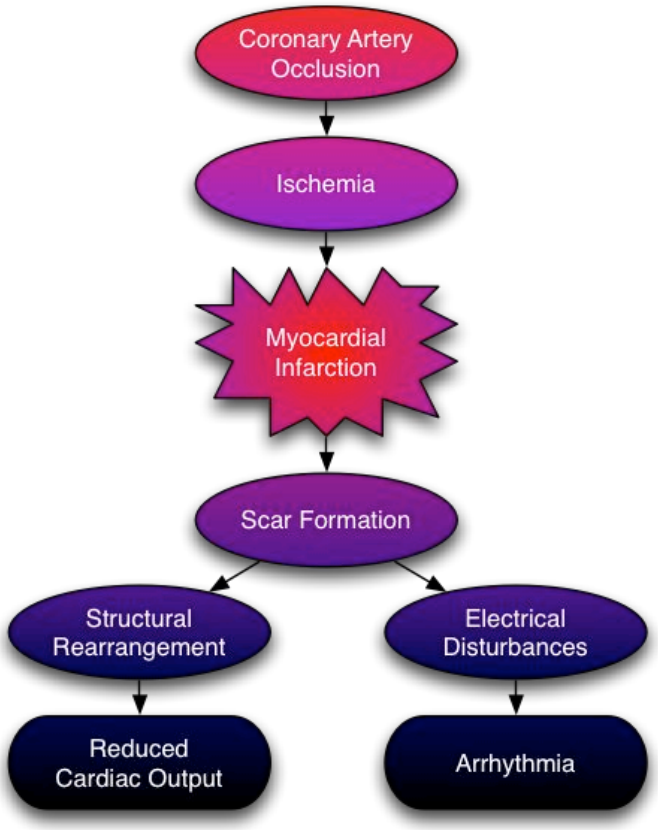

Figure 2: Pathway and Effects of Myocardial Infarction

2.2 From Ischemia to Myocardial Infarction

Coronary heart disease is one condition that can interrupt the flow of blood to the myocardium [14]. This loss of blood flow is called ischemia and can lead to cell death if the myocardium is starved of oxygen for too long [2]. The three common ways that ischemia occurs are through atherosclerosis, embolism, and an artery spasm [14]. Once blood flow to the myocardium is severely restricted there are immediate physiological and metabolic changes that occur within seconds, as depicted in Figure 3 [14, 15]. Eight seconds after occlusion ATP production in the myocardium switches from an aerobic mechanism to an anaerobic mechanism. This happens as a result of oxygen and glucose deficiency in the tissue and causes the production of ATP to fall rapidly. Since the myocytes have less ATP to use for energy, the muscle begins to lose its ability to contract. After approximately 30 seconds the supply of creatine phosphate, which is used as an energy reserve for ATP production, is 90% depleted. As anaerobic glycolysis

continues, hydrogen ions accumulate as a byproduct and after 10 minutes the intracellular pH of the myocytes decreases by about one pH unit [14]. This causes osmotic flooding of water into the myocytes. Edema occurs as the heart tissue continues to swell, and by 20-30 minutes following blockage, irreversible damage and cell death occurs in the myocardium. Within weeks to months scar formation takes place as fibroblasts infiltrate the infarct area and deposit fibrous collagen. Macrophages, monocytes, and neutrophils migrate to the scarred area as part of the inflammatory response and matrix metalloproteinases (MMPs) released from the neutrophils causes further infarct expansion and myocyte collagen degradation [16].

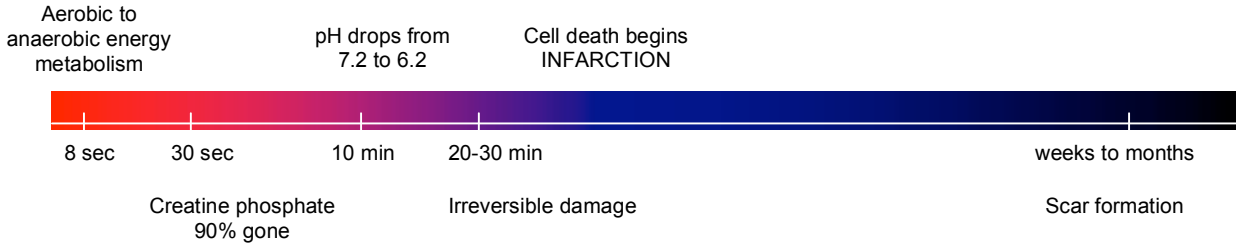

Figure 3: Timeline: Artery Occlusion to Scar Formation

The region of infarcted tissue in the heart muscle has two main effects; electrical function is disturbed and cardiac output is reduced [3, 17]. The infarction, now scar tissue, becomes a region of unproductive myocardium that can block the normal electrical conduction pathways of the heart. Depending on the location of the infarct, the function of the sinoatrial node, atrioventricular node, or the His bundle branches can be compromised due to tissue damage, resulting in an altered and unnatural heart rhythm [17]. This arrhythmia typically causes the heart to either beat slower than normal (bradyarrhythmia), leading to fatigue and cardiac arrest, or faster than normal (tachyarrhythmia), causing pain and discomfort [18].

Aside from arrhythmias, the unproductive region of myocardium also causes structural rearrangements. Slippage between myocyte bundles decreases the number of cells in the ventricular wall [19] causing the ventricle to slowly remodel itself as the infarcted myocardium thins and elongates due to the high ventricular pressure on the wall. This affects the overall shape of the heart, changing it from an elliptical shape to a more spherical shape [3]. Thinning of the infarcted region continues, triggering the unaffected myocytes to compensate by undergoing

hypertrophy in the attempt to increase healthy muscle mass [3]. This ventricular remodeling causes cardiac output parameters such as ejection fraction and stroke volume to change because they are directly dependant on the size and muscle strength of the ventricle [4]. The ejection fraction, the fraction of blood within the ventricle that is ejected with each stroke, declines with infarct size, but compensatory responses work to maintain normal stroke volume. As a result of the extra pressure and volume generated by these compensatory responses, stresses in the ventricular walls increase. Without treatment this can lead to serious complications such as aneurysm and ventricular rupture [4].

2.3 Cardiac Patches in the Clinical Environment

Following a myocardial infarction, the first step toward improving health is to reperfuse the blocked coronary artery, restoring blood flow to the infarcted region. This primary treatment must be administered as soon as possible in order to minimize ischemic death of the myocardium. The longer the myocardium is deprived of blood flow, the more serious the damage will become [20]. Typically, medications are the first attempt to dissolve the clot. Surgical treatments may be necessary if medications do not accomplish the desired outcome, or if the infarction has progressed long enough that it has inflicted serious damage to the myocardium. In conditions where the development of an aneurysm is a primary concern, surgical intervention is necessary to remove the weak, infarcted region and remodel the ventricle. Ventricular remodeling involves removing the infarcted region of the ventricle and reshaping the heart from a spherical shape, to a more efficient elliptical shape [21]. This can be done using either direct linear closure or endocardial patch plasty.

Direct linear closure is a procedure by which the infarcted section of the ventricle is removed, and the remaining heart tissue is sutured together [5]. This procedure provides a method for restoring the size and shape of the infarcted heart however, sometimes there is not enough myocardial tissue to restore the ventricle to the correct dimensions [5]. The Dor procedure, also known as endoventricular patch plasty, has been used since 1984. This is an alternate surgical technique that removes the infarcted myocardium and restores heart structure using a patch that is sutured into the opening as seen in Figure 4 [22]. In this procedure the heart is completely arrested and coronary revascularization is performed, normally by using the

internal left mammary artery [22]. Then an incision is made in the center of the infarcted area and any thrombi that are present are removed and the infarcted scar is cut out. A balloon is inflated inside the left ventricle and a suture is used to tighten the ventricle to the shape of the balloon. Once the suture it tightened, a patch is sutured in the remaining gap in the myocardium. The use of a patch ensures the restoration of ventricular volume and prevents further ventricular distortion [5, 22].

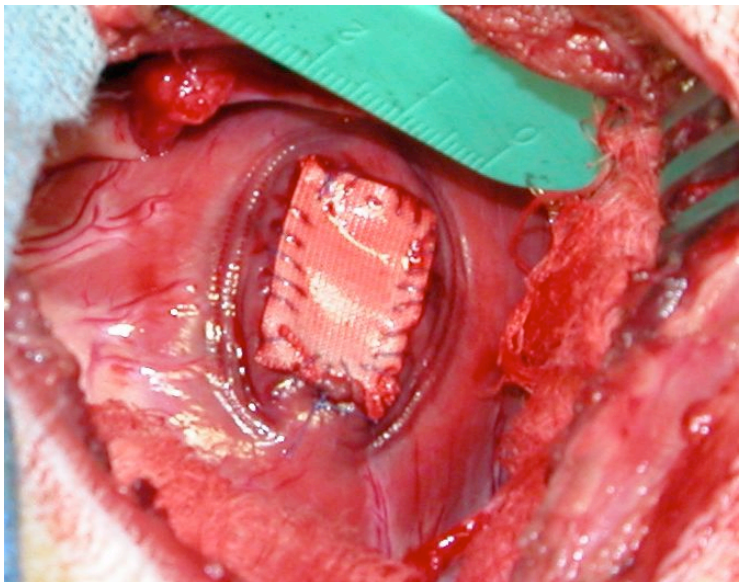

Figure 4: Dacron patch being sewn into the heart (Courtesy of G.R. Gaudette)

Synthetic patches available for clinical use in endocardial patch plasty in the United States are made of polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE). A summary of the clinically available patches approved for use in the United States is presented in Table 1. The technology to form these polymers into fibers was developed in 1939 by DuPont, Inc. and are commercially known as Dacron and Teflon respectively. When these materials are used in cardiovascular patches the fibers can be arranged into either velour, knitted, or woven configurations depending on the desired porosity [23]. Due to the high porosity of the knitted form, the material must be coated with a sealant such as collagen or albumin in order to prevent bleeding through the patch. Coatings are also sometimes used to improve the suture retention strength and help to induce endothelialization. One example, the Gore-Tex® Acuseal Cardiovascular Patch, is a composite material made of expanded PTFE with a middle layer of an

elastomeric fluoropolymer. Use of this patch has been shown to significantly reduce suture-hole bleeding compared to other products [24].

In addition to these synthetic materials, an extracellular matrix derived from biological membrane is also commonly used [6]. This membrane, bovine pericardium, is treated to remove cells and cross-linked with chemicals such as gluteraldehyde to strengthen the mechanical properties [7].

Although these materials are adequate for restoring ventricular geometry and maintaining ventricular pressure, they do not address the basic issue of regenerating myocardial tissue that has been lost. The wide-spread use of these materials is largely due to the fact that they are relatively inert and that they do not degrade over time, thus making the interaction between the material and its biological surroundings very predictable [23]. The result is the development of a region of acellular scar tissue instead of a region of viable myocardium that can contribute to the work of the heart.

Table 1: Commercially available heart patches

MATERIAL	PRODUCT	FEATURES
Expanded Polytetra-fluoroethylene (ePTFE)	Gore-Tex® ACUSEAL cardiovascular patch (Gore Medical Products, Inc.)	Significant reduction in suture hole bleeding
	Cardiovascular Patch (Bard Peripheral Vascular, Inc.)	Multi-directionally oriented node-fiber structure accommodates cellular ingrowth
Polyethylene terephthalate (PET)	DeBakey® cardiovascular fabrics (Bard Peripheral Vascular, Inc.)	Wide range of configurations from which the surgeon can select for each individual case
	Hemashield Double Velour Fabrics (Boston Scientific, Inc.)	Collagen impregnation provides blood tight seal and optimal tissue ingrowth
	HemaPatch® Knitted (DataScope, Inc.)	Coated with cross-linked type I bovine collagen
Glutaraldehyde treated bovine pericardium	PeriGuard® (Synovis Surgical, Inc.)	Improved suturability and handling over synthetic patches
	SJM Pericardial Patch with EnCap® technology (St. Jude Medical, Inc.)	Proprietary coating minimizes calcification and promotes endothelial covering

2.4 Research in Myocardial Regeneration

In the field of cardiac tissue engineering the topic of cardiac patches is being researched extensively. The current trend in this research is to take a biomaterial scaffold, seed it with cardiac cells and implant it in the heart. With these experiments researchers are trying to determine if a scaffold can be used to induce regeneration of contractile myocardium that will replace the infarcted region of the heart and contribute to its overall workload. A summary of some of this research can be found in Table 2.

One important point to highlight is that myocardial regeneration has become a highly controversial topic because of recent research that contradicts long-held beliefs about properties of myocardium. For years the accepted dogma has been that ventricular myocytes are terminally differentiated cells and are unable to divide in the adult heart. This stipulates that the heart is only capable of growing through myocyte hypertrophy [25]. Researchers who support this point of view cite as evidence the fact that the heart does not repair itself after infarction [25].

Recently, other researchers have found evidence that contradicts the long-believed dogma. Studies in animal and human hearts have shown that myocytes express growth-related genes immediately after infarction [26]. These myocytes are found in the infarct border zone where they have access to a sufficient blood supply. They also exhibited a 3 to 4 fold higher activity level one week after infarction [26]. The origin of these replicating myocytes is unknown, but it is suggested that they may come from a pool of resident cardiac stem cells or from circulating hematopoietic stem cells that have migrated to the heart through the blood [26]. It is important to note however, that because this natural replication and regeneration is restricted to areas where the replicating cells have access to a blood supply, the infarcted region is never functionally regenerated [25]. The scaffolds being researched are being designed to create a conducive environment in the infarct region so that regeneration, whether it occurs naturally or not, can take place in the infarct zone.

2.4.1 Use of Scaffolds in Myocardial Regeneration

Porcine urinary bladder matrix (UBM) is currently being researched as a direct replacement for the non degradable patches used clinically today. Robinson et al. [6] implanted a 4 layer UBM patch in the left ventricles of porcine models and after 3 weeks found that it had resorbed and been replaced by tissue growth. This tissue tested positive for alpha smooth muscle actin myofibroblasts and cardiomyocyte specific filament proteins. This suggests the possibility of regeneration of the myocardial tissue. However, the authors are unsure of the origin of the cells that make up this new tissue.

Park et al. [27] tested three scaffolds against a series of goals that would help create functional cardiac tissue: the scaffold must be highly porous to facilitate mass transport, hydrophilic to enhance cell attachment, structurally stable to hold mechanical loads, degradable to be biocompatible, and elastic to comply with contraction. A PLGA/collagen type 1 composite, a collagen sponge, and a PLGA sponge were seeded with cells isolated from the left ventricle of neonatal rats and cultured for eight days in bioreactors. The perfused environment of the bioreactor improved the maintenance of a high and spatially uniform cell density. Both the composite and collagen sponge had localized contractions and synchronous contractions in

response to electrical stimuli after 1 to 2 weeks. Also, the composite had the highest presence of connexin-43 gap junction protein in the scaffold.

Alginate gels and sponges are natural materials that are used extensively in cartilage regeneration therapy. Leor et al. [28] seeded alginate sponges with cells isolated from the ventricles of embryonic rat hearts and attached them to the epicardium of rat ventricles for nine weeks. After removal the authors found that both the seeded scaffold and the unseeded control were partially degraded, the cells were uniformly distributed throughout the scaffold, and the scaffold was covered with connective tissue and neovascularization. There was evidence of differentiation into mature myocardial fibers, gap junctions, and parallel arrangement of myofibers.

Gelatin sponges are also being researched as potential cardiac scaffolds. Li et al. [29] seeded three dimensional Gelfoam sponges with fetal rat cardiomyocytes and investigated in vitro cell growth and in vivo interactions. In vitro cell growth was able to occur, increase, and the cells survived in culture for two months. In vivo, it was found that the sponges partially dissolved after five weeks of being implanted subcutaneously. The cells organized themselves into a pattern resembling normal cardiac tissue and medium sized blood vessels formed indicating significant angiogenesis from the muscle into the graft. Spontaneous regular contractions were observed but there was no overall improvement in heart function. Also this sponge was found to be too thrombogenic for direct contact with blood so its use in the heart would be limited to non endocardial applications.

Fibrin glue is a routinely used surgical adhesive and sealant that can also be used as a scaffold to support myocardial regeneration. Christman et al. [30] seeded fibrin glue with skeletal myoblasts, and injected it into the infarcted area of left ventricles of rats. Initial cell culture testing demonstrated that myoblasts were capable of surviving in fibrin glue for seven days in culture. After five weeks in vivo the myoblast density had increased and the cells were found at the infarct border zone and within the infarct scar. Overall the size of the infarct had been reduced to about 20% and there was evidence of new blood vessel formation in the scar area.

Table 2: Summary of materials being researched for cardiac repair applications

Material	Experimental Model	Advantages	Disadvantages	Reference
UBM	4 layer, implanted as full thickness wall patch in left ventricle of pigs	• Promoted myofibroblast recruitment • Supports cell proliferation • Degradable • Retains matrix elements • Regeneration has been observed	• Questionable mechanical properties • Not currently used in humans	[6]
Collagen Sponge	neonatal rat heart cells suspended in Matrigel were seeded onto Ultrafoam sponges and cultured	• Main protein of ECM • Biocompatible • Enhance properties by crosslinking • Naturally degraded by MMPs • Excellent for cell attachment and proliferation • Elastic modulus conducive to supporting contractile myocytes	• Poor mechanical properties • Lack of structural stability • Large degree of swelling • Thrombogenic	[27]
Tissue Fleece	neonatal rat cardiomyocytes seeded into Tissue Fleece, electrically stimulated for contraction force measurements	• Currently used for tissue grafts in Europe • Significant elasticity • Compliant	• potentially thrombogenic	[31]
Alginate Gel/Sponge	fetal rat cardiac cells grown within alginate scaffolds to form multi-cellular contracting aggregates, implanted over myocardial scar on left ventricle of rats	• hydrophilic • easily wetted • highly porous structure • transparent • soft consistency • integrates with adjacent tissue • extensive neovascularization with cell seeding • degradable • easy to handle • injectable	• no cell adhesion sites • crosslinking agent typically Ca, could be toxic to heart tissue	[28]
Fibrin Glue	rat skeletal myoblasts in fibrin glue were injected into wall of infarcted left ventricle in rats	• readily available • can be augmented easily with growth factors • injectable • use in heart with mesenchymal stem cells • contains RGD binding sites	• clotting risk • scar formation potential	[30]

Polyanaline	H9c2 cardiac myoblasts cultured on conductive polyanaline substrate	• electrically conductive • highly studied polymer • biocompatible – no effect on cell proliferation, morphology	• non degradable • effect of matrix electroactivity has not been determined for cardiac cells	[8]
Gelatin Sponge	Cardiomyocyte-enriched cell inoculum from fetal rat ventricular muscle seeded into Gelfoam and implanted into subcutaneous tissue of rat leg or over myocardial scar in rat heart	• Biodegradable • Growth in three dimensions • Blood vessel growth • Supports contraction of myocytes	• Thrombogenic • Too porous	[29]

2.4.2 Stem Cells in Myocardial Regeneration

The heart is generally thought to have no endogenous ability to regenerate new myocardium after suffering a myocardial infarction. There is some controversy as to whether this always the case [32], but if the heart does have any endogenous regenerative ability, the mechanism is poorly understood. Accordingly, many studies that have sought to regenerate myocardium have done so by attempting to transplant cells into the heart, or to recruit them from other sources within the body and induce them to differentiate into cardiac myocytes [33].

Because of the ability of stem cells to differentiate into many different types of cells, there is hope that some types of stems cells have the ability to differentiate into myocytes [34]. This sparked a number of studies that sought to transplant various types of stem cells into the heart. Notably, the BOOST and TOP-AMI clinical trials showed increased ejection fraction in patients that received stem cells [35, 36]. Other studies have called into question the mechanism through which this occurs and whether the cells are actually differentiating into myocytes [33]. It has been hypothesized that the improvement due to injection of these cells results from passive mechanical support, but that true regeneration required the electrical coupling of cells through gap junctions in order to support synchronous contraction [37].

Some of the most promising studies involve the use of bone marrow cells or bone marrow-derived stem cells. Bone marrow aspirate contains heterogeneous populations of many different cell types which can be separated to obtain an assortment of progenitor cells, such as

mesenchymal stem cells and multipotent adult progenitor cells [36, 38]. Studies involving these types of cells, such as one done by Orlic et al., have found evidence of myocardial regeneration following injection of bone marrow-derived cells [39]. A recent study by Potapova, et al. [40] showed that bone marrow-derived mesenchymal stem cells were capable of producing cardiac lineage markers under *in vitro* conditions. Implanting these cells on UBM scaffolds into full-thickness defects in canine hearts resulted in increased regional stroke work, evidence of functional regeneration.

2.4.3 Use of Growth Factors in Myocardial Regeneration

One approach to myocardial regeneration that has garnered much publicity and controversy is the use of growth factors such as granulocyte colony-stimulating factor (G-CSF). This is used to mobilize autologous bone marrow cells within the patient which may regenerate myocardial heart tissue, as discussed above. G-CSF is a cytokine growth factor that is commonly used as an adjuvant to mobilize neutrophils from bone marrow in patients with abnormally low neutrophil levels, such as those undergoing certain types of cancer treatment [41]. Orlic et al. [42] conducted a study in which bone marrow cells mobilized with G-CSF and stem cell factor (SCF) were injected into the border regions of infarction in mice, followed by intravascular administration of G-CSF and SCF. The treated mice showed a significant degree of tissue regeneration and improved cardiac function. Several other studies have also demonstrated promising results for the use of G-CSF mobilized cells in cardiac applications [43-45], however some studies have shown limited evidence of any regenerative activity [43]. Thus far, G-CSF treatments aimed at myocardial regeneration have involved either systemic administration of G-CSF, or injection of cells the had been mobilized with G-CSF directly into the myocardium, but G-CSF itself has not been administered locally to the heart.

The precise mechanism by which G-CSF improves cardiac function remains unknown and controversial. When G-CSF is delivered systemically, it is hypothesized that cardiac regeneration occurs through mobilization of stem cells from the bone marrow. These bone marrow-derived stem cells have a high affinity for the injured tissue in the myocardial infarction. Thus it is hypothesized that the mobilized stem cells circulate to the site of the infarction, where they migrate into the tissue and differentiate into myocytes [46]. However, at least one

conflicting study has demonstrated that prevention of cardiac remodeling by G-CSF occurs because G-CSF protects still-viable cardiomyocytes instead of recruiting stem cells [47]. It is known that G-CSF mobilized mesenchymal stem cells have a high affinity for fibrin, and fibrin microbeads have been used to isolate mesenchymal stem cells from G-CSF mobilized bone marrow cells [48].

Chapter 3: Project Approach

After a comprehensive literature review of published works, we focus on developing the present device, a scaffold for regenerating myocardium. Before launching into the design process we wish to further define our project by identifying the project hypothesis and enumerating the overall assumptions and goals.

3.1 Project Hypothesis

Conventional approaches to treatment of myocardial infarction, as described above, are aimed primarily at preventing any future deterioration of the heart. However, there is currently no method of treatment that seeks to actively heal the myocardium and restore heart function that has been lost. Conventional treatments do nothing to regenerate dead myocardium and grow new, functional heart muscle tissue. We hypothesize that identifying materials with the appropriate mechanical and regenerative properties will enable the formation of a composite scaffold to replace the currently used patches. This approach would supplant the practice of replacing infarcted tissue with a synthetic patch during endoventricular patch plasty and ventricular restoration. The regenerative scaffold must fulfill the primary function of a synthetic patch by providing mechanical support to the ventricle wall in addition to its regenerative capability.

3.2 Project Assumptions

For any engineering project it is important to explicitly set forth the assumptions that will be made in order to carry out the project. These assumptions give a basis for simplifying the complex problem of heart disease and myocardial infarction to a problem that is within the scope of a senior design project. The primary assumptions that have been made in order to carry out the present project are as follows:

- Regeneration of myocardium following myocardial infarction will lead to improved heart function.
- Bone marrow derived stem cells migrate to the infarcted region and play a beneficial role in myocardial regeneration.

- In vitro cell growth can be used to model myocardial regeneration.

Although the validity of these assumptions are subject to debate and controversy, for the purpose of this project, we are working toward fulfilling goals that will lead to myocardial regeneration and improved cardiac function only under these assumptions. Verification of the validity of these assumptions is beyond the scope of this project.

3.3 Project Goals

As previously identified, the purpose of this project was to create a scaffold that will induce myocardial regeneration in the infarcted heart. The specific goals of this project are to:

- Develop a conceptual design and prototype composite scaffold that is clinically applicable and cost effective for myocardial regeneration
- Select appropriate candidate materials with the mechanical and regenerative properties to replace a section of ventricular myocardium
- Characterize mechanical properties of candidate materials
- Characterize the ability of candidate materials to promote stem cell migration and viability

Chapter 4: Design

At the beginning of this project the design team received the following initial client statement:

> *"Design and develop a clinically applicable scaffold for cardiac applications that will induce cell migration essential in angiogenesis and myocardial regeneration."*

In order to develop this statement into a feasible design, we followed an iterative engineering design process. First, the primary stakeholders of the design process were identified. Based on input from these stakeholders, the objectives, functions, constraints, and specifications of the design were developed in order to clearly state what the design must do and what limitations it must be designed within. Finally, a detailed revised client statement was developed, which incorporated all the major attributes of the design process and provided a basis for developing design alternatives.

4.1 Stakeholders

The stakeholders were categorized as designers, clients, and users as shown in Figure 5.

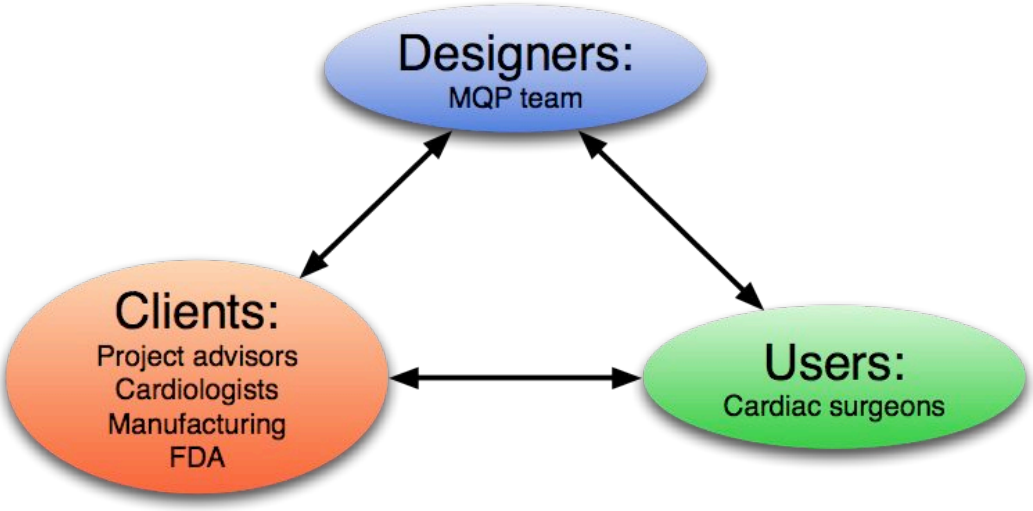

Figure 5: Project Stakeholders

The main stakeholders in this project are the project advisors, George Pins and Glenn Gaudette, and the design team. It is these individuals that will be defining the immediate objectives and functions of the design of the scaffold so that it satisfies the project requirements as well as establish a strong base as an initial step in a complex design project.

The primary users of this scaffold will be cardiac surgeons. Through talking with Dr. Nicola Francalancia (Appendix A: Interview with Dr. Nicola Francalancia), a cardiac surgeon, we discovered that the main concerns of these stakeholders are the survival and long term clinical improvement of their patients. Surgeons are interested in a short and simple surgery in order to minimize complications and are generally more interested in the clinical outcomes than the mechanism by which they occur. The ultimate test of the feasibility of the scaffold designed in this project will be to convince cardiac surgeons that it is superior enough to replace currently used treatments.

One group of clients that must be considered in the design process are cardiologists. When a patient first exhibits signs of heart failure, they are often referred to a cardiologist by their primary care physician. The cardiologist then prescribes a treatment for the patient which can range from medication to surgical intervention. Because cardiologists determine the treatment for the vast majority of the patients with myocardial infarctions, their opinion of any new treatment will be very important. If cardiologists can be convinced that this approach to myocardial regeneration will result in significant cardiac functional improvement, then the clinical acceptance of this design approach will be greatly enhanced.

Another important client group consists of those who represent the business interests involved in producing this scaffold and developing it into a marketable product. Without an efficient, profitable means for producing this product and making it clinically available, it will never be able to reach the patients that it is designed to help. The businesses and investors that would be involved in this process are thus keenly concerned with the ease with which the product can be made clinically available. Easing the product-to-market transition cuts costs and allows for less time and money to be spent in development and regulatory approval. These stakeholders are also concerned with issues such as manufacturing, storing, and packaging that are not major issues in a research lab and can be easily overlooked. It is important to carefully consider how design decisions made in this project will affect such issues in the future.

Closely tied to the business interests are regulatory considerations. Medical devices that enter the market in the United States are closely regulated by the Food and Drug Administration (FDA). Manufacturers must demonstrate that their product does not introduce harmful materials into the human body. The level of stringency with which the FDA examines devices increases with their complexity and obtaining FDA approval can become large percentage of the overall cost of product development.

4.2 Objectives, Functions, and Constraints

Based on consultations with the stakeholders of this design project and a review of the relevant literature, we generated design objectives to define the parameters of our device. The parent and sub level objectives are listed below:

OBJECTIVES

1) Be safe for the patient
    - Have reliable mechanical properties
    - Have predictable biomaterial-tissue responses
2) Be clinically applicable
    - Be practical for use in surgery
    - Be marketable
    - Be easy to produce
3) Have similar properties to healthy myocardium
    - Be contractile
    - Be regenerative

Regardless of how good the concept behind a medical device is, it will never be used in the clinic if it is not safe for the patient. In being safe for the patient the scaffold must have reliable mechanical properties so that it is consistently functional once implanted in the heart. Reliable mechanical properties are important because the implanted scaffold will have to withstand systolic ventricular pressure, and cannot fail mechanically under various cyclic loads and tensions related to the contraction and relaxation of the beating heart. It also needs to have

predictable biomaterials-tissue responses so that it does not induce any immune or biocompatibility response in the body. Since the scaffold will be directly in contact with the ventricular blood flow, not only will it have to be biocompatible, but it will also have to be hemocompatible so as not to induce thrombus and embolus formation.

The next major objective involves the clinical applicability of the design. To this end, a critical component of this objective to be practical for use in surgery. Surgeons will be the primary users of this device and they are more likely to accept a new device if it involves limited variation from procedures that they already perform. The application of the scaffold should therefore conform to currently used surgical procedures. Marketability is another important aspect of being clinically applicable. This patch needs to be marketable with respect to cost and other business interests so that health care providers are drawn to purchase and use it. Finally, the ease of production of the patch will also play a role in how it is incorporated into the clinical field since it must be easily manufactured in order to make it out into the clinical environment.

The last objective stipulates that we must engineer the scaffold to have similar properties to healthy myocardium. Since it will be incorporated in the heart, the scaffold must reflect the properties of the native tissue in order to create a suitable environment for regeneration of contractile myocardium. In order to be similar to the native myocardium, the scaffold must be compliant enough to allow for contraction of new myocytes and to avoid compliance mismatch with the native myocardium.

Subsequent to the development of main-level and sub-level objectives, an objectives tree was formed in order to map out the relationship of the objectives relative to each other and to pave the way for the development of functions and constraints. Before we identified the desired functions of the scaffold, the objectives were weighted in order to better understand their relative importance in the design process. The our project advisor stakeholders and the design team completed pairwise comparison charts to rate the importance of each objective (Appendix B: Pairwise Comparison Charts). The input from the different stakeholders was weighted in accordance with each stakeholder's relevant expertise in the subject of cardiac tissue regeneration and function. Dr. Glenn Gaudette's responses were given the highest weight at 50% because of his expertise and current research in cardiac patches, Dr. George Pins' responses were weighted at 30% since he is our primary expert for the biomaterials and tissue engineering

aspects of the project, and the design team's responses were weighted at 20% based on the research conducted by the team for this project. The pairwise comparison charts were analyzed and the respective weight of each stakeholder's opinion was applied to create the weighted objectives tree that is shown in Figure 6. The first number represents the weight with respect to the parent objective while the second number represents weight with respect to other objectives on the same level.

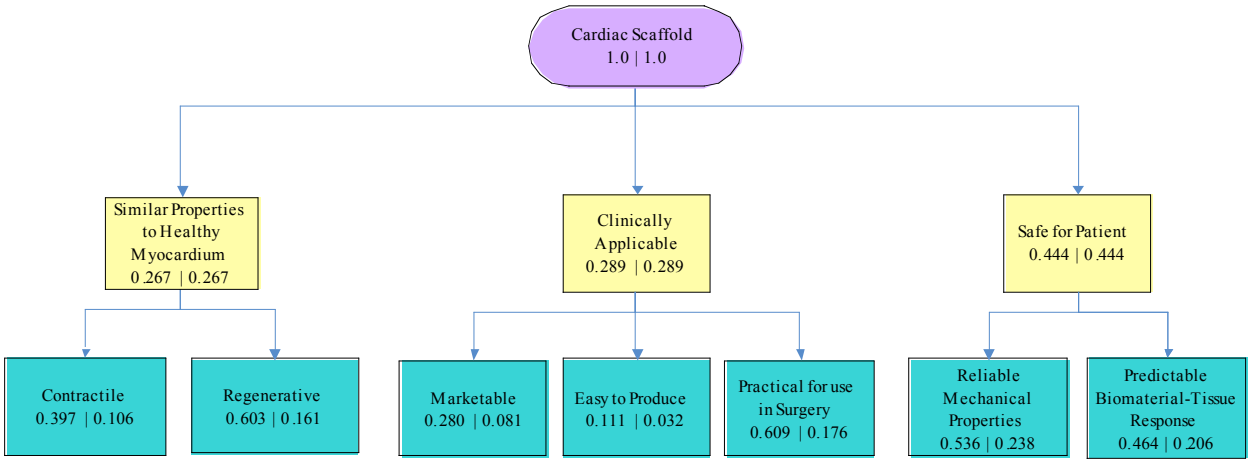

Figure 6: Weighted Objectives Tree

These weighted objectives created a preferential order for our design priorities. This order is as follows:

- Patient safety
- Clinical applicability
- Exhibition of properties similar to native myocardium

Using this information, several functions and constraints were identified that help to further define our design and reflect the weighted objectives shown above. The following list summarizes the primary functions, in order of importance, that the scaffold must perform in order to meet the established objectives.

FUNCTIONS

- Hold heart pressure
- Regenerate contractile myocardium
- Provide a perfused environment

- Facilitate surgical simplicity

Since this scaffold will replace the infarcted myocardium, it must withstand the pressure and forces that occur within the ventricular myocardium. This is essential to prevent catastrophic failure of the device. What sets our patch design apart from current clinically used endocardial patches is that it must also have the ability to regenerate contractile myocardium. Our hypothesis states that new myocardium will improve cardiac function, so this would require the patch to induce and support the growth of myocytes. In order to regenerate new myocardial tissue, it is important for the patch to have a perfused environment for the transport of cells, nutrients, gases and waste products. These are the main functions that support our hypothesis as stated in the project approach. Furthermore, surgeons will use this scaffold as a patch in endoventricular patch plasty, therefore it must be able to maintain and restore the shape of the heart during these procedures.

Along with the primary functions of our design, we developed a series of constraints to restricted our design space. These constraints were as follows:

CONSTRAINTS

- Must be biocompatible
    - Must be composed of nontoxic material
    - Must have nontoxic degradation products
- Cannot induce thrombus or embolus formation on endocardial surface
- Cannot cause arrhythmias
- Non-load bearing components must be degradable
- Must be cost effective for both producers and the consumers
    - Cannot cost more than the project budget of $624
- Must be finished by April 2007

These constraints were made in order to filter out undesirable design alternatives later on in the design process. Briefly, the scaffold must be biocompatible so as not to induce an

undesirable inflammatory response. Besides being biocompatible, the scaffold must also be blood compatible. The endocardial region of the scaffold is in direct contact with the blood, which requires strictly nonthrombogenic and nonembologenic properties. The scaffold also cannot cause arrhythmias that would be detrimental to the function of a healthy heart. Since the scaffold is intended to induce myocardial regeneration and is designed to be a provisional tissue replacement until new tissue is formed, it must be biodegradable to allow new tissue ingrowth [28]. Finally, the design must be within the budget of the design team, and must be completed by April 2007.

4.3 Specifications

Once the objectives and functions of the project were clearly defined, design specifications were developed to detail the specific engineering criteria that the device must meet. These design specifications were developed in accordance with the functions and dictate methods which can be used to assess the scaffolds ability to perform its functions. The specifications are given in Table 3 through Table 6. These tables summarize each individual specification and an appropriate testing method. The details of each testing method are described in the Methodology chapter.

Table 3: Specifications - Hold Heart Pressure

Specification	Description
Strength/Strain	The scaffold must be able to withstand a tensile force of 1.37 N/mm with corresponding strain between 5 and 15%[49-51]
Suture Retention	The scaffold must resist suture pullout under a force of 2.74 N/Suture
Fatigue Strength	The scaffold should have a failure strength that is not statistically different before and after cyclic loading of 276 cycles/minute for 100,000 cycles at 1.37 N/mm [49, 50]

The above specifications focused on the mechanical strength of the scaffold in order to hold heart pressure. All calculations include a safety factor of 5 since failure in these parameters would cause catastrophic failure of the device and probably loss of life. The ventricular pressure,

from a patient with abnormally high ventricular pressure [50], was used to calculate the average wall tension on the ventricle, and was normalized as tensile resistance by dividing by the ventricle wall thickness (Appendix C: Strength/Strain Protocol Calculations). The suture retention force per suture was calculated to ensure the sutures would not tear out of the material after implantation in the heart (Appendix D). Fatigue strength is critical for the scaffold as it is subjected to cyclic loading caused by the beating of the heart. The scaffold must retain its tensile strength following cyclic loading that mimics an accelerated heart rate.

Table 4 shows the specifications that the device must meet in order to regenerate contractile myocardium.

Table 4: Specifications - Regenerate Contractile Myocardium

Specification	Description
Viability	Human mesenchymal stem cells (hMSCs) seeded on a scaffold should maintain viability as shown by increasing in cell number by 100% within 6 days [52]
Migration	hMSCs seeded in fibrin gel should show statistically significant migration laterally into the scaffold within 9 days [53-56]

As explained earlier, our hypothesis involves mobilizing bone marrow stem cells and inducing them to differentiate into contractile myocytes. Mobilization of these cells first involves migration into the scaffold from the surrounding area. Once the cells are in the scaffold they must proliferate in order to fully populate the scaffold. Human mesenchymal stem cells (hMSCs) are commercially available and are of bone marrow origin, thus the specifications are designed to test their ability to migrate and proliferate in the scaffold.

Table 5 shows the specifications that the device must meet in order to provide a perfused environment.

Table 5: Specifications - Provide a Perfused Environment

Specification	Description
Viability	Human vascular endothelial cells (hVECs) seeded on a scaffold should maintain vaibilty as shown by increasing in cell number by 100% within 48 hours [57]
Migration	hVECs seeded in fibrin gel should show statistically significant migration laterally into the scaffold within 9 days [53-56]

A perfused environment, as mentioned earlier, is critical for transport of cells and nutrients. These specifications are aimed at predicting angiogenic behavior *in vivo* that will provide a perfused environment for the scaffold material.

To facilitate use in surgery, the patch should be easy to handle and work with as shown in Table 6. Suturability is a critical factor during an open heart surgery as the surgeon must be able to suture the scaffold quickly and easily. A suture insertion force of 5N was identified as a currently acceptable insertion force in clinically used materials (see Appendix E: Suture Insertion Force Calculation).

Table 6: Specifications - Facilitate Surgical Complicity

Specification	Measurement
Force required to penetrate the scaffold with a needle should be less than 5N [58]	Puncture test

4.4 Revised Client Statement

The objectives, functions, constraints, and specifications developed above define a much clearer picture of the design space in which the scaffold is to be created. With a more defined idea of the goals of our project, the client statement is revised as follows:

Design and develop a clinically applicable scaffold for cardiac applications that will induce cell migration essential in angiogenesis and myocardial regeneration to replace full-thickness infarctions in heart attack victims. Materials for the scaffold must be evaluated for patient safety by being able to withstand a tensile force of 1.37 N/mm and retain sutures at a force of 2.74

N/suture. Implantation of the scaffold should result in immediate heart remodeling in order to improve ventricular efficiency, followed by regeneration of contractile myocardium. Materials must be evaluated for their regenerative ability by promoting viability and migration of mesenchymal stem cells. The scaffold must be clinically applicable by being easy to implant using existing surgical techniques, customizable in shape, and having a suture penetration force of less than 5 N. The scaffold must also degrade in proportion to new tissue growth.

4.5 Developing Design Alternatives

This section describes the techniques used to develop various design alternatives, a basic overall design, and evaluation of the materials used for the basic design. The process began by identifying various means to fulfill the functions, then combining the compatible means into design alternatives. A basic overall design was selected, followed by the evaluation of different possible materials. Finally, a preliminary design was proposed in order to begin testing.

4.5.1 Morphological Chart and Evaluation Matrices

The morphological chart, shown in Table 7, lists all the functions along with the possible means to carry out each function that were produced by brainstorming (Appendix F: Design Alternatives)

Design alternatives were formed using combinations of the above means. Only alternatives that were rational and feasible were considered in evaluation. The following list details a number of the possible alternatives formed from these means:

- Whole wall thickness, growth factors, porous
- Whole wall thickness, seeded, porous
- Sew endocardial edges together, injectable gel
- Endocardial patch, growth factors, injectable gel
- Endocardial patch, seeded, injectable gel
- Endocardial patch, growth factors, void
- Endocardial patch, seeded, void

Table 7: Morphological Chart

FUNCTIONS	MEANS					
Hold heart pressure	Whole, wall thickness, scaffold is one strong piece	Sew endocardial edges together	Endocardial patch			
Regenerate contractile myocardium	Growth factors for myocyte migration and proliferation	Material properties for myocyte migration and proliferation	Scaffold seeded with mesenchymal stem cells	Growth factors for MSC migration and proliferation	Material properties for MSC migration and proliferation	
Provide a perfused environment	Injectable gel that promotes angiogenesis from surrounding tissue	Leached out channels for oriented angiogenesis	Leave void	Porous material for angiogenesis	Concentration gradient in scaffold to direct mass transport	Attach a blood vessel to scaffold
Facilitate surgical simplicity	Etched directional lines	Rough surface on one side	Colored markings			

These alternatives were organized into a numerical evaluation matrix and weighted according to how well they fulfilled the constraints and objectives. A list of detailed metrics can be seen in Appendix G: Metrics and Matrices for Evaluation of Design Alternatives. Each objective was broken down and defined on a scale of 0-2, 0 being the least favorable and 2 being the most favorable in fulfilling the objective. For example, the objective 'predictable biomaterial-tissue responses' was given a score of 2 if the design had very high potential for controlling the response of the tissue to the implanted scaffold, and a score of 0 was given if the design did not plan for any control over tissue response.

After these metrics were established, an evaluation matrix was made to evaluate each candidate design. An excerpt of this table can be seen in Table 8.

Table 8: Evaluation Matrix to Evaluate Design Alternatives

Design / Objectives & Constraints	Weight	Design 1	Design 2	Design 3
C: Constraint 1	Y/N			
O: Objective 1	(0.238)			
Total Score				

The top row lists the various candidate designs, and the left column lists the design objectives (O) and constraints (C). The second column shows the objective weights that were established earlier from the weighted objectives tree. The designs were evaluated by scoring them against the metrics (0, 1, or 2) and writing the corresponding metric for each material along the row. After doing this, each metric was multiplied by its corresponding objective weight and the final score for the design was obtained by adding the weighted metric score for each objective. The maximum score any design could get was 2, which would mean that it met all stated objectives.

The top design candidates can be seen in Table 9. The full list of evaluated designs and total scores, along with a description of each design and justification of scoring can be found in Appendix G: Metrics and Matrices for Evaluation of Design Alternatives.

Table 9: Evaluation of Design Alternatives

Design Alternatives	**Total Score**
Endocardial patch, growth factors, injectable gel	1.414
Endocardial patch, seeded, injectable gel	1.125
Whole wall thickness, seeded, porous	1.110
Whole wall thickness, growth factors, porous	1.062
Sew endocardial edges together, injectable gel	0.984

For holding heart pressure, an endocardial patch is ideal because it is an established surgical repair method. The whole wall thickness design was rated lower because it would make it difficult to reach the suture line, and direct linear closure was eliminated because it would not provide any volume for myocardial regeneration to take place nor would it allow for restoration of the original shape of the heart.

Regeneration of contractile myocardium using adult myocytes was decided against because of the difficulty involved in their isolation and in vitro culture. Seeding a scaffold with cells prior to implantation is advantageous in that the damaged region is replaced with an already developed tissue structure. However, the geometry of seeded scaffolds is limited by the thickness through which oxygen and nutrients can diffuse to support the cells. This limitation in thickness is not compatible with the thicknesses of myocardium that must be regenerated, and thus seeded scaffolds were rated lower in the different alternatives. Growth factors are also a powerful method for inducing the migration and proliferation of cells, however their use may be limited by their high cost.

For providing a perfused environment, an injectable gel is ideal due to its ease of implantation and use in other clinical applications. For facilitating surgical simplicity, all the means were considered because they all would aid the surgeons in identifying and using the patch.

4.5.2 Basic Design

Based on the evaluations above, a basic design was developed in accordance with the design alternative that scored the highest. This basic design consists of two components: an endocardial patch sutured to the endocardial region of the ventricular wall, and a filler material that would be placed above the endocardial patch filling the wall thickness. Figure 7 shows an illustration of a cross section of the myocardium in which the infarcted region would be removed and replaced by this composite scaffold.

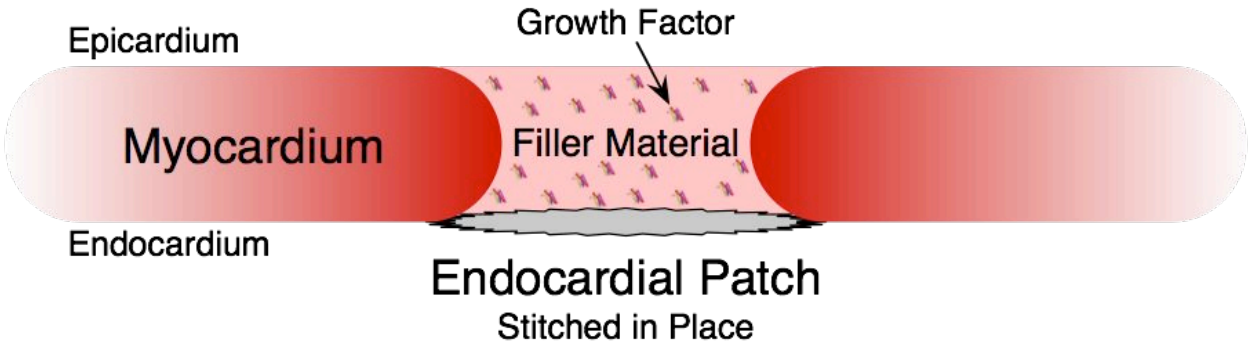

Figure 7: Illustration of the Basic Patch Design

The endocardial patch provides mechanical support to hold heart pressure and can be used for cardiac remodeling similar to current patches. The filler material provides a provisional matrix for controlled myocardial regeneration and angiogenesis. Another feature of this design is the incorporation of growth factors such as G-CSF. We have hypothesized that G-CSF has a similar chemotactic affect on bone marrow-derived mesenchymal stem cells as on polymorphonuclear leukocytes so we plan to incorporate G-CSF into a scaffold filler material for myocardial regeneration. In this way we hypothesize that stem cells will be mobilized from the bone marrow, and migrate into the filler material due to both a high affinity for fibrin and the chemotactic affect of G-CSF. These stem cells will then be able to proliferate and differentiate into myocytes to form new, functional heart tissue.

This device has several advantages associated with it, one of the most important of which is that it replaces the full thickness of the removed myocardial region. The filler material is designed to promote the re-growth of new myocardium, which is an improvement over the formation of uncontrolled scar tissue in the current patches. In addition, this device would be acellular which would make it relatively easy to manufacture and be in accordance with FDA regulations. The endocardial patch would be the pressure bearing component. Furthermore, the whole patch can be designed to be bioresorbable and would be completely replaced by new tissue.

4.5.3 Evaluating Candidate Materials for Basic Design

After the basic design composite scaffold design was established, several candidate materials for each component were evaluated against the design objectives using another set of

metrics. An explanation of these metrics can be seen in Appendix H. Each objective was broken down and defined with relevance to material selection on a scale of 0-2, 0 being the least favorable and 2 being the most favorable in fulfilling the objective. For example, the objective 'predictable biomaterial-tissue interactions' was given a score of 2 if the material had literature references indicating an absence of fibrotic response to the material, and a score of 0 was given if the material had a past history of extensive fibrotic response when used in cardiac applications.

After these metrics were established, an evaluation matrix was made to evaluate each candidate material for the endocardial patch and filler. An example of this table can be seen in Table 10.

Table 10: Evaluation Matrix to Evaluate Candidate Materials

Objectives →	*Reliable mechanical properties*	*Predictable biomaterial-tissue interactions*	*Practical for use in surgery*	*Regenerative*	*Contractile*	*Marketable*	*Easy to produce*	***Total Score***
Objective Weights →	0.238	0.206	0.176	0.161	0.106	0.081	0.032	
Patch 1	2	0	1	0	0	2	2	0.878
Filler 1	-	1	1	1	2	1	0	0.836

The left column lists the various candidate materials, and the top row lists the design objectives. The second row shows the objective weights that were established earlier from the weighted objectives tree. The materials were evaluated by scoring them against the metrics (0, 1, or 2) and writing the corresponding metric for each material along the row. After doing this, each metric was multiplied by its corresponding objective weight and the final score for the material was obtained by adding the weighted metric score for each objective. The maximum score any material could get was 2, which would mean that it met all stated objectives.

The top candidates for endocardial patch materials and filler materials can be seen in Table 11 and Table 12. The full list of evaluated material and total scores, along with a description of each material and justification of scoring can be found in Appendix H.

Table 11: Evaluation of Patch Materials

Patch Material	*Total Score*
UBM	1.531
Veritas	1.531
Trimethylene Carbomate (TMC) – D,L-Lactide (DLLA) copolymer	1.338
Tissue Fleece	1.081
Poly(glycolide-co-caprolactone) (PGCL) copolymer	1
Dacron	0.878
ePTFE	0.878
Cross linked Bovine Pericardium	0.878

Table 12: Evaluation of Filler Materials

Filler Material	*Total Score*
Fibrin Glue	1.524
Alginate Gel	1.492
Platelet Rich Plasma	1.46
Gelatin Sponge (Gelfoam)	1.348
Collagen Sponge (Ultrafoam)	1.316
Alginate Sponge	1.104
Collagen Hydrogel	1.036

4.6 Proposed Final Design

Based on the results of the evaluation matrices for the endocardial patch and filler material, porcine urinary bladder matrix (UBM) and Veritas were chosen as test materials for the endocardial patch, and fibrin glue was chosen as the filler material. Another material, collagen membrane, was also selected for testing as one of the candidate materials based on the suggestion of Dr. George Pins since these are currently being developed in his lab. These films are made by cross linking a thin layer of collagen solution on a plastic stencil. The cross linking method used for fabricating the collagen membranes for this project was by using carbodiimide (EDC) as the cross linking agent. EDC cross linking was used to fabricate the collagen membranes because the membranes had favorable elastic modulus, swelling ratio and fibroblast migration rates [59]. The collagen membranes are stored as thin brittle sheets that become flexible and viscoelastic when hydrated with saline solution. As part of the project, the collagen membranes would be characterized to determine if they have sufficient strength and strain properties for their use as an endocardial patch.

Urinary bladder matrix, as explained in section 2.4.1, is a lyophilized extracellular matrix taken from a porcine urinary bladder. Figure 8 shows the characteristic features of the 4 layer UBM material. The solid markings on the material are due to the processing of the material as the 4 layers are lyophilized to form a thicker material. Figure 9 shows the single layer UBM. The

collagen fibers are oriented in different directions which increases the strength of this biological material. It is currently used in veterinary applications like tissue remodeling and regeneration after wounds [6].

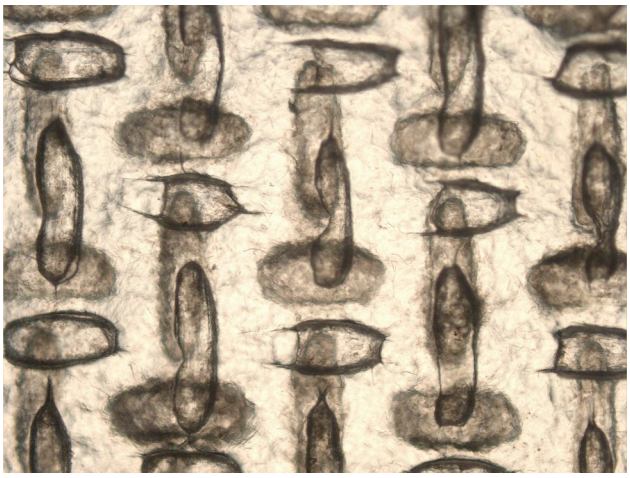

Figure 8: Four layer lyophilized UBM (5X)

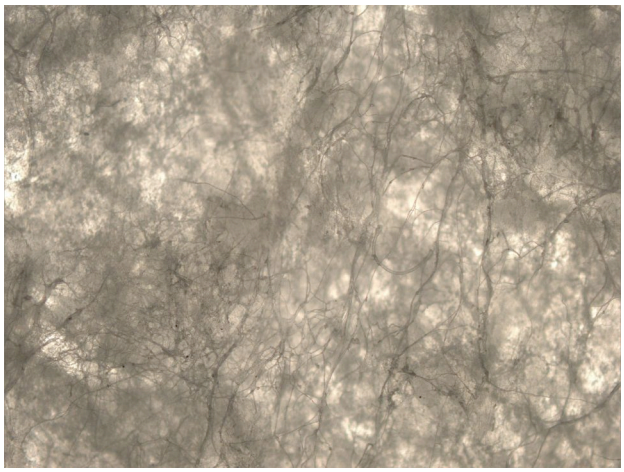

Figure 9: Single layer UBM (5X)

Veritas is currently under investigation by Dr. Gaudette for use in cardiac applications. Veritas is a non crosslinked bovine pericardium that is used in pelvic reconstruction surgeries. Because of the nature of these surgeries, repair materials used must have similar mechanical requirements as compared to the conditions in the heart [60]. It is also advantageous for use in these surgeries because it integrates into the surrounding tissue and supports new tissue growth. Other qualities include exceptional tensile strength, biocompatibility, suture retention, and angiogenic responses [61]. Figure 10 shows the structure of the Veritas collagen membrane. Similar to UBM the collagen fibers are multidirectionally oriented which increases the strength and suture retention [62].

Figure 10: Veritas Collagen Matrix (5X)

The final endocardial patch material that was tested is a woven fabric (Hemashield, Boston Scientific Corp.). This material will serve as the high end control since it is the standard for cardiac patches. Figure 11 shows the woven pattern of a typical Dacron patch material. The fibers are weaved orthogonally and form a strong structure.

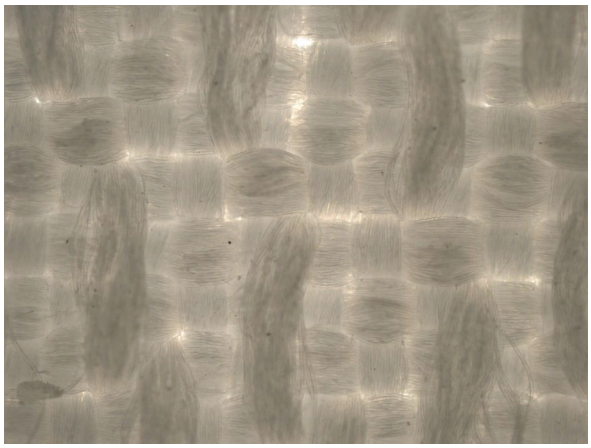

Figure 11: Dacron woven material (5X)

Figure 12 and Figure 13 show the woven polyester vascular graft which was the synthetic material actually used during testing. The structure of the woven polyester vascular graft is very similar to the structure of the Dacron material shown in Figure 11. The inner view of the vascular graft shows a much smoother weave as compared to the outer view. Also, the woven structure of

the vascular graft is much looser in order to allow for increased flexibility and longitudinal stretch.

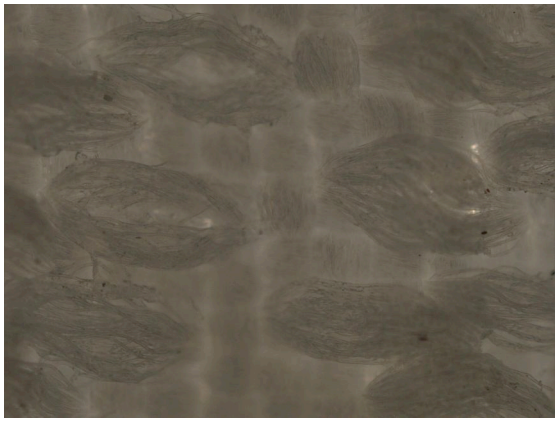

Figure 12: Woven polyester graft, inner view (5X)

Figure 13: Woven polyester graft, outer view (5X)

Chapter 5: Methodology

This chapter discusses the preliminary testing methods that are used to determine which endocardial patch materials would be appropriate to use. The production of the filler material as well as the testing methods used to characterize both the filler and patch materials will also be discussed. A diagram of the overall testing plan can be seen in Figure 14.

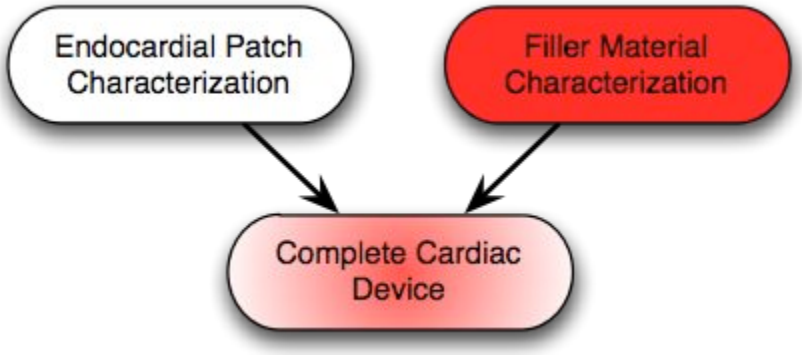

Figure 14: Overall Testing Plan

5.1 Preliminary Testing of Endocardial Patch

The preliminary testing phase was designed to eliminate those materials which had been identified for possible use as the structural component of our device but which may not meet the specifications that were established. The two specifications used to screen materials in this phase are as follows:

- The ability of the material to hold the required load while exhibiting the appropriate stretch.
- The suture retention strength of the material.

These specifications were chosen as the focus of preliminary testing because inadequate performance in either of these categories would result in catastrophic failure of the device. The details of the testing methods for each of these tests are described in the following sections. Four materials were tested in this phase, UBM, Veritas, collagen membranes, and woven polyester vascular graft as a high end control.

5.1.1 Strength/Strain

The strength of the material was measured uniaxially using an Instron In-Spec 2200 Benchtop Portable materials testing machine controlled by LabView 7.1. A detailed testing protocol can be found in Appendix I: Strength Strain Testing Protocols. Uniaxial testing does not completely quantify the strength of the material since the heart wall experiences multiaxial loading however it is a comparatively simple method that yields data sufficient for an initial screening of materials.

The samples were cut to into the dog bone shape specified by ASTM Standard Designation E8M-04 (Appendix J: Drawings of Dog Bone and Suturability Base). Markers were placed at the center of each sample for image analysis and each sample was secured to the upper and lower grips as seen in Figure 15. The grips were advanced at a strain rate of 0.20/s to mimic one heart beat at average heart rate. The test was run until failure or until the maximum capabilities of the Instron were reached.

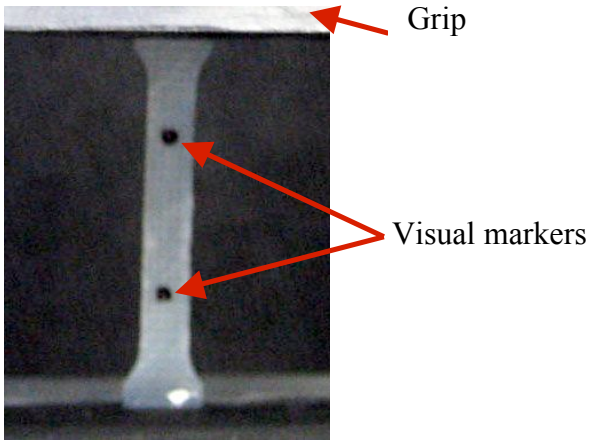

Figure 15: Strength Strain Sample

Raw data was obtained in the form of force in grams and extension in millimeters. Force is converted to Newtons using a factor of 0.0098 N/g. Crosshead strain was calculated by dividing the extension by the original gauge length. Since the samples are cut into a dog bone shape, an analysis of local strain was also performed in order to establish its relationship to the crosshead strain. Local strain of the sample was measured by acquiring images of two markers placed vertically on the central portion of the sample. Images are taken 0.5s apart throughout the

duration of the test. The relative displacement of the markers was then analyzed using ImageJ (National Institutes of Health) (Appendix K: ImageJ Strain Measurement). The relationship between crosshead strain and local strain was established by plotting the two against each other and creating a line of best fit. The equation of this line was then used to convert crosshead strain, as given in the raw data, to give a better approximation of local strain.

5.1.2 Suture Retention

The suture retention test was performed based on the methods described in American National Standards Institute standard VP20-1994, which addresses cardiovascular implants and vascular prostheses. The detailed testing protocol can be found in Appendix L: Suture Retention Testing Protocols. Each material was cut into 2 x 1cm samples and placed in the bottom grip of an Instron In-Spec 2200 Benchtop Portable materials testing machine. A 6-0 prolene suture, which is commonly used in cardiac surgery, was threaded through the center of the sample at a 2mm bite depth according to the standard, see Figure 16. The remainder of the suture was secured to the upper grip which was advanced at a crosshead speed of 2mm/s (0.05/s strain rate). The test was allowed to run until either material failure or suture failure, or until the maximum capabilities of the Instron were reached.

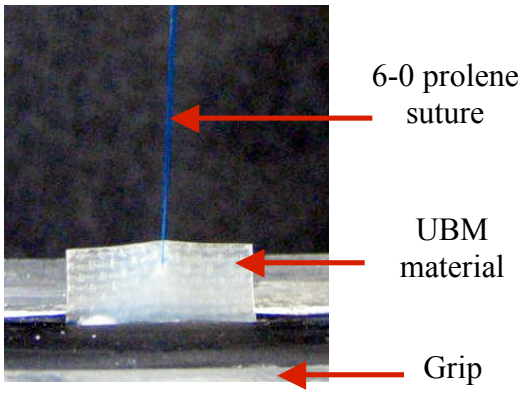

Figure 16: Suture Retention Sample

Raw data was obtained in the form of force in grams and extension in millimeters. Force was converted to Newtons using a factor of 0.0098 N/g. The failure of the sample was assessed by measuring force at failure and visually inspecting for signs of tearing at the suture insertion

point. Materials that did not show signs of failure at specification were considered to have acceptable suture retention strength.

5.2 Characterization Testing of Endocardial Patch

Once preliminary testing has established which materials have acceptable critical properties to be used as the structural component of our design, further testing was performed to more fully characterize our endocardial patch. These tests examined the following specifications:

- The ability of the material to hold the required load while exhibiting the appropriate stretch (repeated from preliminary testing at larger sample size)
- The suture retention strength of the material (repeated from preliminary testing at larger sample size)
- The force required to puncture the material with a suturing needle

5.2.1 Strength/Strain

This test was performed using the same protocol as section 5.1.1. One minor change was made to ensure the samples would not slide out from between the grips. Sandpaper was used to create a tougher surface on the grip faces to more securely anchor the patch materials as seen in Figure 17.

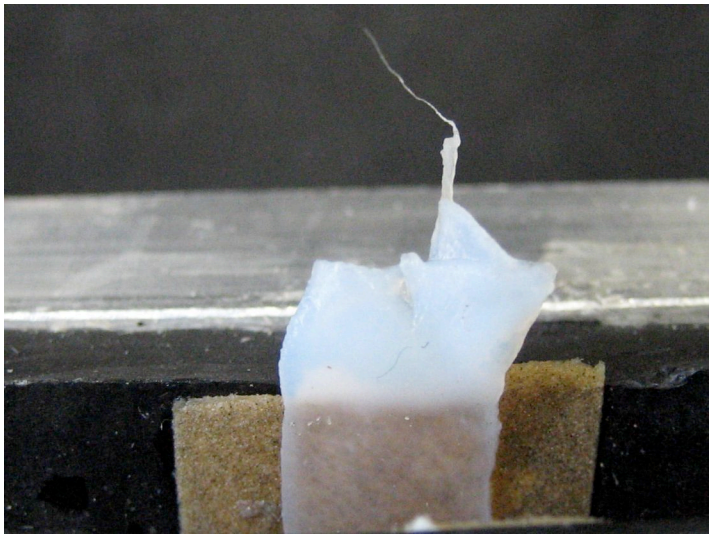

Figure 17: Sandpaper Lined Grip Face

5.2.2 Suture Retention

This test was performed using the same protocol as described in section 5.1.2. One change was made to ensure the loose ends of the suture would not slide out of the grips. The suture was knotted in a square knot around a ring, as seen in Figure 18, so that slippage would be eliminated. Sandpaper was also used on the lower grip to prevent the sample from slipping out.

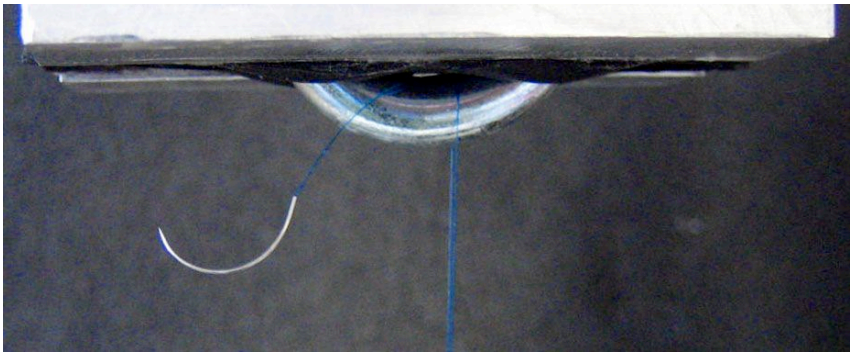

Figure 18: Ring for Suture Retention Test

5.2.3 Suturability

Suturability testing measures the force required to puncture the patch with a suturing needle, see detailed protocol in Appendix M: Suturability Testing Protocol. Samples were placed over a custom made base to support the material during testing as seen in Figure 19 and Figure 20. A suturing needle is placed in the top grip of the Instron testing machine and was advanced downwards at a rate of 2mm/s. Force data was acquired until the suture needle penetrated the sample as determined by a sharp decrease in the measured force along with visual inspection. The maximum force recorded before this sharp decrease is recorded as the patch penetration force.

Figure 19: Suturability Base Top View

Figure 20: Suturability Base Side View

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5.3 Cell Culture

The migration and proliferation experiments in the following sections were performed using human mesenchymal stem cells (hMSCs; Cambrex). Cells between passages 9 and 12 were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen). The cells were incubated in T75 tissue culture treated flasks (Falcon) at 5% CO2 and 37° C and the media was replaced every 3-4 days. To detach the cells from the tissue culture flasks, 5mL of 0.25% trypsin (Invitrogen) was added for approximately 5 minutes or until the cells were visibly detached. The proteolytic action of the trypsin was stopped by adding 5mL of DMEM and thoroughly rinsing the surface of the flask with this solution to ensure that all cells were dislodged. The cell suspension was then transferred to a 15mL conical tube and centrifuged for 5 minutes at 1000 rpm. The supernatant was decanted and the cell pellet was resuspended in DMEM. To determine the number of cells, 10µL of the cell suspension was added to 50µL trypan blue (Invitrogen) and 40µL phosphate buffered saline solution and the number of viable cells was counted using a hemocytometer. Additional DMEM was added to the cell suspension to achieve the desired concentration of cells.

5.4 Production and Testing of Filler Material

Fibrin, the material that we have chosen to use as our filler material, can exist in many different forms and has a wide range of properties based on the methods of production. In order to develop the optimal form of fibrin with the properties best suited for the regeneration of myocardium, we are producing fibrin using several different methods. Each method will be evaluated based on the migration and proliferation of cells in the material as defined by our specifications.

5.4.1 Manufacturing of Fibrin Glue

The method for producing fibrin gel was based primarily on methods used by Tuan et al. [63] and Silver et al. [64]. Fibrinogen from bovine plasma ~60% protein (~95% clottable) and thrombin lyophilized powder from bovine thrombin, 40-300 NIH units/mg were purchased from Sigma-Aldrich. Anhydrous calcium chloride was acquired from Sigma-Aldrich. FibriJect fibrin adhesive applicators were generously donated by Micromedics, Inc. Step-by-step protocols can be found in Appendix N.

Thrombin was resuspended in a 9:1 solution of HEPES buffered saline (HBS) and H_2O to a concentration of 40 U/ml and sterilized by passing through a 0.22 μm syringe filter. Thrombin was stored at -20ºC until use. Upon use, the thrombin was warmed and mixed 1:1 with a sterile 40 mM $CaCl_2$ solution to yield the final concentrations of 20 U/ml thrombin and 20 mM $CaCl_2$ in HBS. The thrombin concentration was chosen because low thrombin concentrations produce gels with thicker fibrin strands and larger pores than higher thrombin concentrations [65]. This is desirable to encourage cell migration through the gel. Lower thrombin concentrations also contribute to an increased ultimate tensile stress and stiffness [66]. A final concentration of 20 U/ml is still active enough to provide a relatively rapid gelling time [64]. The 20 mM $CaCl_2$ serves to catalyze the fibrin polymerization reaction.

Fibrinogen was resuspended in HBS with a pH of 7.4 at a fibrinogen concentration of 30 mg/ml. The solution was prefiltered by passing through a 0.45 μm syringe filter to remove large particulates, followed by a 0.22 μm tissue culture vacuum filter. Fibrinogen was stored at -20ºC until use; final dilutions of fibrinogen were diluted using HBS. The final fibrinogen/fibrin concentrations that were tested were 3, 5, and 9 mg/ml. 3 mg/ml was chosen because it is close

the normal physiological concentration of fibrinogen in the plasma [67]. Brown et al. [68] also found that a fibrinogen concentration of 3 mg/ml allowed fibroblasts to migrate optimally. Ho et al. [52]found that a fibrin concentration of 5 mg/ml provided maximal proliferation of mesenchymal stem cells in a 3D clot formation, while a separate study by Benaïd et al. [69] found that proliferation of seeded mesenchymal stem cells was optimized at 9mg/ml. Although these two studies did not take migration into account, proliferation of mesenchymal stem cells is also vital to the success of this project.

Due to the limitations of this project we were unable to obtain the synthetic peptides or other resources necessary to conjugate heparin to a fibrin matrix. We were also unable to obtain G-CSF due to budget constraints for our project.

5.4.2 Migration

A migration assay was performed based on the methods described by Clark et al. [70]. The migration of fibroblasts from normal dermal tissue into a clot region was modeled by seeding fibroblasts into a collagen gel to represent the dermis and surrounding it with an unseeded fibrin gel to represent the clot. The cells were not stained and the assay was designed to be non-terminal to allow limited resources to be used for as many samples as possible. This method was adapted for our uses to model the migration of cells from the bloodstream into the fibrin gel implant. The fibrinogen concentration of the seeded gel was the same as the lowest concentration used to measure migration, and close to the physiological concentration in human blood [67] at 3 mg/ml fibrinogen. An unseeded fibrin gel was used to model our regenerative filler material. The concentration of this gel was varied in order to find an optimized concentration. In this adapted scenario, rather than measuring cell migration from one material to a different material, migration was measured between different concentrations of the same material.

hMSCs were chosen to model to mobilized bone marrow cells for the migration assay. Please refer to Appendix O: Migration Testing Protocols for the complete protocol. Both Greiling et al. [71] and Clark et al. [70] seeded collagen scaffolds with cells at a concentration of 10^6 cells/ml, therefore the same concentration was used for our assay. Another important consideration is the thickness of the sample, since this affects the perfusion of oxygen and

nutrients, the amount of material required, and the ease with which the sample can be imaged. Greiling et al. [71] used 0.6 ml samples that were approximately 2.4 mm thick when they were first formed. Although these dimensions are for the gel before it contracted, they provide a good approximation of what thicknesses are appropriate and we will therefore use them for our procedure. Refer to Appendix P: Calculations for Migration Assay.

To form the inner and outer gels, 8 mm circles were scored in the bottom of standard 16 mm diameter 12-well tissue culture plates using a etching tool and a circular template. The plate was then thoroughly washed in 70% ethanol and sterile H_2O to remove any plastic particles. The fibrinogen solution and thrombin solution were each loaded into one syringe of a FibriJect fibrin adhesive applicator (Micromedics). An equal volume of each solution was then dispensed simultaneously through the blending applicator tip. A total of 800 µl mixed fibrinogen/thrombin solution was dispensed into the outer ring of the well. The outer ring of fibrin was allowed to polymerize, and 300 µl of the seeded solution was dispensed into the inner ring.

The samples were analyzed by measuring the distance from the boundary between the seeded and unseeded gels to the farthest cell at 5 randomly selected locations. A limitation of this approach is that it is not an accurate measure of the actual migration of the cells since it is not possible to determine if the cells in the unseeded gel migrated from the seeded gel or if they are the result of the proliferation of cells which previously migrated into the unseeded region. A more accurate way to express this data would be as the spreading of cells from the seeded gel into the unseeded gel. Although an assay which strictly measures migration would be preferred from an experimental point of view, cell spreading will be sufficient for our purposes because we are interested in characterizing the ability of cells to populate the implanted gel.

Samples were analyzed using light microscopy with a Nikon Eclipse TS100 inverted microscope at 10X magnification. Four images of each gel were acquired at each time point using a RT Color Spot camera system and Spot imaging software (Diagnostic Instruments, Inc.). The locations for the images were randomly chosen and were independent at each time point. The focal depth for all analyzed images was set at 184 µm above the score mark for each well. The distance in pixels from the external edge of the score mark to the centroid of the farthest cell in each image was measured using ImageJ software. This distance was then converted to micrometers using a scale determined by observing a micrometer at the same magnification.

5.4.3 MTS Assay: Viability

The MTS assay (CellTiter 96® AQueous One Solution Reagent; Promega) is a colorimetric method based on the reduction of a tetrazolium compound into a purple formazen dye by mitochondria. The concentration of this dye is thus directly proportional to the total metabolic activity of the cells. The manufacturer's protocol specifies that MTS solution be added directly to the cell culture media of each sample at a ratio of 1:5 and incubated for 1-4 hours before reading the absorbance at 490nm.

To determine the incubation time that would be best suited to these experiments, 15,000 hMSCs were placed in 8 wells of a 96-well plate and cultured for 3 hours to ensure that they were confluent. Short incubation times increase the ease with which the experiment can be performed, and thus 1 and 2 hour incubations were compared. MTS solution was added to all of the wells, and the absorbencies of four of the wells were recorded after 1 hour and the absorbencies of the other four wells were measured after 2 hours. A qualitative assessment of the absorbencies at both time points was made to determine whether the increase in absorbance signal at a longer incubation time was appreciable enough to warrant its use.

To develop a conversion between absorbance and cell number a standard curve was created by seeding hMSCs into a 96-well plate at a range of different concentrations as shown in Table 13. Three wells of each concentration were made. After a 3 hour incubation in media, 20μL of MTS solution was added and the samples were incubated for 2 hours before recording the absorbance at 490nm.

The absorbance for each concentration was reported as the mean ± standard deviation of the three wells. The absorbance values were plotted against the known cell number and fit to a linear equation to determine a general relationship which would allow a conversion between absorbance and cell number.

Table 13: Range of concentrations used for standard curve

Plate Well	Concentration (cells/mL)	Cells/well (0.1mL)
A	500,000	50,000
B	375,000	37,500
C	250,000	25,000
D	187,500	18,750
E	125,000	12,500
F	62,500	6,250
G	31,250	3,125
H	0	0

Fibrin gels were formed as described previously by placing 25μL of thrombin solution in the appropriate wells of a 96-well plate. Twenty-five microliters of fibrinogen solution were then added and pipetted up and down several times to ensure that the two solutions were completely mixed. Seeded fibrin gels containing 15,000 hMSCs were formed as well as gels containing no cells. The final fibrinogen concentrations of 3 mg/mL, 5 mg/mL, and 9 mg/mL were investigated. The gels were allowed to solidify for 2 hours before adding media. The day 0 gels were incubated in media for 1 hour before adding MTS solution and incubating for 2 hours. One hundred microliters was then transferred from each well into a new 96-well plate and the absorbance was recorded at 490nm.

Veritas and UBM were seeded with hMSCs by placing a 30μL drop containing 15,000 cells onto the center of 1cm x 1cm samples in a 24 well plate. Positive control wells containing only hMSCs were also made. The samples were placed in the incubator for 2 hours to allow the cells to attach before adding 300μL of DMEM. After an additional 1 hour incubation, 60μL of MTS solution was added directly to the media. The samples were incubated in the MTS solution for 2 hours before transferring 100μL of solution from each sample to a 96-well plate and recording the absorbance at 490nm.

Correction for background absorbance was performed by recording the absorbance at each time point in control wells containing only culture medium or samples with no cells.

Corrected absorbance was determined by subtracting the average background absorbance of each material from the absorbance recorded for each experimental data point. Absorbencies were reported as the mean ± standard deviation of the corrected absorbencies and converted to cell number using the standard curve.

5.5 Statistical Analysis

Statistical difference between groups was analyzed using ANOVA with a Bonferroni post hoc correction (SPSS). Significance was established for $p < 0.05$.

Chapter 6: Results and Analysis

This chapter describes the outcomes of the procedures outlined in the Methodology chapter.

6.1 Endocardial Patch Preliminary Testing

The results and analysis of the preliminary testing provide an initial screen of materials to assess whether or not each material meets or fails the critical specifications. Those materials that pass this round of testing will continue to Characterization Testing where they will be further tested against the remainder of the specifications.

6.1.1 Suture Retention

The collagen membranes were eliminated from testing due to their qualitatively fragile properties. Placing a suture through the membrane caused it to immediately tear and the membranes fell apart into multiple pieces upon cutting them out of their support frames. Based on these results it can be concluded that the collagen membranes, in their present state, would not act as a good cardiac patch and thus were not tested any further.

The ability of the Veritas, UBM, and Hemashield to retain a suture under force is shown in Figure 21. All three materials passed the specification force of 2.74 N as shown by the solid specification line. Table 14 summarizes the maximum force and modulus for each material. Two UBM samples were tested, however, due to a software error data from only one sample was available for preliminary analysis.

Table 14: Suture Retention Results

	Maximum Force (N)	Modulus (N/mm)	Mode of Failure
Specification	2.76	-	-
Veritas	4.58+0.54	0.61+0.12	all samples: suture slipped from grip
UBM*	4.05	0.34	all samples: material slipped from grip
Hemashield	4.53+0.29	0.55+0.04	all samples: suture slipped from grip

*data is presented as mean + stdev, data available from only one UBM sample

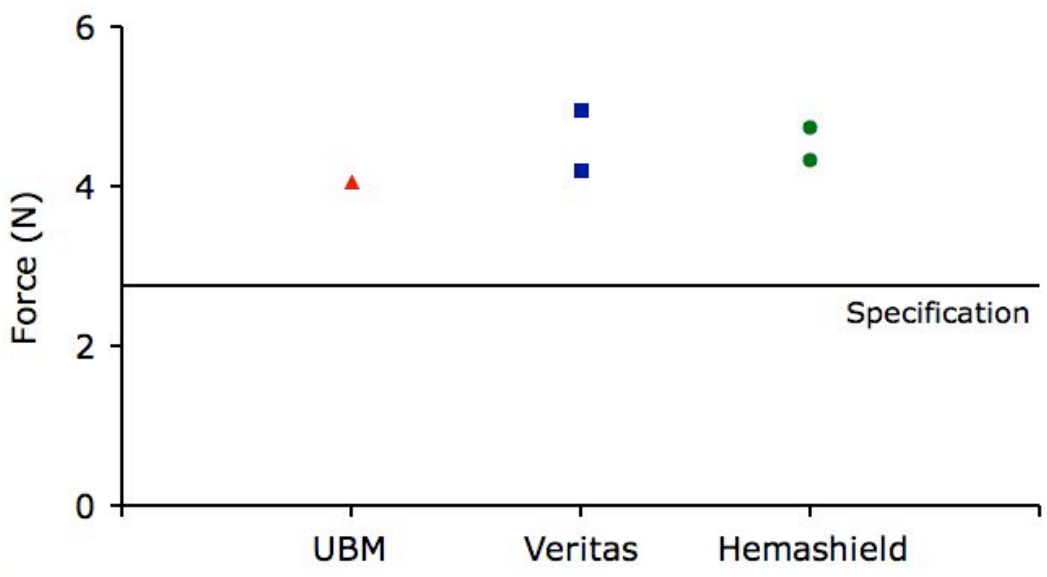

Figure 21: Suture Retention Preliminary Data

The maximum force in Table 14 shows a convergence of all materials at a force between 4 and 5 N. This is due to a defect in the testing method. The loose ends of the suture were secured in the top grip in testing setup as seen in Figure 22. During testing the suture ends began to slide out from between the grip between 4 and 5N, therefore no sample experienced more force than this. Once the suture began to slip, the force fluctuated around this range. Even with this defect, the preliminary data showed that all three materials passed the specification since the material retained the suture beyond the specification line even while the suture was slipping from the top grip. The procedure was altered for characterization testing to ensure that the true suture retention strength of the materials can be measured.

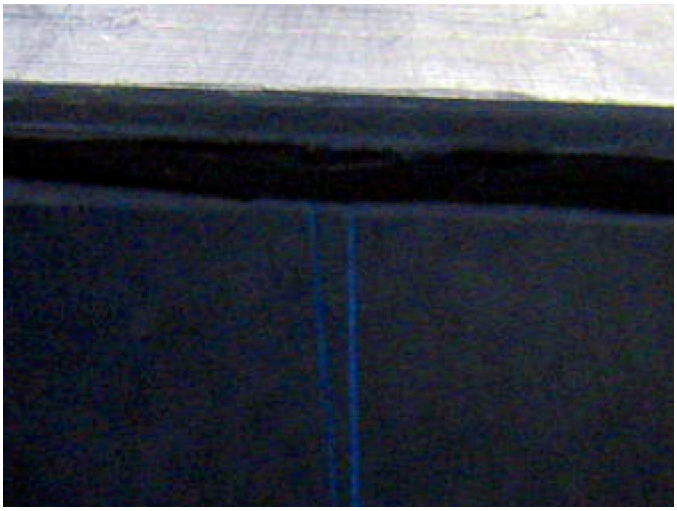

Figure 22: **Gripping of Suture in Preliminary Testing**

The moduli in Table 14 represent the slopes of the graph of each material between 2mm and 8mm extension. Since the stretching of the suture accounts for most of the strain that was observed, these moduli are fairly similar to each other. Any difference that exists is due to the small amount of material that is stretching as the suture applies force to it. The elastic modulus calculated for UBM is lower than that of Hemashield and Veritas. This is likely due to there being only one UBM sample, thus preventing a truly representative sampling, and that this material slid out of the lower grip during testing as opposed to having the sutures slide from the top grip.

6.1.2 Strength/Strain

Figure 23 depicts the force versus strain data for each material in the strength/strain preliminary test. Crosshead strain was normalized to local strain using image analysis (Appendix Q: Transformation of Strength/Strain Preliminary Data). Table 15 summarizes the important parameters, including the ultimate tensile strength and strain at the specification force.

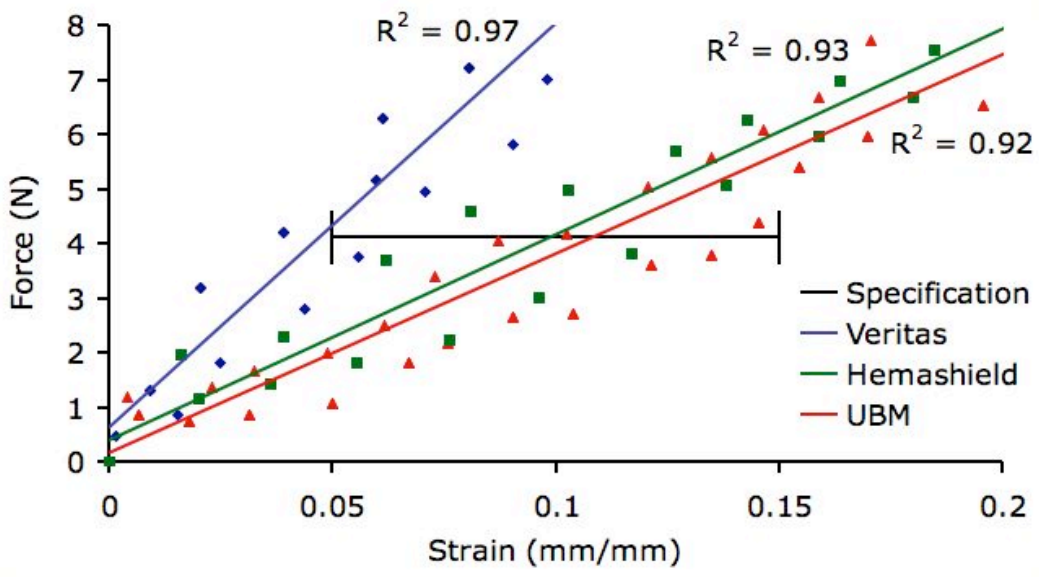

Figure 23: **Strength Strain Preliminary Results**

Table 15: Summary – Strength/Strain Preliminary

Material	Maximum Force (N)	Strain at Specification Force (%)
Specification	4.11	5 to 15
UBM	7.98±1.30	12±3
Veritas	12.7±1.6	5±2
Hemashield	18.6±7.4	10±3

All materials passed the force specification of 4.11 N and are generally within the stretch specification range (5 to 15%). The Veritas, in blue, is very close to the low end of the specification range but it will be kept for further testing since one of the samples was within the range.

6.2 Endocardial Patch Characterization Testing

The results obtained from the characterization testing of each material will allow us to obtain a more thorough understanding of how the material will respond in the cardiac environment. This testing covers the following three tests:

- Suture retention

- Uniaxial strength and strain
- Suturability

6.2.1 Suture Retention

The suture retention of all materials is summarized in Table 16 and Figure 24. The test method was successfully modified from the preliminary methodology in that all samples experienced either material failure or failure of the suture. All materials again passed the force specification of 4.11 N as expected, however the failure strength is not statistically different based on analysis of variance (Appendix R: Suture Retention Statistical Analysis).

Table 16: Summary - Suture Retention Characterization

Material	Max Force (N)	Mode of Failure
Specification	2.76	-
UBM	7.47±1.48	2 samples: suture broke, 1 sample: material failure
Veritas	6.12±0.94	all samples material failure
Hemashield	7.69±0.69	all samples suture broke

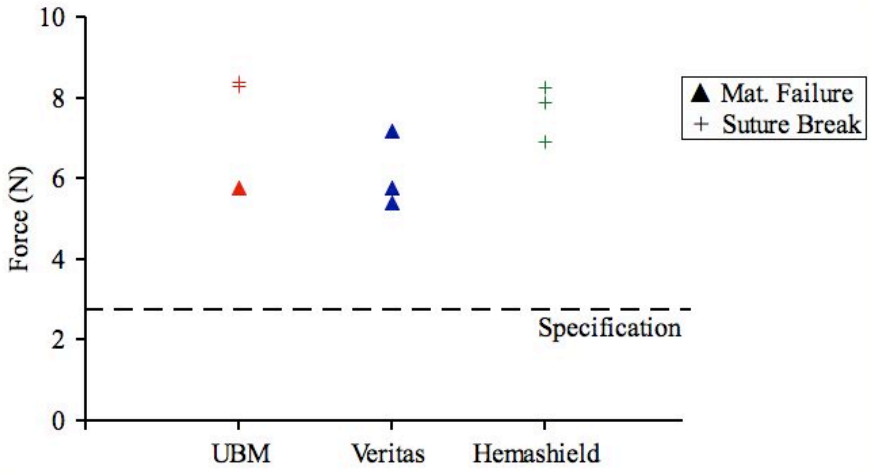

Figure 24: Suture Retention Characterization

6.2.2 Strength/Strain

Figure 25 depicts the force versus strain data for each material in the strength/strain characterization test. The solid lines show an average linear fit of all samples of each type of

material (n=6 for UBM and Veritas, n=3 for woven fabric). Crosshead strain was normalized to local strain using image analysis (Appendix S: Transformation of Strength/Strain Characterization Data). Table 17 summarizes the important parameters, including the ultimate tensile strength and strain at the specification force.

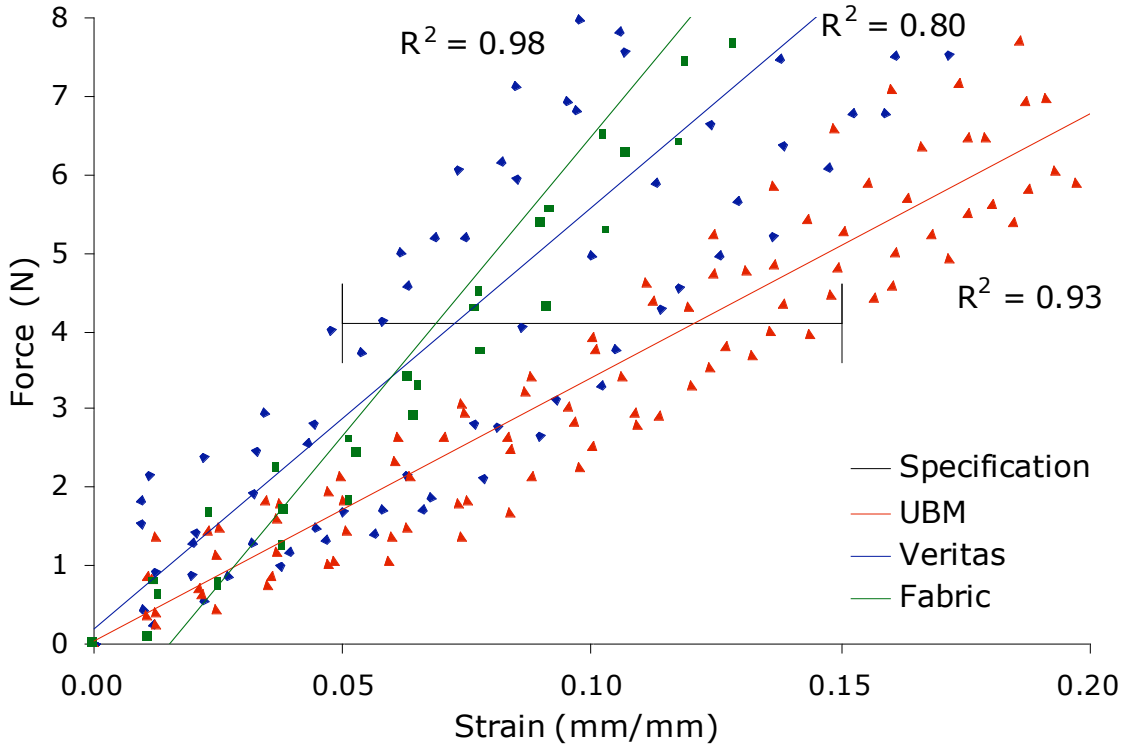

Figure 25: Strength Strain Characterization

Table 17: Summary – Strength Strain Characterization

Material	Maximum Force (N)	Strain at Specification Force (%)
Specification	4.11	5 to 15
UBM	7.7±0.7	11.0+1.8
Veritas	10.5±0.7	6.5+0.3
Hemashield	37.7±4.1	6.4+0.7

All materials passed the force specification of 4.11 N as expected and are within the stretch specification range (5 to 15%). Statistically, there is no difference between the strain at specification for Veritas and Hemashield as determined by ANOVA with a Bonferroni post-hoc test (Appendix S: Transformation of Strength/Strain Characterization Data).

6.2.3 Suturability

The maximum recorded force needed to insert the suture needle in the patch materials is summarized in Table 18. The force needed to insert the suture needle into the patch materials was established as the maximum recorded force until the needle completely passed through the patch material. The maximum force required to insert the suture needle in the patch materials was lower than 5N for each patch material.

Table 18: Summary - Suturability

Material	Maximum Force (N)
Specification	5
UBM	0.95±0.08
Veritas	0.71±0.23
Hemashield	0.76±0.14

6.3 Filler Material

The results obtained from the testing of each formulation of fibrin gel was intended to test the suitability of fibrin gel as a filler in the composite scaffold design. We also wished to optimize the formulation of the fibrin gel for this application by testing gels containing several different fibrinogen concentrations.

6.3.1 Migration

Initially we planned to run preliminary assays to optimize a fibrinogen concentration for hMSC and endothelial cell migration and proliferation. Following this, we planned to conduct studies using different concentrations of G-CSF in the fibrin gels. However, we were unable to obtain G-CSF for the project due to budgetary constraints. Media for culturing endothelial cells was ordered but did not arrive in time to be utilized in this project.

The initial hMSC migration assay was intended to provide only preliminary data and was conducted with a sample size of three. The results of this migration assay are shown in Figure 26 and are expressed as the average distance +/- the standard error of means (SEM) of the farthest cell from the score mark at various time points. An experiment was run with time points taken at days 1, 3, 6, and 9. This assay showed no significant migration of cells at the time points observed for any of the fibrinogen concentrations tested (ANOVA, $p > 0.05$). A full

characterization assay was begun with sample size of five (data not shown), however bacterial contamination was observed after one day and the experiment was terminated. In the initial experiment (Figure 26), measurements were not taken at day 0, leaving the possibility that cells were migrating between day 0 and day 1, but not after day 1. To test this possibility, a third experiment was initiated with sample size of five to measure migration distance starting at day 0. A fibrinogen concentration of 5 mg/ml was used since this concentration showed the greatest distances in the initial experiment. Figure 27 shows the measured distance of hMSCs from the score mark in fibrin gel at a fibrinogen concentration of 5 mg/ml at days 0 and 1. On day 1 of this experiment contamination was observed once again, likely fungal in nature, however we were still able to obtain measurements from unaffected areas and uncontaminated wells for day 1. The results revealed no significant migration between days 0 and 1 ($p < 0.05$). At this point, the decision was made not to pursue the hMSC migration experiments any further.

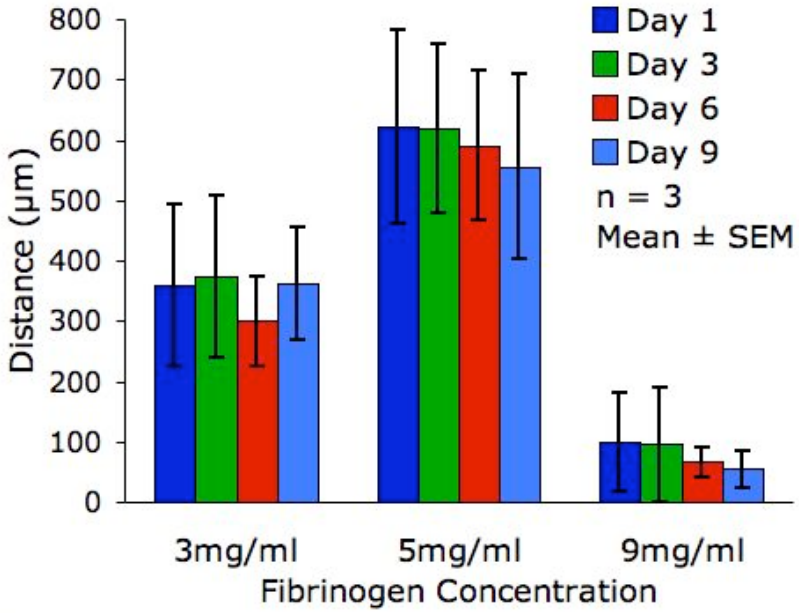

Figure 26: hMSC distance from score mark in fibrin gel at days 1-9

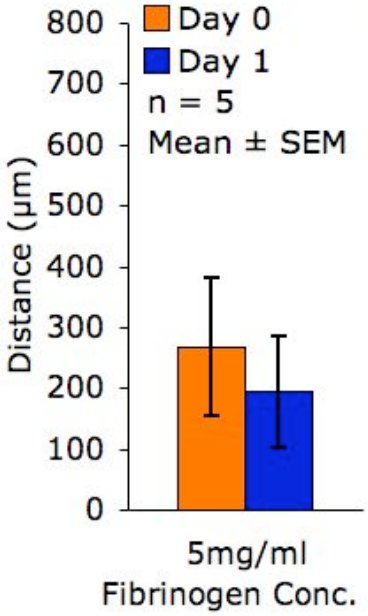

Figure 27: hMSC distance from score mark in fibrin gel at days 0-1

6.3.2 MTS Assay

This section validates the use of the MTS assay in this study, reports the results obtained, and discusses their significance. Figure 28 compares the absorbance from wells containing hMSCs at 1 and 2 hours. The absorbance for the hMSCs increases 60% from 0.928 ± 0.043 at 1

hour to 1.488 ± 0.130 at 2 hours. Because of this substantial increase in absorbance, an MTS solution incubation time of 2 hours will be used.

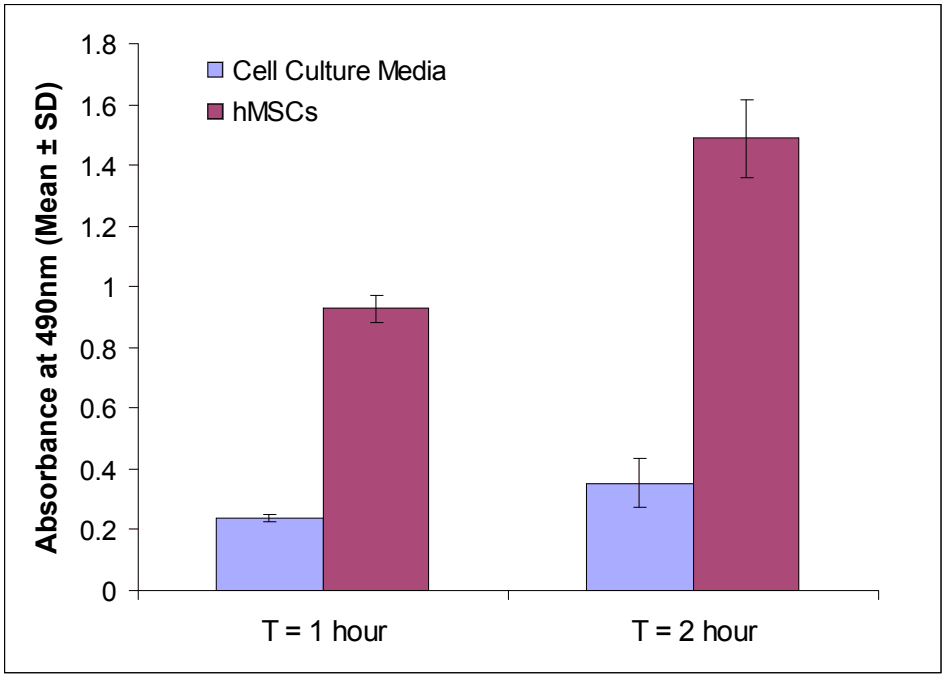

Figure 28: Optimization of Incubation Time (n = 4)

The absorbencies from wells containing known numbers of hMSCs are plotted in Figure 29. This curve is one representative of the standard curves which are created for each new experiment which was performed. The equation of the trend line which is fit to the data points was used to calculate the number of cells corresponding to each recorded absorbance.

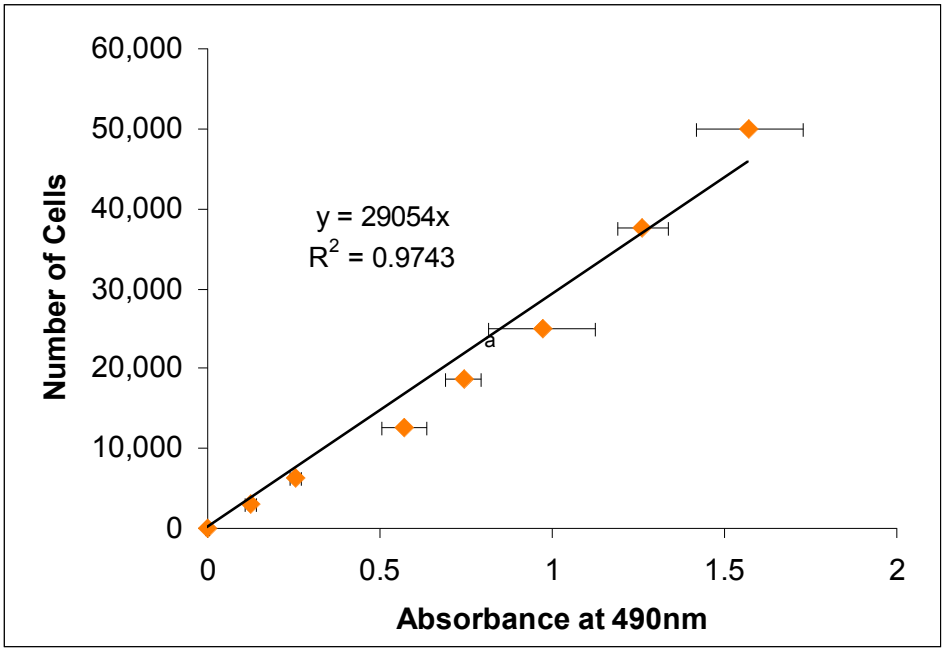

Figure 29: Standard Curve (n = 3)

Figure 30 shows the number of cells estimated from the absorbencies recorded at 0, 1 and 4 days. Data is presented as mean ± SD.

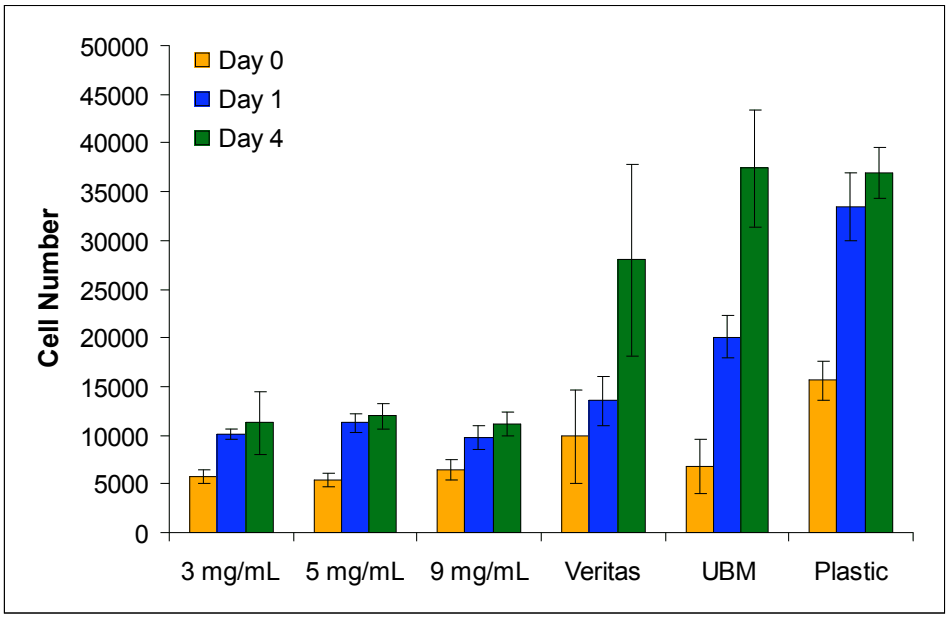

Figure 30: Cell viability (n = 3)

A representation of this data as the percent increase in number of viable cells from the number measured at day 0 is given in Figure 31. Data is presented as mean ± SD and the (*) indicates significant statistical difference between Day 1 and Day 4. This figure shows that both Veritas and UBM had a statistically significant increase in the number of viable cells at Day 4.

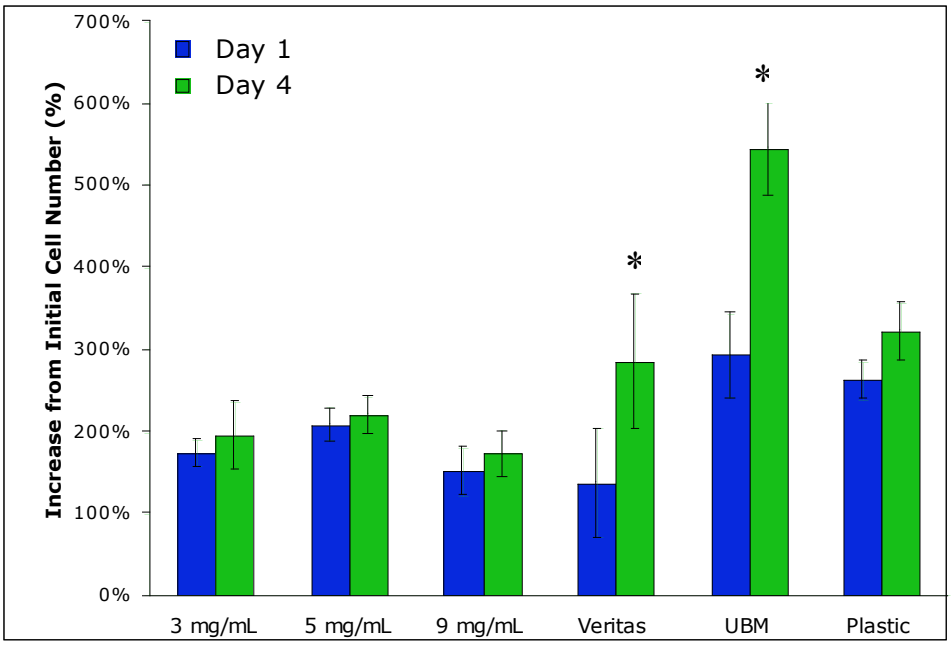

Figure 31: Increase in number of viable cells (n=3)

The statistically significant differences between the materials are indicated in Table 19.

Table 19: Comparison of percent increase in number of viable cells

	Day 1						*Day 4*					
	3mg/mL	**5mg/mL**	**9mg/mL**	**UBM**	**Veritas**	**Plastic**	**3mg/mL**	**5mg/mL**	**9mg/mL**	**UBM**	**Veritas**	**Plastic**
3mg/mL	…			*		*	…				*	
5mg/mL		…		*	*			…			*	
9mg/mL			…	*		*			…		*	
UBM				…	*					…	*	
Veritas					…	*					…	*
Plastic						…						…

The results from a further experiment which examined the number of viable cells in fibrin gels over a time period of two weeks are shown in Figure 32. Data is presented as mean ± SD.

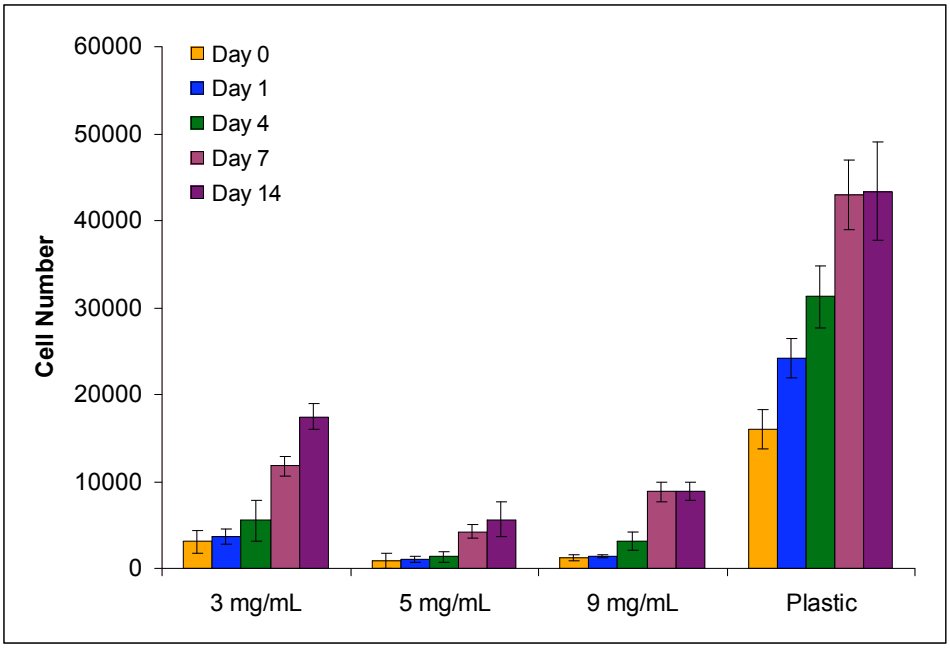

Figure 32: Cell viability in fibrin gels (n = 4)

A representation of this data as the percent increase in number of viable cells from the number measured at day 0 is given in Figure 33. All materials exhibited a statistically significant increase in number of viable cells at Day 7. The fibrin gels containing 9mg/mL of fibrinogen also had a statistically significant increase at Day 4. Data is presented as mean ± SD and the (*) indicates significant statistical difference from previous time point.

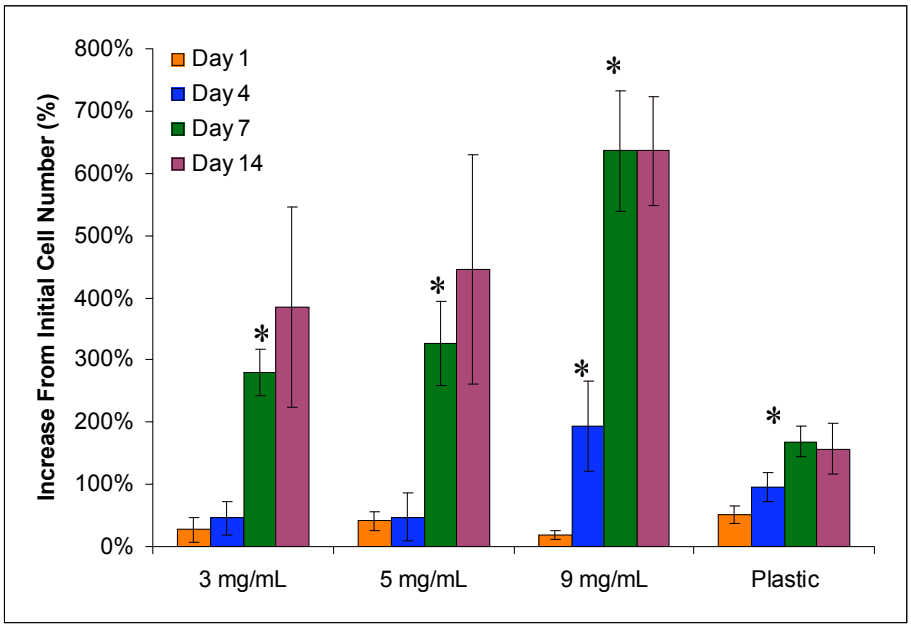

Figure 33: Increase in number of viable cells in fibrin gels (n = 4)

Table 20 shows the statistically significant differences between the materials at each of the time points.

Table 20: Comparison of percent increase in number of viable cells

	Day 1				*Day 4*				*Day 7*				*Day 14*			
	3mg/mL	**5mg/mL**	**9mg/mL**	**Plastic**	**3mg/mL**	**5mg/mL**	**9mg/mL**	**Plastic**	**3mg/mL**	**5mg/mL**	**9mg/mL**	**Plastic**	**3mg/mL**	**5mg/mL**	**9mg/mL**	**Plastic**
3mg/mL	…				…		*		…		*		…			
5mg/mL		…				…	*			…	*	*		…		
9mg/mL			…	*			…				…	*			…	*
Plastic				…				…				…				…

Chapter 7: Discussion

This chapter serves to interpret results of both the endocardial patch testing and filler material testing. The analysis of both of these components will aid in assembling the best possible composite scaffold with appropriate properties for myocardial regeneration.

7.1 Endocardial Patch

The mechanical testing performed in this project begins to characterize each of the candidate materials to quantitatively assess their ability to serve as the patch component of a composite scaffold.

No statistical difference existed between the suture retention failure strengths of any of the three materials tested. Photographs of a sample of UBM and Veritas from this test are shown in Figure 34A-F. Images A and D were taken at the specification force, B and E were taken at failure, and C and F are detailed views of the sample after failure. These two materials exhibited different mechanisms of failure. The suture created a large hole in the UBM until either the suture or the UBM broke. Veritas, however, conformed to the suture until maximum force, at which the suture simple rips out of the material. Figure 34E shows that at failure, parts of the Veritas still cling to the suture even after the suture has pulled out of its original insertion point. Since the UBM plastically deforms to create large holes, it is important to understand at what point this begins to happen. It is not readily apparent from the pictures that were taken whether or not there is any hole present at the specification force. Therefore a more through assessment would be necessary before implantation in patients, especially since there have been incidents of single layer UBM failing in the heart in animal studies [6].

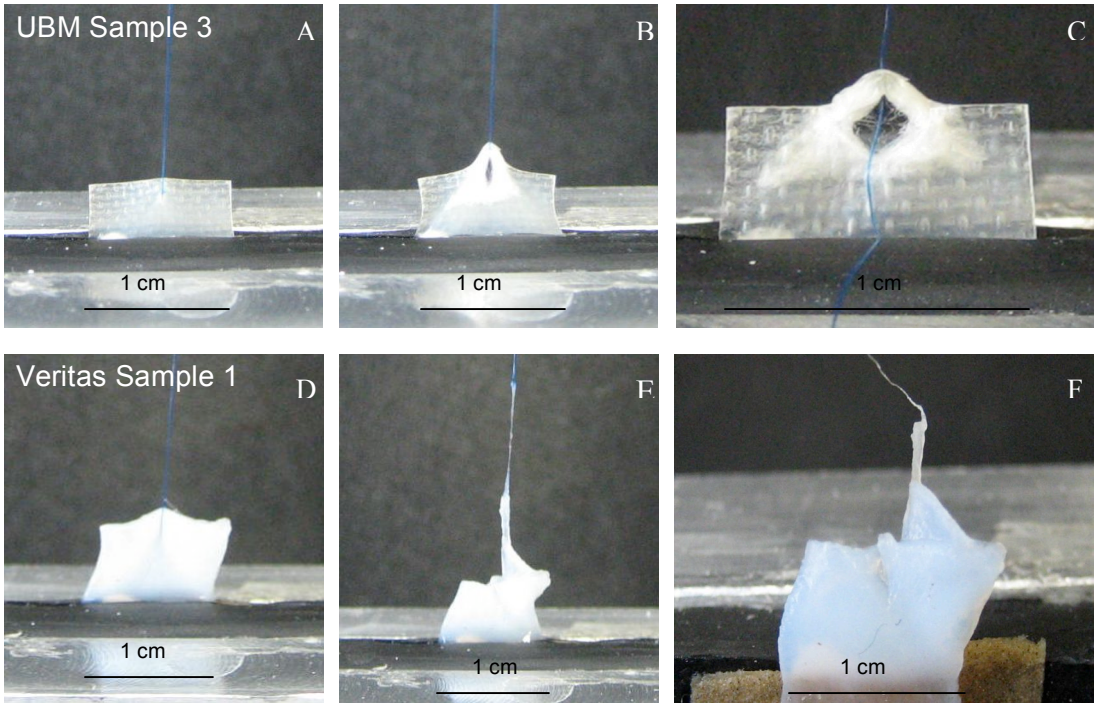

Figure 34: Suture Retention Modes of Failure: UBM and Veritas

The strength/strain characterization test confirmed the results of the preliminary testing in that all of the materials met the predefined specifications. There are differences in the two data sets, but this can be attributed to improved testing methods during characterization testing as opposed to preliminary testing. There was no slipping of the material between the grips when they were lined with sandpaper and the gage length of the sample was more accurately measured. The Hemashield samples did not fail, which matched our expected results since this is a very strong woven material. However, the results did not show the level of stiffness that was expected of a woven synthetic heart patch. One possible explanation for this is that the specific Hemashield material that was tested was designed to be a vascular graft.

Veritas demonstrated a strain response that fell completely within the specification, but on the lower end of the range. It was also not statistically different from the high end control Hemashield. In order for the endocardial patch component of this design to improve upon existing endocardial patches, the material should be more compliant than current synthetic materials. UBM was statistically different from both Veritas and Hemashield with 11% stretch at the specification force. However, the question still remains whether or not strain at the

specification force, as measured in this test, will translate to the correct material properties *in vivo*. The specification force incorporates a safety factor of 5, a higher force than a typical endocardial patch would experience during loading in the heart. Further testing would have to be conducted to understand the properties of this material at realistic contractile forces.

The suturability results demonstrated that all the patch materials, UBM, Veritas and Hemashield, had needle insertion forces significantly lower than the set specification of 5N (Appendix T). The order of magnitude of the measured insertion forces was very small and the force measurements made during this experiment had a low signal-to-noise ratio because the load cell was not sensitive enough to accurately measure the required forces. This force measurement noise can be seen in Figure 35. This data cannot be used to accurately describe the actual needle puncture force but it does demonstrate that all materials passed the specification.

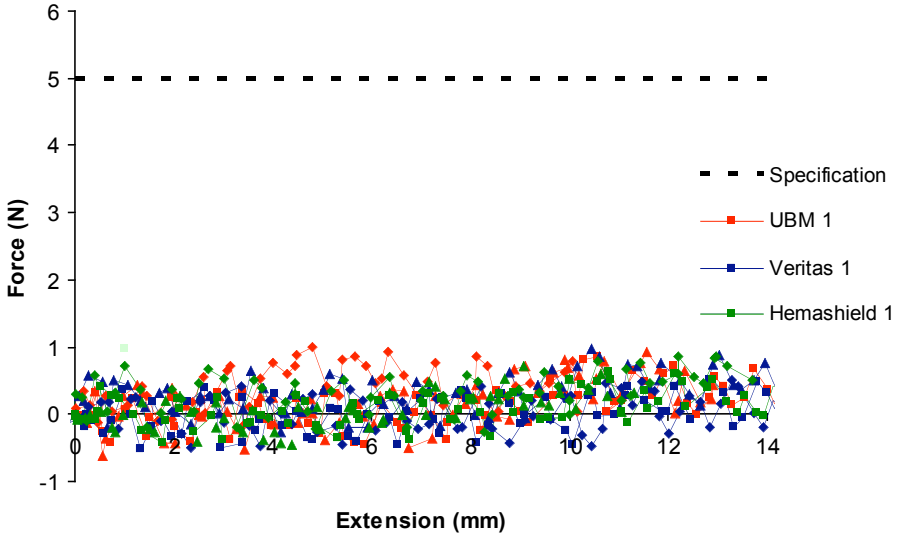

Figure 35: Suturability Data

7.2 Filler Material

The results of the migration assay showed a remarkable uniformity of distance measurements within each fibrinogen concentration, but high baseline variability between different concentrations. One possible explanation for this is that the concentration of fibrinogen affected the viscosity of the fibrinogen solution as it was injected into the well, thus altering the

manner in which the gels initially formed and the resulting conformation within the well. However, this would lead one to expect that the 3 mg/ml sample would be the least viscous, and would therefore flow the farthest outside of the score mark. This is not what was observed experimentally, as the 5 mg/ml samples generated the greatest baseline distance from the score mark to the farthest cell. At this point we are unsure of the exact cause of this baseline variability. The results from the third experiment (Figure 27) were inconsistent with the high distance response observed at 5 mg/ml fibrinogen in the initial assay. This is likely due to the complexity of the procedure.

The data obtained from the MTS viability assay show that cells remain viable in fibrin gels for up to 14 days and is consistent with previous studies [72]. The lack of increase in cell number between days 7 and 14 in the 9 mg/ml sample is not surprising and is consistent with the behavior of hMSCs grown on tissue culture plastic which normally reach confluency in 7-10 days. At day 0 the number of cells in the fibrin gels measured by the MTS assay was significantly less than the actual number of cells placed in the material. This difference in number of viable cells was not observed on the tissue culture plastic and might be brought about by an initial lack of adherence of the cells in the fibrin gels. Another possible cause for this might be that the fibrin gel itself interferes with the diffusion of the MTS solution and the formazen dye that it is converted into. Normally the cells are directly in contact with the media and MTS solution, therefore when it is converted into formazen dye, the dye is again directly transferred into the media. However, when the cells are in fibrin gels, the MTS solution must go through the fibrin gel before contacting the cells. So then the formazen dye must also go through the fibrin gel to get back out to the media where it can be detected. This may be responsible for the decrease in formzen dye in the media rather than the lack of viable cells. However, the cells in the fibrin gels subsequently had a significant increase in cell number with final counts over 6 times that of the initial cell number. In contrast, the final cell number for hMSCs on plastic was about twice that of the initial cell number. Despite the fact that the increase in cell number was greater for the fibrin gels than for the plastic, the overall number of viable cells on plastic was higher at all time points. The parameter of most interest to us in this project was not the overall number of cells present in the material but the rate of increase in cell number over time. Thus fibrin gel was considered to have a superior cell response compared to tissue culture plastic since

the increase in number of viable cells was greater.

Chapter 8: Conclusions

Based on the testing and analysis outlined in the previous chapters, several conclusion can be formed that summarize our efforts towards the design of a composite cardiac scaffold.

Both Veritas and UBM have appropriate suture retention, strength/strain, and suturability properties for cardiac patch applications as defined by our specifications. The only significant differences between the materials exist in their strain properties. This is an important quality of an endocardial patch material and merits further examination to determine if both materials are indeed appropriate for the environment of the heart.

From the data shown in Figure 26 and Figure 27, we conclude that hMSCs do not migrate in fibrin gel alone at fibrinogen concentrations of 3-9 mg/ml. This suggests that fibrin gel may not be an ideal scaffold filler material for this design, and that other materials should be tested in order to compare the results with those from the fibrin gel. Another approach would be to add growth factors to the fibrin gel in an effort to increase migration. It must also be considered whether hMSCs are a good model for the mobilized bone marrow cells that we hope to produce under actual *in vivo* conditions. It is possible that clinical results could differ significantly from our observations using this limited model for cellular migration.

Fibrin gels with any of the fibrinogen concentration tested here are well suited to support the viability of mesenchymal stem cells. This finding supports their use for the filler material application to promote viability of bone marrow cells. Given the strong viability data, but the absence of any evidence of migration, one possible approach would be to test the fibrin gel material as a carrier for cells to be seeded onto the surgical site. Instead of having to migrate into the gel, the cells would be able to proliferate inside, utilizing the high cell-binding affinity of fibrin gel.

Overall this testing has proven the initial capabilities of the selected materials. A significant amount of testing remains to be completed before full clinical applications can be realized, however this characterization presented here serves as a very important initial step towards the design of a composite cardiac scaffold.

Chapter 9: Recommendations

The work presented here describes just the beginning of the characterization of a heart patch that takes a vital first step towards regeneration of myocardium. There are many areas that can be further investigated in order to continue the development of this design so that it can completely fulfill its functionality and make a difference for patients suffering from myocardial infarction.

The results from testing the endocardial patch materials demonstrated that Veritas® and UBM had sufficient strength to hold heart pressure and stretched within the strain specifications. However, the tensile testing done in this project was limited to uniaxial testing. In order to ensure that the patch material has sufficient strength and strain properties when implanted in the heart, it is essential to perform multi-axial testing. This type of testing would ensure that the patch material is able to withstand the many different orientations of forces applied to it. Suggested multi-axial test methods include biaxial tensile testing and burst testing. It would also be advisable to perform fatigue testing to confirm the ability of the material to withstand the repetitive contractions it would experience once implanted in the heart.

The results of the migration assays revealed no significant migration of hMSCs in fibrin gel. The next step in this area would be add G-CSF to the fibrin gels and see if migration is affected in any way. Past studies have shown that G-CSF acts as a chemotactic agent for human polymorphonuclear leukocytes [73] and have suggested the same for neutrophils specifically[74], however, to our knowledge no studies have examined the chemotactic effect of G-CSF on bone marrow or mesenchymal stem cells. It should be noted that one study has shown that G-CSF has been implicated in increased risk of ischemia for patients who suffer from coronary artery disease [75]. Future research may also seek to examine the possibility of seeding the fibrin gel with bone marrow or mesenchymal stem cells, or testing other candidate materials for applications in a composite scaffold.

Seeing as this is a composite scaffold, it is also be important to study the interactions between the patch and filler component of this device. Degradation studies would estimate how long the patch and filler are able to stay at the site of implantation, as well as test if any toxic byproducts are released. Tests should also be done to characterize the ability of the filler material

to adhere to the patch material. Mechanical tensile testing would characterize the strength and strain in the composite and ensure that the filler material does not adversely effect mechanical properties of the patch material, such are reducing its elongation capacity.

The ultimate test to demonstrate the efficacy of this design would be *in vivo* testing of the combined patch and filler device. In vivo testing could be done on an animal model such as a rat, by excising a full thickness portion of the ventricle wall and replacing it with the composite device. The patch would first be sutured similar to current patches, and the filler would be injected on top of the patch to fill the full thickness of the myocardium. Upon explantation after at least eight weeks, the patch and heart should be examined for several different characteristics. Angiogenesis, the formation of new blood vessels, is essential to new tissue growth, so the patch should be stained to measure the microvessel density and observe the presence of new blood vessels [28, 30]. Degradation of the device components should be analyzed macroscopically to see how much degradation occurs over the time of implantation, with special attention paid to degradation as it relates to the mechanical stability of the implant [28]. Scar tissue formation should also be analyzed macroscopically for abscess formation, fibrotic encapsulation, and calcification, all of which would be detrimental to the development of new heart tissue [6]. Thrombus formation could also be detected by examining the endoventricular side of the patch for blood clots. The presence of blood clots would indicate the need to further investigate the blood compatibility of the materials [6]. Finally, the inflammatory response can be measured by staining for the presence of inflammatory cells such as macrophages, lymphocytes, and fibroblasts [28, 30].

Upon the successful evaluation of this device in rats, it could then be implanted in larger animal models such as a dog or pig heart, as these more closely mimic the human heart. This study would concentrate on two main outcomes: regional contractility and the presence of myocytes. Regional work is an important assessment because it measures whether or not the implanted patch is contributing to the work of the heart during contraction. This can be measured by placing markers on the patch and analyzing the change in area of the patch region as compared to the native myocardium [9]. Finally, staining for the presence of myocytes indicates how the patch is inducing regeneration of the myocardium. By staining with Connexin-43, the gap junctions of the myocytes will be visible and their orientation and presence in the device can

be analyzed.

Overall, the results from the patch and filler material characterization in this project were a crucial stepping stone towards the development of a new composite cardiac scaffold. While research done in this project is not a complete validation for using this composite scaffold in a clinical setting, it provides a strong foundation for future development of a therapy for ventricular restoration and myocardial regeneration.

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Appendix A: Interview with Dr. Nicola Francalancia

1. Cardiothoracic surgeon, PSU, JHU, 1997
    a. Research and surgery, sternum project
2. our project background
    a. patch for aneurysm excision surgery – actually help regenerate myocardium
3. aneurysm is a small piece of what cardiology field is
4. under what circumstances would you cut hole
    a. ventricular aneurysm: unusual thin
    b. HF more common
    c. Look for some treatments for HF
        i. Ventricular restoration – failing in all other ways
        ii. Cutting out doesn't always work
        iii. Need to correct geometry to get anything to work
        iv. Cut out and replace and need to maintain new geometry
            1. Dacron patch – endoventricular patch and then reconstruct the outside
    d. Patch inside, close outside, remodel
5. overall reluctance: big jump from value op to cutting ventricle
    a. time and risk
    b. heart failure is still a medically treated disease
6. mechanical, modeling, vasculature, myocardial regeneration
    a. ladder
7. ventricular restoration
    a. infarcted – hypokinetic, a kinetic
    b. will replace areas no with no aneurysm, just infarct
    c. viable tissue – new blood flow supply, doesn't work, recovers hibernating myocardium
    d. patch inside to maintain inner volume
    e. suture to endocardial surface
        i. prolene sutures
        ii. cut patch
    f. free wall rupture, pericardium contained
    g. aneurysm – old, easy to suture to
    h. key is getting geometry back
    i. Baptiste – not specific to diskinetic region
        i. Muscle weakening – whole heart – cut out a lot and put it back together
        ii. Reduce the whole thing
    j. Denton Cooley – Jarvic
    k. Endoventricular patch – Dor
8. Decision to cut out infarct
    a. Patient with heart failure – amiable to improving function then will
    b. Will go in only to cut out an infarct – ventricular restoration
9. suturing – cut off blood supply to where it is attached
    a. strangulation: edge not going to do too well, want to distribute tension

b. want it to be connected
c. glues – not always reliable for pressure for bonding
    i. glue contacting blood going to brain = not good
    ii. use glue in patients with clotting problems, glue holds
    iii. or for things not in direct contact with the blood stream

10. electrical connections
    a. aneurysm surgery: arrhythmia management
    b. arrhythmia: border zone, electrophysiology, get rid of this section that is causing arrhythmia
    c. infarct over conductive
11. patch procedure suturing
    a. hold – how patch?
    b. Cut out oval shape, patch – Dacron, sew all the way, ½ cm from edge sutures pulling
    c. Felt pieces cover next hold
12. patch
    a. Hemashield – Dacron
    b. albumin – less porous
13. Dacron: easy to use, super important to be able to cut, sew, bend
    a. Strength
    b. Biocompatibility
    c. Blood compatibility: endothelialize
14. bovine pericardium
    a. packaged in rectangular sheets, cut it how you want
    b. easy to work with
    c. small pieces to stick on
    d. not flexible but more handelability
        i. no crunching or cracking
        ii. hold sutures
    e. Dacron better – right kind of spring to it
        i. Pericardium: use when need something small
            1. 2 surfaces, shiny on one side
15. cells – acceptance
    a. if it fills some need, better than it can
    b. hurdles
        i. biomaterial: FDA stuff
        ii. extraneous cells on something – active blood stream and growing
        iii. carcinogenic worries
    c. if it is useable and has demonstrated advantages
        i. improved ventricular function
        ii. have to show survival benefit – randomized clinically controlled trials
        iii. better than just plain without cells
16. growth factors
    a. show that it makes a difference

i. blood vessels or is that worse/better than fixing geometry

17. suture: how close together

18. produce patch with monitoring
    a. don't have a lot of applicability: studying natural history of patch – research
    b. no used In people
    c. markers
    d. follow patient with conventional methods – echocardiogram
        i. don't distort MRI
        ii. can see which walls are moving and which aren't
        iii. not high priority to see where you put it
19. redo operations more complicated
20. most important thing is functional results – want something that will be there
21. region is electrically isolated, can come in over the top layer of tissue that was place back
    a. doesn't have to conduct
22. contraction
    a. biopatch that would beat – clinically no more desirable
    b. talking about having something beating, making it better through geometry not making spot beat again
    c. beating not priority but interesting for down the line applications
23. secondary – incorporates into vascular tissue well
    a. put patient on blood thinners, worry of clotting
24. next factors: use related, does it work with currently used remodeling tools
25. deploy patch without surgery
26. mitral valve regurgitation – when heart enlarging
27. porosity – not very porous so doesn't bleed through
    a. matters when not covering the other side with tissue because it can't leak into the pericardium
28. features over currently used
    a. various thickness and flexibility
    b. posterior have to lift heart
    c. easier way than suturing – method of attachment involving less surgery
29. Dalin Tang FEM patch size
    a. Large parts without contraction making the rest of the heart work harder
30. .ctsnet.org modified endoventricular
    a. Ventricular restoration: donato, Marisa
    b. Menicanti
    c. Show on eco that ventricular geometry is beginning to distort
31. myocardial regeneration would have to be attributing to functional improvement
    a. regenerate muscle mass – help out
    b. regrown but still have decreased ventricular function long term: regenerate heart tissue to keep shape

Appendix B: Pairwise Comparison Charts

Table 21: Pairwise Comparison Chart - Pooled

Goals	Marketable	Easy to Produce	Practical for Use in Surgery	Contractile	Regenerative	Reliable Mechanical Properties	Predictable Biomaterial-Tissue Interactions	Score	Score + 1	(Score + 1)/40.1
Marketable	••••	1.3	0.25	0.4	0.3	0	0	2.25	3.25	0.08
Easy to Produce	0.3	••••	0	0	0	0	0	0.3	1.3	0.03
Practical for Use in Surgery	1.35	1.6	••••	1.25	1.25	0.25	0.35	6.05	7.05	0.18
Contractile	1.2	1.6	0.35	••••	0.1	0	0	3.25	4.25	0.11
Regenerative	1.3	1.6	0.35	1.5	••••	0.1	0.6	5.45	6.45	0.16
Reliable Mechanical Properties	1.6	1.6	0.85	1.6	1.5	••••	1.4	8.55	9.55	0.24
Predictable Biomaterial-Tissue Interactions	1.6	1.6	1.25	1.4	1	0.4	••••	7.25	8.25	0.21
									40.1	1

Table 22: Pairwise Comparison Chart - Professor Gaudette

Goals	Marketable	Easy to Produce	Practical for Use in Surgery	Contractile	Regenerative	Reliable Mechanical Properties	Predictable Biomaterial-Tissue Interactions	Score	Score + 1	(Score + 1)/28
Marketable	••••	1	0	0	0	0	0	1	2	0.07
Easy to Produce	0	••••	0	0	0	0	0	0	1	0.04
Practical for Use in Surgery	1	1	••••	0.5	0.5	0.5	0.5	4	5	0.18
Contractile	1	1	0.5	••••	0	0	0	2.5	3.5	0.13
Regenerative	1	1	0.5	1	••••	0	0.5	4	5	0.18
Reliable Mechanical Properties	1	1	0.5	1	1	••••	1	5.5	6.5	0.23
Predictable Biomaterial-Tissue Interactions	1	1	0.5	1	0.5	0	••••	4	5	0.18
									28	1

Table 23: Pairwise Comparison Chart - Professor Pins

Goals	Marketable	Easy to Produce	Practical for Use in Surgery	Contractile	Regenerative	Reliable Mechanical Properties	Predictable Biomaterial-Tissue Interactions	Score	Score + 1	(Score + 1)/27
Marketable	••••	1	0.5	0	0	0	0	1.5	2.5	0.09
Easy to Produce	0	••••	0	0	0	0	0	0	1	0.04
Practical for Use in Surgery	0.5	1	••••	1	1	0	0	3.5	4.5	0.17
Contractile	1	1	0	••••	0	0	0	2	3	0.11
Regenerative	1	1	0	1	••••	0	0.5	3.5	4.5	0.17
Reliable Mechanical Properties	1	1	0	1	1	••••	1	5	6	0.22
Predictable Biomaterial-Tissue Interactions	1	1	1	1	0.5	0	••••	4.5	5.5	0.20
									27	1

Table 24: Pairwise Comparison Chart - Design Team

Goals	Marketable	Easy to Produce	Practical for Use in Surgery	Contractile	Regenerative	Reliable Mechanical Properties	Predictable Biomaterial-Tissue Interactions	Score	Score + 1	(Score + 1)/90
Marketable	••••	2.5	0.5	2	1.5	0	0	6.5	7.5	0.08
Easy to Produce	1.5	••••	0	0	0	0	0	1.5	2.5	0.03
Practical for Use in Surgery	3.5	4	••••	3.5	3.5	0	0.5	15	16	0.18
Contractile	2	4	0.5	••••	0.5	0	0	7	8	0.09
Regenerative	2.5	4	0.5	3.5	••••	0.5	1	12	13	0.14
Reliable Mechanical Properties	4	4	3	4	3.5	••••	3	21.5	22.5	0.25
Predictable Biomaterial-Tissue Interactions	4	4	3.5	3	3	2	••••	19.5	20.5	0.23
									90	1

Appendix C: Strength/Strain Protocol Calculations

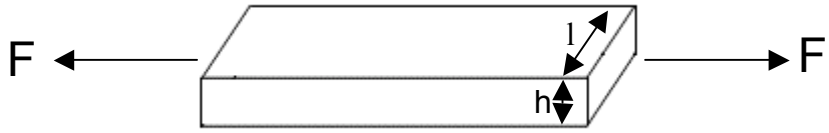


Figure 36: Strip of Heart Wall Showing Meridional Forces

Source for cardiac data and dimensions: [50]

Meridional wall stress in patients with high left ventricular pressure, $\sigma_w = 16.1\,\text{kPa}$

F = Wall tensile force

A = Wall cross - sectional area

$$\sigma_w = \frac{F}{A} = \frac{F}{l \times h}$$

Average wall thickness for patients with high left ventricular pressure, h = 17 mm = 0.017 m

Safety Factor (SF) = 5

Wall resistance in N/mm $(F/l) = \sigma_w \times h$

Wall resistance with safety factor in N/mm

$$= \sigma_w \times h \times SF = (16.1\,\text{kPa} \times 0.017\,\text{m} \times 5)\left(\frac{1\,\text{m}}{1000\,\text{mm}}\right) = \underline{\underline{1.37\ \text{N/mm}}}$$

Appendix D: Suture Retention Protocol Calculations

Source for cardiac data and dimensions: [50]
Source for distance between sutures: [76]

Meridional wall stress in patients with high left ventricular pressure, $\sigma_w = 16.1\,\text{kPa}$

Average wall thickness for patients with high left ventricular pressure, $h = 17\,\text{mm}$
Safety factor (SF) = 5

Estimated distance between suture, $l_s = 2\,\text{mm/suture} = 0.002\,\text{m/suture}$

Force per suture, $F_s = \sigma_w \times h \times l_s \times SF =$

$$= (16.1\,\text{kPa})(0.017\,\text{m})(0.002\,\text{m/suture}) \times 5 = \underline{2.737\,\text{N/Suture}}$$

Crosshead speed: between 50 and 150 mm/min

$$\frac{100mm}{\min} x \frac{1\min}{60s} = 1.67mm/s$$

Set speed to ~2mm/s

Appendix E: Suture Insertion Force Calculation

Source: [58]

Force from DeBakey woven fabric = 510 grams

Assuming gravitional acceleration = 9.81 m/s^2

Peneration force = mass × acceleration = 0.510 kg × 9.81 m/s^2 = <u>5 N</u>

Appendix F: Design Alternatives

Design Alternative 1

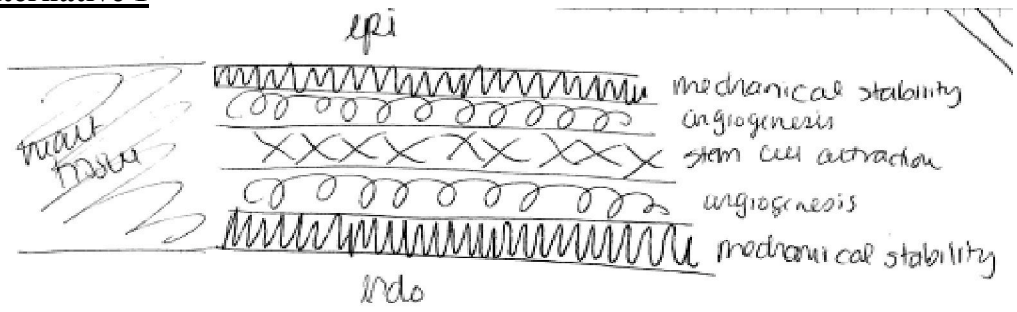


Figure 37: Design Alternative 1

Components:

- Endocardial Patch:
  - An endocardial patch sutured to the endocardial region, and is degraded once the differentiated myocytes can form a strong matrix to hold heart pressure.
- Middle Squirtable Layers:
  - Hydrogel layer for angiogenesis induced from surrounding tissue
  - Middle layers is another hydrogel layer embedded with growth factors (G-CSF) for stem cell differentiation into myocytes
  - This layer should also degrade as new tissue id formed
- Epicardial Patch:
  - Placed to hold the gel layers in place
  - Can be substituted with any left over Epicardial tissue

Pros:

- Stratified layers analogous to native tissue
- Degrades to leave only natural tissue

Cons:

- Complex architecture
- May be difficult to apply in surgery

Design Alternative 2

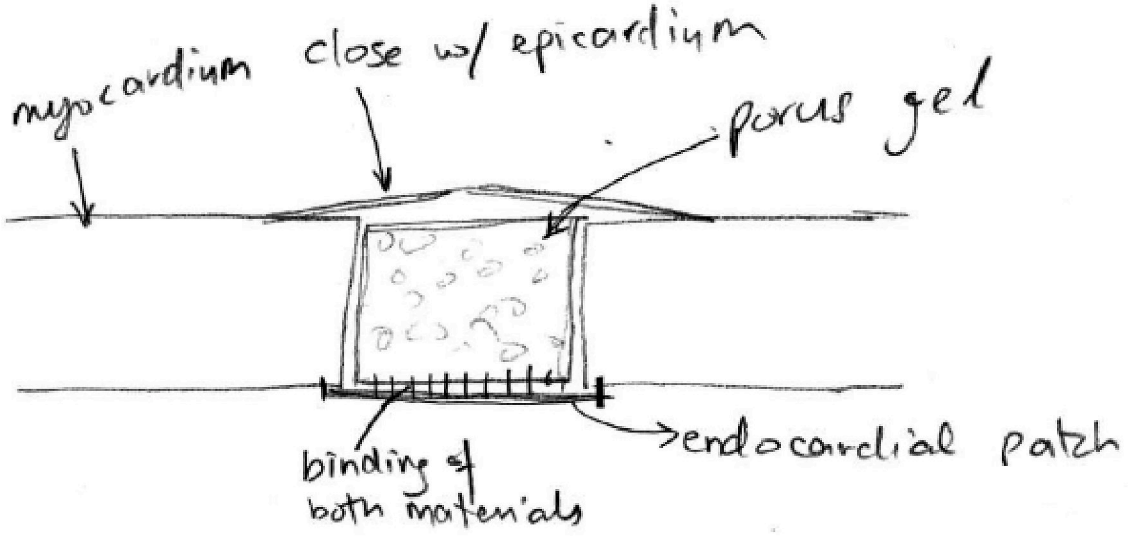


Figure 38: Design Alternative 2

Components:

- Endocardial Patch:
  - An endocardial patch sutured to the endocardial region to hold heart pressure and remodel the shape of the ventricle. Its elastic modulus is similar to the myocardium to allow for flexibility.
- Porous Filler:
  - Full thickness porous material miller to allow for angiogenesis and stem cell migration and differentiation. Pore size about 100um for stem cell and endothelial proliferation and migration
- Epicardial closure:
  - The scaffold can then be closed up using the left over epicardium

Pros:

- Scaffold is able to fully interface with surrounding tissue
- Many highly characterized scaffold materials to choose from

Cons:

- Shape of three-dimensional scaffold is not easily customized during operation
- Compliance mismatch may cause motion of the scaffold within the ventricular wall and tissue damage

Design Alternative 3

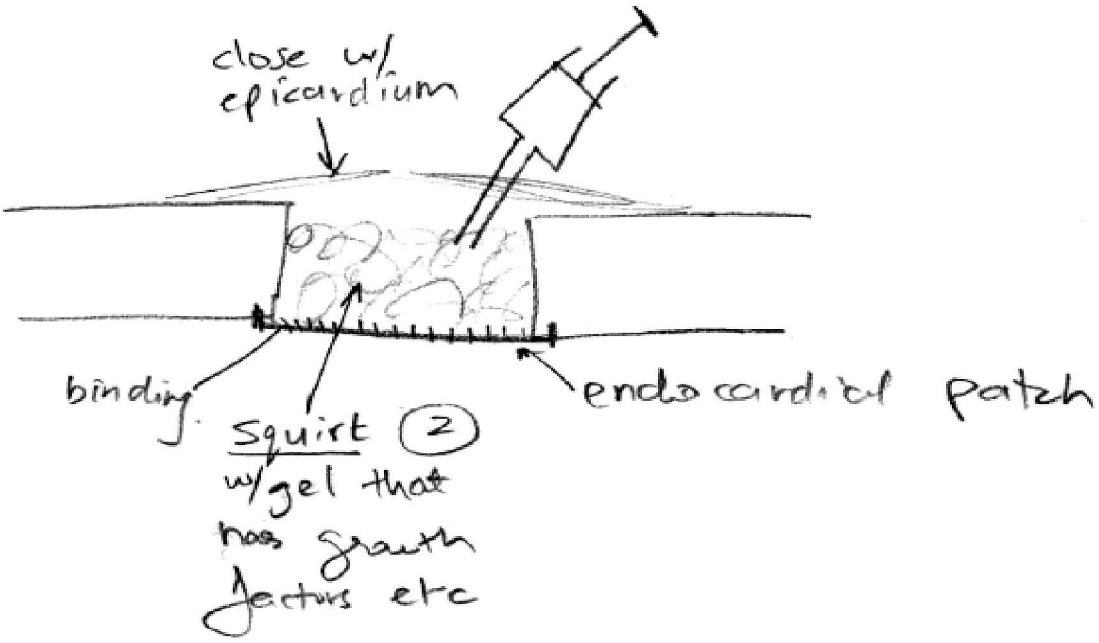


Figure 39: Design Alternative 3

Similar to Design Alternative 2, but instead of a porous filler, there can be an injectable hydrogel. This would allow for better spread of filled material in the void. The hydrogel can be made of fibrin glue and can incorporate growth factors for stem cell differentiation into myocytes.

Pros:

- Hydrogels are customizable to suit the purpose
- Can include RDG binding domains, growth factors, etc. in the gel
- Injectable design makes it easy to implant during surgery

Cons:

- Adhesion strength of the material may be limited

Design Alternative 4

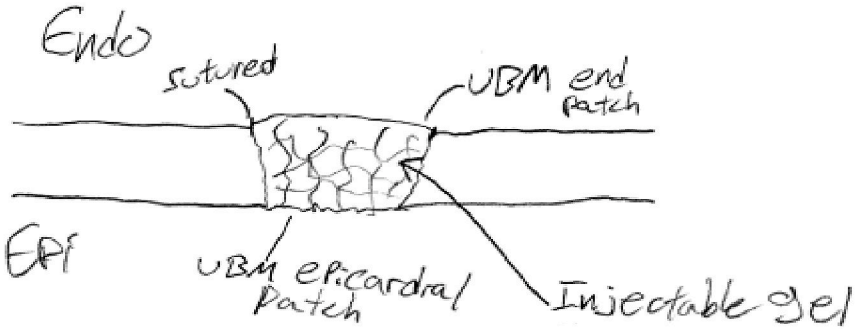


Figure 40: Design Alternative 4

Components:

- UBM Endocardial Patch:
  - UBM patch sutured in for holding heart pressure and structural remodeling
- Injectable Filler:
  - Injectable polymer hydrogel with growth factors and cell adhesion cites.
  - Degradable
- UBM Epicardial Patch:
  - UBM patch sutured in for closing up the injectable gel filler.

Pros:

- Synthetic materials are easier and cheaper to mass produce
- Complex polymer design
- Injectable design makes it easy to implant during surgery

Cons:

- Synthetic materials do not have as high an affinity for cell ingrowth

- **Design Alternative 5**

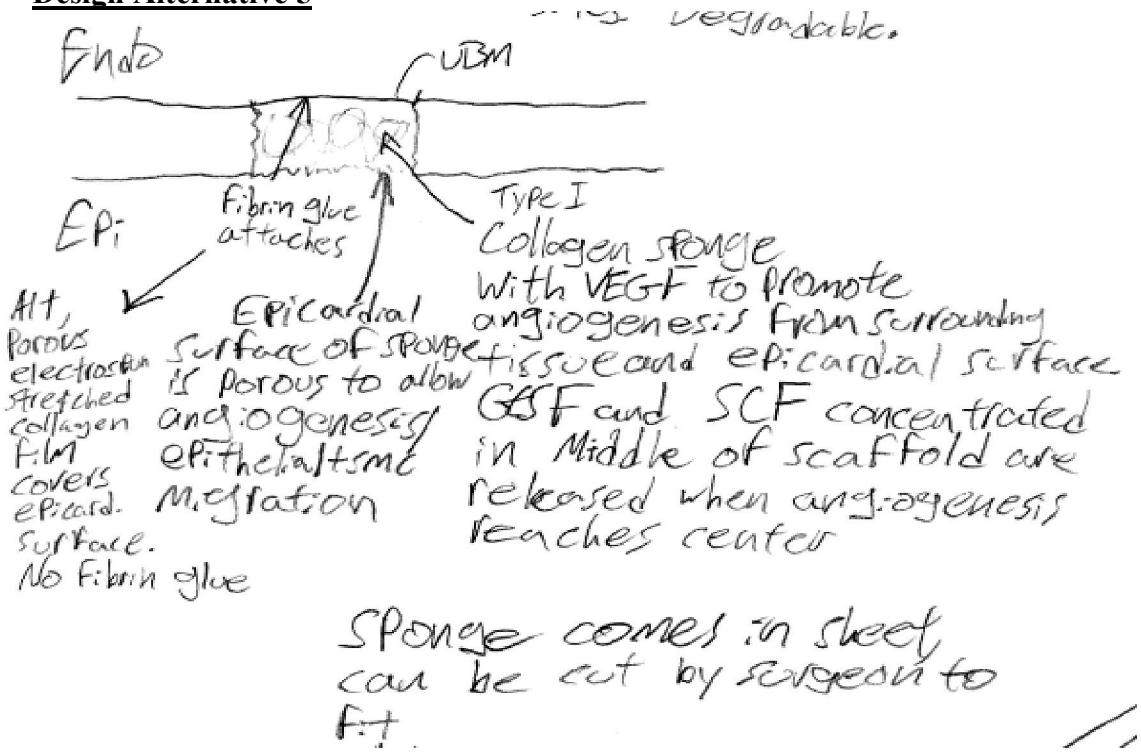


Figure 41: Design Alternative 5

Please refer to figure for detailed explanation

Pros:

- Natural materials have natural cell-binding domain

Cons:

- Many growth factors complicates design, increases expense, and complicated passage through the FDA
- Complex scaffold design may be difficult to manufacture

Design Alternative 6

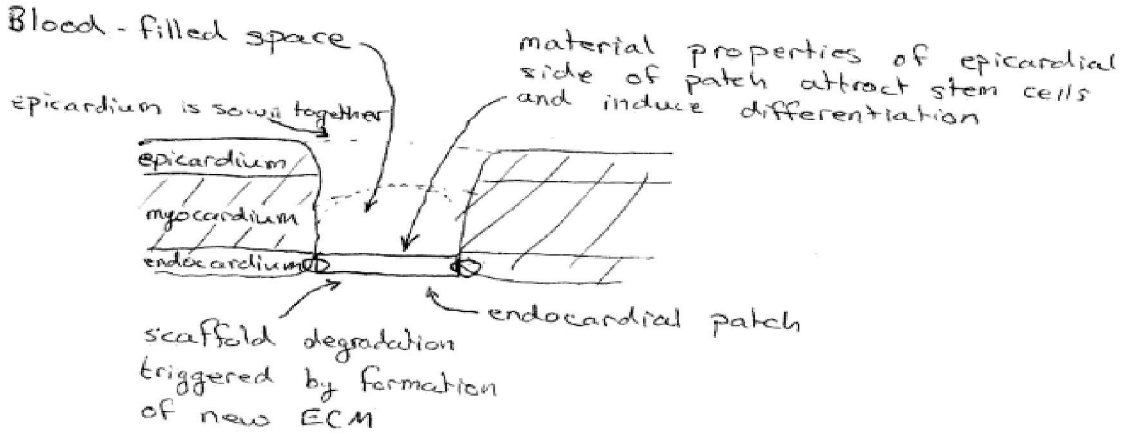


Figure 42: Design Alternative 6

Components:

- Endocardial Patch
  - Holds heart pressure and aids in remodeling
  - Epicardial side of the patch has material and chemical properties needed to attract stem cells and differentiate them into myocytes
- Blood-filled space
  - The void over the endocardial patch is filled with blood
  - Epicardium is sewed over to close the void

Pros:

- Simple implantation procedure same as currently used techniques
- One-piece design

Cons:

- Does not control the environment throughout the full thickness of the myocardium
- Clot may lead to scar tissue formation

Appendix G: Metrics and Matrices for Evaluation of Design Alternatives

This appendix details the evaluation of the design alternatives. Constraints and objectives from Section 4.2 that were applicable to the design of the scaffold are explained below in the context of metrics. The metrics create a scaling system to evaluate design alternatives and choose which one best fulfills our design criteria.

Constraints

Constraint: No thrombus or embolus formation
Basis for Comparison: Ability of the design in general to be compatible with blood. Presence of crevices or opportunities to form blood clots would be evaluated as not meeting this constraint.
Metric: Y (yes) or N (no)

Objective: Cost effective
Basis for Comparison: Will the design be in a reasonable cost range to produce and use? Incorporation of expensive techniques or complicated assembly would be evaluated as not meeting this constraint.
Metric: Y (yes) or N (no)

Main Objective: Similar Properties to Healthy Myocardium

Objective: Contractile
Basis for Comparison: Ability of the design to be conducive to redeveloping an area of contractile myocardium
Metric: 0 for no ability, 1 for provides some ability, 2 for definite ability

Objective: Regenerative
Basis for Comparison: Ability of design to support new tissue growth into the replaced region
Metric: 0 for no ability, 1 for provides some ability, 2 for definite ability

Main Objective: Clinically Applicable

Objective: Marketable
Basis for Comparison: Ability of the design to be attractive for marketing
Metric: 0 for no ability, 1 for provides some ability, 2 for definite ability

Objective: Easy to Produce
Basis for Comparison: Ability of the design to be easily manufactured, packaged, and stored.
Metric: 0 for no ability, 1 for provides some ability, 2 for definite ability

Objective: Practical for Use in Surgery
Basis for Comparison: Ability of the design to be easily used in current surgical procedures
Metric: 0 for no ability, 1 for provides some ability, 2 for definite ability

Main Objective: Safe for Patient

Objective: Reliable Mechanical Properties
Basis for Comparison: Ability of the design to provide the necessary mechanical stability to the heart
Metric: 0 for no ability, 1 for provides some ability, 2 for definite ability

Objective: Predictable Biomaterial-Tissue Interactions
Basis for Comparison: Ability of the design to control the growth of new tissue in the implanted region
Metric: 0 for no ability, 1 for provides some ability, 2 for definite ability

Table 25: Evaluation Matrix for Design Alternatives Part 1

Design / Objectives & Constraints	Weight	Endocardial patch, growth factors, injectable gel	Endocardial patch, seeded, injectable gel	Endocardial patch, growth factors, void	Endocardial patch, seeded, void
C: No thrombus or embolus formation	Y/N	Y	Y	N	N
C: Cost effective	Y/N	Y	Y	-	-
O: Reliable mechanical properties	(0.238)	2(0.238)=0.476	2(0.238)= 0.476	-	-
O: Predictable biomaterial-tissue responses	(0.206)	1(0.206)=0.206	1(0.206)=0.206	-	-
O: Practical for use in surgery	(0.176)	2(0.176)=0.352	1(0.176)=0.176	-	-
O: Regenerative	(0.161)	1(0.161)=0.161	1(0.161)=0.161	-	-
O: Contractile	(0.106)	1(0.106)=0.106	1(0.106)=0.106	-	-
O: Marketable	(0.081)	1(0.081)=0.081	0(0.081)=0	-	-
O: Easy to produce	(0.032)	1(0.032)=0.032	0(0.032)=0	-	-
Total	**2**	**1.414**	**1.125**	**-**	**-**

Table 26: Evaluation Matrix for Design Alternatives Part 2

Design / Objectives & Constraints	Weight	Whole wall thickness, growth factors, porous	Whole wall thickness, seeded, porous	Sew endocardial edges together, injectable gel
C: No thrombus or embolus formation	Y/N	Y	Y	Y
C: Cost effective	Y/N	Y	Y	Y
O: Reliable mechanical properties	(0.238)	2(0.238)=0.476	2(0.238)=0.476	2(0.238)= 0.476
O: Predictable biomaterial-tissue responses	(0.206)	1(0.206)=0.206	1(0.206)=0.206	1(0.206)= 0.206
O: Practical for use in surgery	(0.176)	0(0.176)=0	0(0.176)=0	0(0.176)=0
O: Regenerative	(0.161)	1(0.161)=0.161	2(0.161)=0.322	1(0.161)=0.161
O: Contractile	(0.106)	1(0.106)=0.106	1(0.106)=0.106	0(0.106)=0
O: Marketable	(0.081)	1(0.081)=0.081	0(0.081)=0	1(0.081)=0.081
O: Easy to produce	(0.032)	1(0.032)=0.032	0(0.032)=0	2(0.032)=0.060
Total	**2**	**1.062**	**1.110**	**0.984**

Justification of Scores

Design: Whole wall thickness, growth factors, porous

- No thrombus or embolus formation
  - (Y): fills excised area
- Cost effective
  - (Y): reasonable enough to produce, incorporation of growth factors would be expensive
- Reliable mechanical properties
  - (2): provides a whole wall thickness plug, will be stable to hold pressure of heart
- Predictable biomaterial-tissue responses
  - (1): know generally what growth factors should do, but not entirely sure if they will perform as needed
- Practical for use in surgery
  - (0): will need to design procedure in order to implant since it is a full thickness scaffold instead of a patch that can be sutured to the endocardium
- Regenerative

    - (1): porous to allow some cell ingrowth and growth factors should attract cells for new tissue formation
- Contractile
    - (1): porous spaces would allow cells to grow and contract within scaffold, whole scaffold would have to degrade to get entire region contracting
- Marketable
    - (1): attractive as single piece scaffold, unknowns of growth factors may detract from this
- Easy to produce
    - (1): easy as a single piece, material would have to be made porous or come porous, procedure needed for incorporating the growth factors

<u>Design: Whole wall thickness, seeded, porous</u>

- No thrombus or embolus formation
    - (Y): fills excised area
- Cost effective
    - (Y): reasonable enough to produce, incorporation of cells may be expensive and difficult
- Reliable mechanical properties
    - (2): provides a whole wall thickness plug, will be stable to hold pressure of heart
- Predictable biomaterial-tissue responses
    - (1): know generally what seeded cells should do, but not entirely sure if they will perform as needed
- Practical for use in surgery
    - (0): will need to design procedure in order to implant since it is a full thickness scaffold instead of a patch that can be sutured to the endocardium
- Regenerative
    - (2): as long as scaffold supports cell viability there will be new tissue growth since cells are seeded throughout the scaffold
- Contractile
    - (1): porous spaces would allow cells to grow and contract within scaffold, whole scaffold would have to degrade to get entire region contracting
- Marketable
    - (0): attractive as single piece scaffold, seeding cells at time of implantation and unknowns about how cells would react could detract from this
- Easy to produce
    - (0): easy as a single piece, material would have to be made porous or come porous, would have to find a way to store it with cells seeded or seed the cells at the time of implantation

<u>Design: Sew endocardial edges together, injectable gel</u>

- No thrombus or embolus formation
    - (Y): suturing eliminates gaps in area

- Cost effective
    - (Y): injectable gel easy to produce and apply
- Reliable mechanical properties
    - (2): walls of heart muscle are being sewn back together, heart wall is already strong enough to support pressure
- Predictable biomaterial-tissue responses
    - (1): injectable gel will provide some control over new tissue growth but may not be enough
- Practical for use in surgery
    - (0): not always enough muscle tissue to sew heart back together in the right shape, gel will not be strong enough to cover open gaps
- Regenerative
    - (1): gel can provide environment for regeneration but is not controlling exactly what new tissue is growing
- Contractile
    - (0): suturing heart muscle will still leave some dead space that will not contract with any new tissue formed
- Marketable
    - (1): injectable gel is easy to sell, but procedure is not always applicable to everyone because of the need of a certain amount of heart tissue
- Easy to produce
    - (2): injectable gel would be relatively easy to produce and store

<u>Design: Endocardial patch, growth factors, injectable gel</u>

- No thrombus or embolus formation
    - (Y): fills excised area
- Cost effective
    - (Y): reasonable enough to produce a gel and a patch, incorporation of growth factors would be expensive
- Reliable mechanical properties
    - (2): provides an endocardial patch similar to existing procedures, will be stable to hold pressure of heart
- Predictable biomaterial-tissue responses
    - (1): know generally what growth factors should do, but not entirely sure if they will perform as needed
- Practical for use in surgery
    - (2): suturing endocardial patch in is already a surgical procedure, injecting gel afterwards is a simple addition
- Regenerative
    - (1): gel with growth factors should attract cells for new tissue formation and provide conducive environment
- Contractile

    - (1): space in gel would allow cells to grow and contract within scaffold, patch may have to degrade to get entire region contracting
- Marketable
    - (1): attractive as already established surgical procedure and ease of gel injection, unknowns of growth factors may detract from this
- Easy to produce
    - (1): production of different pieces (gel and patch) make production less complex, growth factors can be incorporated into gel

Design: Endocardial patch, seeded, injectable gel

- No thrombus or embolus formation
    - (Y): fills excised area
- Cost effective
    - (Y): reasonable enough to produce a gel and a patch, incorporation of seeded cells may be difficult and expensive
- Reliable mechanical properties
    - (2): provides an endocardial patch similar to existing procedures, will be stable to hold pressure of heart
- Predictable biomaterial-tissue responses
    - (1): know generally what seeded should do, but not entirely sure if they will perform as needed
- Practical for use in surgery
    - (1): suturing endocardial patch in is already a surgical procedure, injecting gel afterwards is a simple addition, but having seeded cells in the gel may make things more complex than just growth factors
- Regenerative
    - (1): gel with seeded cells should be conducive new tissue formation and the gel provides stable environment
- Contractile
    - (1): space in gel would allow cells to grow and contract within scaffold, patch may have to degrade to get entire region contracting
- Marketable
    - (0): attractive as already established surgical procedure and ease of gel injection, seeding cells at time of implantation and unknowns about how cells would react could detract from this
- Easy to produce
    - (0): production of different pieces (gel and patch) make production less complex, would have to find a way to store gel with cells seeded or seed the cells at the time of implantation

Design: Endocardial patch, growth factors, void

- No thrombus or embolus formation
    - (N): leaves unfilled area behind patch for clot formation and unknown regeneration

Design: Endocardial patch, seeded, void

- No thrombus or embolus formation
    - (N): leaves unfilled area behind patch for clot formation and unknown regeneration

Appendix H: Metrics and Matrices for Evaluation of Materials

The metrics formed in the previous appendix for design evaluation were further defined to be quantitatively specific to evaluate different types of materials. The scales for ranking each objective are described below.

Main Objective: Similar Properties to Healthy Myocardium
Objective: Contractile
Basis for Comparison: Ranking the stiffness of the material on a scale of 0 (worst) to 2 (best) in comparison with the stiffness of the healthy myocardium
Metric: 0 for stiffness greater than 280 kPa (stiffness of heart wall at the end of systole), 1 for stiffness between 60 kPa (stiffness of heart wall at the start of systole) and 280 kPa (stiffness of heart wall the end of systole), 2 for stiffness less than 60 kPa (stiffness of heart wall at the start of systole).

Objective: Regenerative
Basis for Comparison: Ranking the adherence, migration and proliferation of stem cells on the scaffold on a scale of 0 (worst) to 2 (best)
Metric: 0 is stem cells adhere to the material, 1 if stem cells adhere and migrate in the material, 2 if stem cells adhere, migrate and proliferate in the material

Main Objective: Clinically Applicable
Objective: Marketable
Basis for Comparison: Ranking the cost and FDA compliance scale of 0 (worst) to 2 (best)
Metric: 0 for cost greater that $10000, 1 for cost less that $10000 and use of FDA unapproved materials, 2 for cost less that $10000 and use of FDA approved materials

Objective: Easy to Produce
Basis for Comparison: Ranking the manufacturability on a scale of 0 (worst) to 2 (best)
Metric: 0 for un-established reproducibility of material (eg. creating a new polymer configuration), 1 for established manufacturing techniques with available resources, 2 for use of off the shelf materials

Objective: Practical for Use in Surgery
Basis for Comparison: Ranking the complexity of surgery on scale of 0 (worst) to 2 (best)
Metric: 0 for need for new surgical technique, 1 for moderate complexity and time for surgery (i.e. scaffold must be cut into desirable shape and sutured), 2 for minimal complexity and time for surgery (i.e. Injectable material)

Main Objective: Safe for Patient
Objective: Reliable Mechanical Properties
Basis for Comparison: Ranking the ability of patch material to withstand a high ventricular pressure on scale of 0 (worst) to 2 (best)

Metric: 0 for inability to withstand tensile resistance of 1.37 N/mm, 1 for ability to withstand tensile resistance of 1.37 N/mm but not under cyclic conditions, 2 for ability to withstand tensile resistance of 1.37 N/mm under cyclic conditions

Objective: Predictable Biomaterial-Tissue Response
Basis for Comparison: Ranking the ability of material to resist a fibrotic tissue development on scale of 0 (worst) to 2 (best)
Metric: 0 for extensive fibrotic tissue development, 1 moderate fibrotic tissue development, 2 for negligible fibrotic tissue development

Table 27: Metrics Scoring for Patch Materials

Objectives / Patch Materials	Reliable Mechanical Properties	Predictable Biomaterial-tissue Response	Practical for use in Surgery	Regenerative	Contractile	Marketable	Easy to Produce	**Total Score**
	(0.238)	(0.206)	(0.176)	(0.161)	(0.106)	(0.081)	(0.032)	**2**
UBM	2	2	1	2	0	1	2	**1.531**
Veritas	2	2	1	2		1	2	**1.531**
Trimethylene carbonate (TMC) - DLLA copolymers	2	2	1	1	0	1	1	**1.338**
Tissue Fleece	0	2	1	1	1	2	2	**1.081**
Poly(glycolide-co-caprolactone) (PGCL)	1	1	1	1	1	1	1	**1**
Electrospun natural polymers	0	2	1	2	0	1	0	**0.991**
Dacron	2	0	1	0	0	2	2	**0.878**
ePTFE	2	0	1	0	0	2	2	**0.878**
Bovine Pericardium (crosslinked)	2	0	1	0	0	2	2	**0.878**
polymer poly-4-hydroxybutyrate (P4HB)	0	1	1	1	2	1	0	**0.836**
E-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide fabric (PCLA)	0	1	1	1	0	1	1	**0.656**
Polyaniline			1	2	0	1	0	**0.579**
Alginate/PEO electrospun	0		1		0	1	1	**0.289**

Table 28: Metrics Scoring for Filler Materials

Objectives / Patch Materials	Reliable Mechanical Properties	Predictable Biomaterial-tissue Response	Practical for use in Surgery	Regenerative	Contractile	Marketable	Easy to Produce	**Total Score**
	(0.238)	(0.206)	(0.176)	(0.161)	(0.106)	(0.081)	(0.032)	2
Fibrin Glue	0	2	2	2	2	2	2	1.524
Alginate Gel	0	2	2	2	2	2	1	1.492
Injectable Collagen Scaffold	0	2	2	2	2	2	1	1.492
Fibrin Blend	0	2	2	2	2	2	1	1.492
Platelet Rich Plasma	0	2	2	2	2	2	0	1.46
Gelatin Sponge (Gelfoam)	0	2	1	2	2	2	2	1.348
Collagen Sponge	0	2	1	2	2	2	1	1.316
Alginate Sponge	0	2	1	2	0	2	1	1.104
Collagen Sponge (Ultrafoam)	0	1	1	2	1	2	2	1.036
Collagen Gel (hydrogel)	0	1	1	2	1	2	2	1.036

Justification of Metrics by Material

DACRON

- Reliable Mechanical Properties
  - (2): Its suitable mechanical properties can be inferred from the fact that it is currently used
- Predictable Biomaterials-Tissue Response
  - (0): Dacron exhibits fibrosis [9]
- Practical for Use in Surgery
  - (1): Dacron is sold as large sheets that can be cut
- Regenerative
  - (0): No cardiomyocytes observed on implanted Dacron patches [9]
- Contractile
  - (0): vascular graft materials ~ 690 stiffer than normal canine myocardium [77]
- Marketable
  - (2): Currently used
- Easy to Produce
  - (2): Already established product

ePTFE

- Reliable Mechanical Properties
  - (2): Its suitable mechanical properties can be inferred from the fact that it is currently used
- Predictable Biomaterials-Tissue Response
  - (0) ePTFE exhibited extensive fibrotic encapsulation after being implanted for 3 months [6]
- Practical for Use in Surgery
  - (1): Sold as large sheets that can be cut
- Regenerative
  - (0): No striated cardiac cells observed on implanted ePTFE patches [6]
- Contractile
  - (0): Vascular graft materials ~ 690 stiffer than normal canine myocardium [77]
- Marketable
  - (2): Currently used
- Easy to Produce
  - (0): Already established product

Bovine Pericardium (crosslinked)

- Reliable Mechanical Properties
  - (2): Its suitable mechanical properties can be inferred from the fact that it is currently used
- Predictable Biomaterials-Tissue Response
  - (0): A thick layer of fibrous tissue was attached to bovine pericardium patches 4 weeks after implantation [78]
- Practical for Use in Surgery
  - (1): Bovine Pericardium can be made to any shape
- Regenerative
  - (0) No mesothelial cells found on patch 12 weeks after implantation [78]
- Contractile
  - (0): Tensile modulus of glutaraldehyde crosslinked bovine pericardium was 0.51 +/-0.09 MPa at a load of 20 kPa (low modulus region) and 3.8 +/- 0.8 MPa under 150 kPa (high modulus region) [79]
  - Stiffness of glutaraldehyde fixed bovine pericardium in preferred direction and cross direction were 3.27+/-0.25 MPa and 1.31+/-0.18 MPa respectively [80]
- Marketable
  - (2): Currently used
- Easy to Produce
  - (2): Already established product

UBM

- Reliable Mechanical Properties

    - (2): Maximum force was 1.79 N/mm (tensile strength of 0.3-0.4 MPa) [81, 82]. Tensile strength was ~2 MPa [83]
- Predictable Biomaterials-Tissue Response
    - (2): Tissue replacing UBM patch in canine heart showed had "healing scar" [6]
- Practical for Use in Surgery
    - (1): UBM comes as a sheet that must be cut
- Regenerative
    - (2): Cardiomyocytes account for ~30% of remodeled tissue after 8 weeks in vitro [84]
- Contractile
    - (0): Estimated stiffness was 450 kPa [82]
- Marketable
    - (0): UBM is not approved by the FDA for use in cardiac patch applications (it is approved for other uses though)
- Easy to Produce
    - (2): UBM is a commercially available material

Polyanaline

- Reliable Mechanical Properties
    - (0): Polyurethane/polyaniline blend had tensile strength of 2.5 MPa (Abbati et al., J Appl Sci 2003;89:2516-1521)
- Predictable Biomaterials-Tissue Response
    - (0): Tissue excised from implant region had similar histological characteristics to healthy tissue away from implant site [85]
- Practical for Use in Surgery
    - (1): Can be electrospun into fibers [8]
- Regenerative
    - (2): Adhesion and proliferation of cardiac myoblasts was similar to that observed on tissue culture polystyrene [8]
- Contractile
    - (0): Stiffness of electrospun scaffolds made of 45:55 blend of polyanaline: gelatin was 1384 +/- 105 MPa [8]. Polyurethane/polyaniline copolymer had stiffness of 4 MPa [86]
- Marketable
    - (1): Polyanaline is not approved by the FDA for implantation in humans
- Easy to Produce
    - (0): Can be electrospun, formed into a gel, or formed into other morphologies

Veritas® collagen matrix

- Reliable Mechanical Properties
    - (0): Currently used for lung, gastric, and thoracic surgeries which have similar mechanical environments to the ventricle [87]
- Predictable Biomaterials-Tissue Response

    - (2): Number of acute/inflammatory cells was small [88]
- Practical for Use in Surgery
    - (1): Derived from bovine pericardium
- Regenerative
    - (2): Significant host cell infiltration of the implant was observed [88]
- Contractile
    - (0): No information available
- Marketable
    - (1): Approved by the FDA for lung, gastric, thoracic surgeries but not for cardiac applications
- Easy to Produce
    - (2): Veritas is a commercially available material

Electrospun natural polymers

- Reliable Mechanical Properties
    - (0): Mechanical properties are controlled by copolymerizing with synthetic polymers
- Predictable Biomaterials-Tissue Response
    - (2): Natural materials mimic native extracellular matrix
- Practical for Use in Surgery
    - (1): Can be electrospun or formed into gel
- Regenerative
    - (2): Natural materials mimic native extracellular matrix
- Contractile
    - (0): Mechanical properties are controlled by copolymerizing with synthetic polymers
- Marketable
    - (1): Natural polymers not approved by FDA for cardiac use. Electrospinning is an economical manufacturing process [89].
- Easy to Produce
    - (0): materials must be copolymerized and electrospun or formed into gel

Trimethylene Carbonate (TMC) – D,L-Lacitide (DLLA) copolymer

- Reliable Mechanical Properties
    - (2): TMC-DLLA blends had a tensile strength from 10-50 MPa [90]
- Predictable Biomaterial-Tissue Response
    - (1): All samples showed endothelial cell migration and proliferation on the surface [90]
- Practical for Use in Surgery
    - (1): Polymer is prepared as thin sheets that have to be cut for optimized shape [90]
- Regenerative

    - (0): Only published studies were done with myocytes that, however, did proliferate and migrate [90]
- Contractile
    - (0): Elastic modulus was in the range of 5-2000 MPa [90]
- Marketable
    - (1): TMC is unapproved by the FDA and is a relatively new material for research in cardiac applications [90]
- Easy to Produce
    - (1): This is not an off the shelf material, but has well established manufacturing techniques [90]

Tissue Fleece

- Reliable Mechanical Properties
    - (1): Tissue Fleece had a tensile strength similar to native heart muscle, unsure about actual numbers [31]
- Predictable Biomaterial-Tissue Response
    - (2): All samples were able to successfully culture contractile myocytes [31]
- Practical for Use in Surgery
    - (1): Tissue Fleece needs to be cut into appropriate shape before application [31]
- Regenerative
    - (0): Only published studies were done with myocytes that, however, did proliferate, migrate and contract [31]
- Contractile
    - (1): The stress strain curve was very similar (almost identical) to native rat myocardium. However, this was with myocyte seeded and cultured tissue fleece samples. [31]
- Marketable
    - (1): TMC is approved by the FDA and is currently used in surgical applications [31]
- Easy to Produce
    - (2): Tissue Fleece can be bought off the shelf [31]

Poly(glycolide-co-caprolactone) (PGCL)

- Reliable Mechanical Properties
    - (1): PGCL polymer had about 100% recovery in cyclic loading, with yield strength of about 0.2 MPa [91]
- Predictable Biomaterial-Tissue Response
    - (1): Showed no adverse tissue response when seeded with smooth muscle cells in vitro [91]
- Practical for Use in Surgery
    - (1): Polymer is prepared as thin sheets that have to be cut for optimized shape [91]
- Regenerative

    - (0): Only published studies were done with smooth muscle cells that did proliferate and migrate [92]
- Contractile
    - (1): Elastic modulus was in the range of 100 kPa [91]
- Marketable
    - (1): PGCL is unapproved by the FDA but has been used for research in cardiac applications [91]
- Easy to Produce
    - (1): This is not an off the shelf material, but has well established manufacturing techniques [91]

Fibrin Glue

- Predictable biomaterial – tissue Response
    - (2): no inflammation or fibrosis occurred when fibrin glue was injecting into infarction regions of rat hearts [30, 93].
- Practical for Use in Surgery
    - (2): fibrin glue is an injectable material with a long history of clinical application [94].
- Regenerative
    - (2): has been shown to improve cell transplant survival, reduce infarct expansion, and improve heart function [30, 93].
- Contractile
    - (2): modulus of elasticity is approximately 14.6-27.5 kPa depending on fibrinogen concentration [95].
- Marketable
    - (2): fibrin glues are already FDA approved for use in humans [30, 93].
- Easy to Produce
    - (2): readily available off-the shelf product [30, 93, 96].

Fibrin Blend

Was merged with fibrin glue

Injectable Collagen Scaffold

- Predicatable biomaterial-tissue Response
    - (2): uncrosslinked collagen integrated into native tissue, while crosslinked collagen material induce encapsulation and scar formation [97]
- Practical for Use in Surgery
    - (2): it is injectable and has been used for surgery extensively in the past [96].
- Regenerative
    - (2): seeded human heart cells have proliferated on a collagen matrices in a past study [53].
- Contractile

    - (2): it is a soft, injectable gel [96].
- Marketable
    - (2): it is a currently approved biomaterial for clinical use [96].
- Easy to produce
    - (1): we would have to produce it on our own, but expertise and resources are readily available.

Platelet-rich Plasma

- Predictable biomaterial-tissue Response
    - (2): it has been shown to produce regenerative healing in the equine epidermis and dermis without fibrosis or scar formation [98].
- Practical for Use in Surgery
    - (2): it is injectable and is currently used in clinical applications [99].
- Regenerative
    - (2): it has been shown to produce regenerative healing in the equine epidermis and dermis without fibrosis or scar formation [98].
- Contractile
    - (2): although no data was found regarding the elasticity of platelet-rich plasma, it received a score of 2 for contractile based on the fact that it is generally characterized as a highly compliant gel [100].
- Marketable
    - (2): autologous platelet-rich plasma is used clinically [99],
- Easy to produce
    - (0): the use of autologous materials would complicate the manufacturing process.

Collagen Sponge

- Predictable biomaterial-tissue Response
    - (2): for the same reasons as the injectable collagen scaffold [97].
- Practical for Use in Surgery
    - (1): its shape could be customized by cutting to the correct size.
- Regenerative
    - (2): seeded human heart cells have proliferated on a collagen matrices in a past study [53].
- Marketable
    - (2): it is a currently approved biomaterial for clinical use [101].
- Easy to produce
    - (1): collagen scaffold are available off-the-self, however producing our own collagen sponge would prove to be more difficult.

Alginate Sponge

- Predictable biomaterial-tissue Response
    - (2): thin lucent connective tissue enriched with blood vessels formed over it, integrated well with the infarcted area [28]

- Practical for use in surgery
    - (1): alginate sponge has to be prepared into certain sizes before implantation [28]
- Regenerative
    - (2): human embryonic stem cells formed embroid bodies and populated the alginate scaffolds, two fold increase in cell number within first week in culture [102]
- Contractile
    - (0): tested dry alginate sponges, elastic modulus between 385 and 1136kPa which translates to 0.0115 N/mm [103]
- Marketable
    - (2): can be purchased commercially and is relatively inexpensive [103]
- Easy to produce
    - (1): established protocols to make alginate with commercially available resources [104]

Collagen Sponge

- Predictable biomaterial-tissue Response
    - (1): injectable collagen from bovine dermis has caused high levels of anti bovine collagen antibodies in patients, collagen type IV is very strong in immunogenic character [105, 106]
- Practical for use in surgery
    - (1): collagen sponge can be cut to size and sutured on to/in infarction [27, 31]
- Regenerative
    - (2): collagen is readily available ECM component that allows cell infiltration and remodeling, excellent substrate for cell attachment and infiltration [27, 107]
- Contractile
    - (1): no numbers but shown to allow contraction of myocytes on scaffold [27, 31]
- Marketable
    - (2): commercially available [108]
- Easy to produce
    - (2): buy off the shelf and cut to desired shape [27]

Gelatin Sponge

- Predictable biomaterial-tissue Response
    - (2): saw good biocompatibility, sponge filled with regenerated tissue, minimal inflammatory response and no multinucleated giant cells [109]
- Practical for use in surgery
    - (1): commercially available, cut down to desired shape and suture in [29, 53]
- Regenerative
    - (2): hMSCs proliferated and differentiated on the sponge [109]
- Contractile
    - (2): tensile testing, modulus about 123 Pa, less than 7kPa [53]
- Marketable

    - (2): commercially available [110]
- Easy to produce
    - (2): off the shelf commercially available material [53]

Collagen Gel

- Predictable biomaterial-tissue Response
    - (1): injectable collagen from bovine dermis has caused high levels of anti bovine collagen antibodies in patients, collagen type IV is very strong in immunogenic character [105, 106]
- Practical for use in surgery
    - (2): injectable [106]
- Regenerative
    - (2): collagen is readily available ECM component that allows cell infiltration and remodeling, excellent substrate for cell attachment and infiltration [27, 107]
- Contractile
    - (1): no numbers but shown to allow contraction of myocytes on scaffold [27, 31]
- Marketable
    - (2): commercially available [108]
- Easy to produce
    - (2): established manufacturing techniques [106, 111]

Alginate Gel

- Predictable biomaterial-tissue Response
    - (2): minimum immune response when purified, unpurified causes major encapsulation [104]
- Practical for use in surgery
    - (2): injectable, can tailor viscosity to desired properties [104]
- Regenerative
    - (2): cell migration and proliferation seen in the gel [112]
- Contractile
    - (2): subjective, gel – high compression, low tensile
- Marketable
    - (2): materials can be purchased commercially and is relatively inexpensive [103]
- Easy to produce
    - (1): produced through established techniques [103, 104]

Appendix I: Strength Strain Testing Protocols

	Strength/Strain-Preliminary Testing
Objectives and Specifications	Endocardial patch should withstand a force of at least 1.37N/mm (4.11 N or 419.1 g)
Materials and Equipment	• Instron In-Spec 2200 • Camera with tripod • Computer with ImageJ • Dog bone shaped cutter • Sample size n = 2
Procedure	• Setup Instron according to 'Setup and Test Basics for Instron' • Set crosshead speed to 4 mm/s according to 'Setting Crosshead Speed' • Samples should be cut into 1cm by 10cm dogbone shapes as specified (lab notebook p 33) and hydrated in PBS for 30 minutes before testing • Place two dots on gauge length of dog bone about 1 cm apart, to be used for stretch analysis • Secure material first in the upper grip then in the lower grip • Apply tare load by adjusting position of upper grip so the material is taught between the grips • Zero the displacement and tare according to 'Setup and Test Basics for Instron' • Re-measure and record gage length • Start test and start camera (pictures at ½ second intervals) • Stop test after material fails

Expected Results and Methods of Analysis	**Results**
	• Force vs Displacement curve
	• Strain
	  o Use ImageJ program to calculate actual strain between zero force and 1.37N/mm
	Analysis
	• Force vs Displacement
	  o Pass: sample did not fail at 1.37N/mm (4.11 N or 419.1 g)
	  o Fail: sample failed below 1.37N/mm (4.11 N or 419.1 g)
	• Strain
	  o Should be 5 to 15% at specification

References	[10, 49-51]

Protocol	Cutting Dog Bone Samples for Strength/Strain Testing
Objectives and Specifications	To prepare ASTM standard samples for tensile testing
Materials and Equipment	• Steel punch made according to ASTM standard E8M – 04 • Acrylic board • Clamp • Hammer • Petri dish • Saline solution • Sand paper • X-acto knife
Procedure	• Tightly secure the acrylic board on the bench top using the clamp • Place the material on the board, making sure there are no kinks or folds • Carefully place the metal punch over the material • Holding it securely with one hand, hammer at least 15 times to cut the material • Lift the punch and cut any uncut parts of the material using an x-acto knife. Do not drag knife on material, cut by simply applying downward pressure. • Immerse sample in PBS in a Petri dish for 30 minutes • Smooth out acrylic base and sharpen punch edges using the sand paper • Wipe the acrylic base before reuse

Crosshead Speed:
Sample width: 3mm

$F = 1.37N/mm * 3mm$

$F = 4.11N$

Crosshead Speed for Preliminary Testing:
Average Heart Rate: [10]

Heart beats in one day = 100,000 Beats/Day

$$\text{Beats per Minute, BPM} = \frac{100{,}000 \text{ Beats}}{Day} \times \frac{1 \text{ Day}}{24 \text{ Hrs}} \times \frac{1 \text{ Hr}}{60 \text{ min}} = \underline{\underline{69.4 \text{ BPM}}}$$

Stretch % of heart muscle: around 21%

$$strainrate = \frac{stretch\%}{60s \big/ avgheartrate(bpm)}$$

Crosshead speed = gage length * strain rate
Maximum possible crosshead speed = 4mm/s

$$4mm/s = 0.24/s * gagelength$$
$$gagelength = 16.7mm$$

	Strength/Strain-Characterization Testing
Objectives and Specifications	Endocardial patch should withstand a force of at least 1.37N/mm (4.11 N or 419.1 g)
Materials and Equipment	• Instron In-Spec 2200 • Camera with tripod • Computer with ImageJ • Dog bone shaped cutter • Sample size n = 3
Procedure	• Setup Instron according to 'Setup and Test Basics for Instron' • Set crosshead speed to 4 mm/s according to 'Setting Crosshead Speed' • Samples should be cut into 1cm by 10cm dogbone shapes as specified (lab notebook p 33) and hydrated in PBS for 30 minutes before testing • Place two dots on gauge length of dog bone about 1 cm apart, to be used for stretch analysis • Secure material first in the upper grip then in the lower grip • Apply tare load by adjusting position of upper grip so the material is taught between the grips • Zero the displacement and tare according to 'Setup and Test Basics for Instron' • Start test and start camera (pictures at ½ second intervals) • Stop test after material fails

Expected Results and Methods of Analysis	**Results**
	• Force vs Displacement curve
	• Strain
	  o Use ImageJ program to calculate actual strain between zero force and 1.37N/mm (should be between 5 and 15%)
	Analysis
	• Force vs Strain
	  o Comparison of failure strength to specification

	○ Comparison of stretch at force specification • ANOVA: quantitative comparison of different materials to each other
References	[10, 49-51]

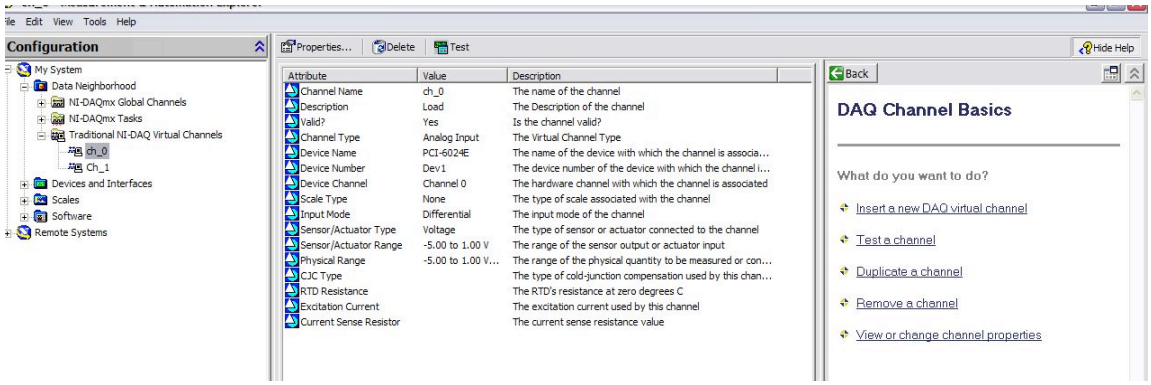
Crosshead Speed for Characterization Testing
Average heart rate: 69.4 bpm
Stretch % of heart muscle: between 5 and 15%

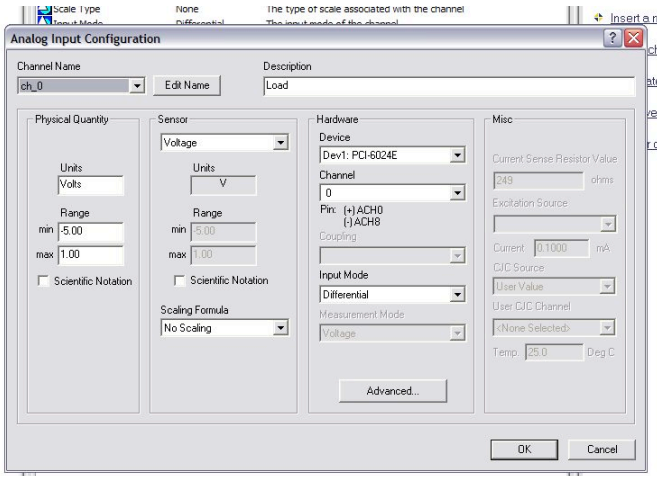
$$\frac{\partial \varepsilon}{\partial t} = \frac{0.1}{60s \diagup 69.4bpm} = 0.12/s$$

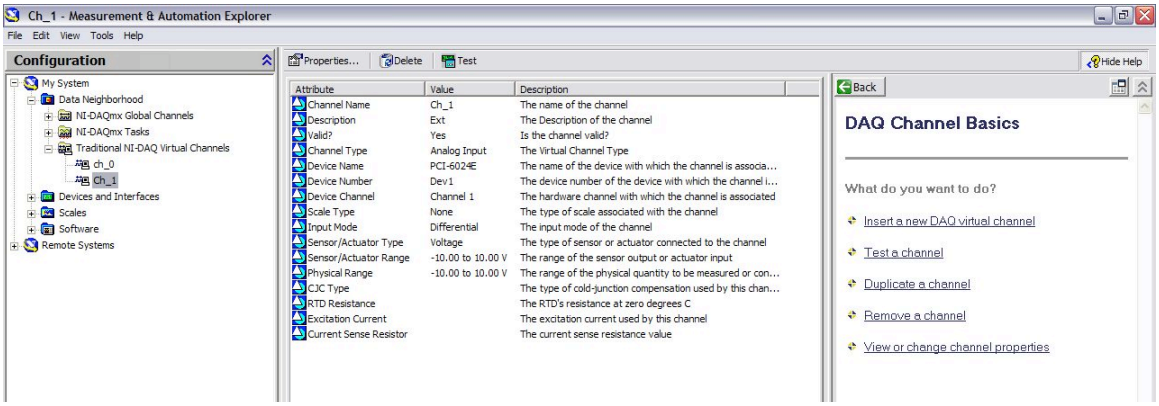
$$Crossheadspeed = 0.12/s * 20mm = 2.4mm/s$$

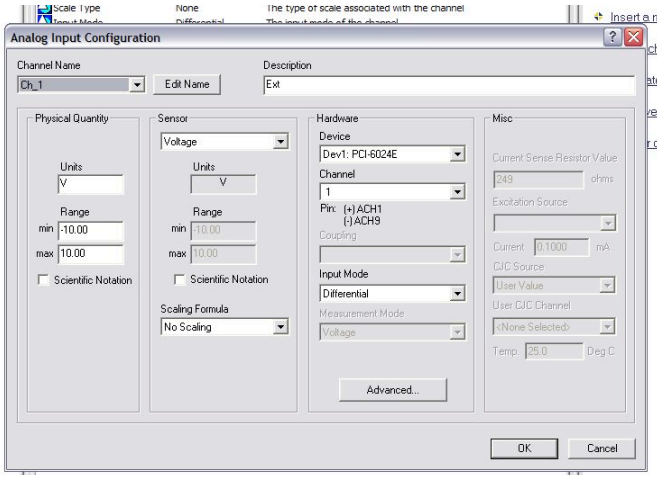
Setup and Test Basics for Instron (In-Spec 2200)

1. Equipment setup
    a. Plug DAQ board into computer (National Instruments BNC 2120)
        i. Secure the cable labeled EXT into ACH1
        ii. Secure the cable labeled LOAD into ACH0
        iii. Ensure settings on DAQ board are set as follows
            1. ACH0 – GS
            2. ACH1 – FS
    b. Plug the power cord from the Instron into an outlet
    c. Turn machine on using power switch at the top
2. Open Measurement and Automation
    a. Data Neighborhood – Traditional NI-DAQ Virtual Channels: Ch 0 and Ch 1
    b. Check that each channel (ch0 is load and ch1 is extension) is set up as seen in Figures 1 through 4


File Edit View Tools Help
Configuration
My System
Data Neighborhood
NI-DAQmx Global Channels
NI-DAQmx Tasks
Traditional NI-DAQ Virtual Channels
ch_0
Ch_1
Devices and Interfaces
Scales
Software
Remote Systems
Properties...
Delete
Test
Hide Help
Attribute
Value
Description
Channel Name
ch_0
The name of the channel
Description
Load
The Description of the channel
Valid?
Yes
Is the channel valid?
Channel Type
Analog Input
The Virtual Channel Type
Device Name
PCI-6024E
The name of the device with which the channel is associa...
Device Number
Dev1
The device number of the device with which the channel i...
Device Channel
Channel 0
The hardware channel with which the channel is associated
Scale Type
None
The type of scale associated with the channel
Input Mode
Differential
The input mode of the channel
Sensor/Actuator Type
Voltage
The type of sensor or actuator connected to the channel
Sensor/Actuator Range
-5.00 to 1.00 V
The range of the sensor output or actuator input
Physical Range
-5.00 to 1.00 V...
The range of the physical quantity to be measured or con...
CJC Type
The type of cold-junction compensation used by this chan...
RTD Resistance
The RTD's resistance at zero degrees C
Excitation Current
The excitation current used by this channel
Current Sense Resistor
The current sense resistance value
Back
DAQ Channel Basics
What do you want to do?
Insert a new DAQ virtual channel
Test a channel
Duplicate a channel
Remove a channel
View or change channel properties


Analog Input Configuration
Channel Name
ch_0
Edit Name
Description
Load
Physical Quantity
Units
Volts
Range
min -5.00
max 1.00
Scientific Notation
Sensor
Voltage
Units
V
Range
min -5.00
max 1.00
Scientific Notation
Scaling Formula
No Scaling
Hardware
Device
Dev1: PCI-6024E
Channel
0
Pin: (+) ACH0
(-) ACH8
Coupling
Input Mode
Differential
Measurement Mode
Voltage
Advanced...
Misc
Current Sense Resistor Value
249
ohms
Excitation Source
Current 0.1000 mA
CJC Source
User Value
User CJC Channel
<None Selected>
Temp 25.0 Deg C
OK
Cancel


Ch_1 - Measurement & Automation Explorer
File Edit View Tools Help
Configuration
My System
Data Neighborhood
NI-DAQmx Global Channels
NI-DAQmx Tasks
Traditional NI-DAQ Virtual Channels
ch_0
Ch_1
Devices and Interfaces
Scales
Software
Remote Systems
Properties...
Delete
Test
Hide Help
Attribute
Value
Description
Channel Name
Ch_1
The name of the channel
Description
Ext
The Description of the channel
Valid?
Yes
Is the channel valid?
Channel Type
Analog Input
The Virtual Channel Type
Device Name
PCI-6024E
The name of the device with which the channel is associa...
Device Number
Dev1
The device number of the device with which the channel i...
Device Channel
Channel 1
The hardware channel with which the channel is associated
Scale Type
None
The type of scale associated with the channel
Input Mode
Differential
The input mode of the channel
Sensor/Actuator Type
Voltage
The type of sensor or actuator connected to the channel
Sensor/Actuator Range
-10.00 to 10.00 V
The range of the sensor output or actuator input
Physical Range
-10.00 to 10.00 V
The range of the physical quantity to be measured or con...
CJC Type
The type of cold-junction compensation used by this chan...
RTD Resistance
The RTD's resistance at zero degrees C
Excitation Current
The excitation current used by this channel
Current Sense Resistor
The current sense resistance value
Back
DAQ Channel Basics
What do you want to do?
Insert a new DAQ virtual channel
Test a channel
Duplicate a channel
Remove a channel
View or change channel properties



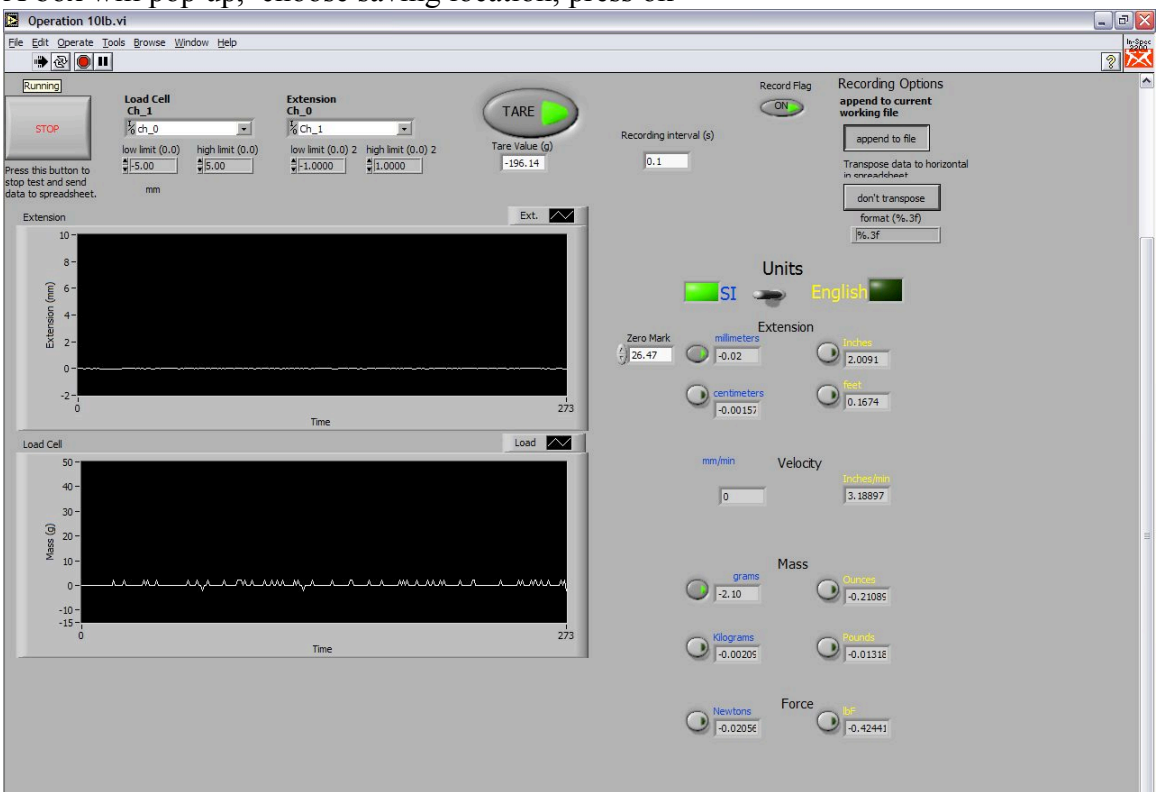
c. Right click over channel number and select test, a second window will open
    i. Load: apply force to load cell, observe green line, it should move upwards the more force that is applied
    ii. Extension: press start button on Instron, green line should move up if upper grip is moving up and down if upper grip is moving down

3. Open 10lb.llb (must have LabVIEW version 7.1 or program will not work)
    a. Double click on red In-Spec 220 icon, LabView user interface will open in a new window
    b. Using dropdown menus at top of screen, set load cell to ch_0 and extension to Ch_1
    c. Go to Window-Show Block Diagram, check that the slope and intercept on the load cell part of the diagram are correctly set (Page 39 in lab notebook)
    d. Press the arrow button on top left hand side of toolbar (run)
    e. Tare (Part 1)
        i. Put 0 in the Tare value box, press green arrow
        ii. record exact reading from grams box under Mass
    f. Set up sample in grips according to desired procedure
    g. Tare (Part 2)
        i. Take exact reading from grams box under Mass and subtract it from the reading recorded in step e part ii
        ii. Add 205.5 g to this value and put it in the Tare value box
        iii. Press enter
        iv. White line will not relocate to zero on the Load Cell graph until the green button on the Instron is pressed to start the test. After taring the value in the grams box should be around -4400ish grams.
    h. Zero Extension:
        i. Put 0 in the Zero Mark box and press enter
        ii. Take the number in the millimeters box to the right and put it in the zero mark box
        iii. A white line will relocate to the zero mark on the extension/time graph

i. Press Red stop sign button on the toolbar at the top of the screen (NOT the big button with the word STOP on it)
j. Right click in the area of the extension chart, select data operations, then clear chart, white lines will disappear
k. Repeat for load cell chart. This clears all the base setup data out so that it is not used in the data analysis
l. Press the arrow button on the top left hand side of the toolbar again (run)
m. Press green start button on front of Instron
n. Allow test to run according to desired procedure

Press square STOP button at end of test (press green button on face of Instron to stop the machine as well)

A box will pop up, choose saving location, press ok



Appendix J: Drawings of Dog Bone and Suturability Base

Dimensions all in inches

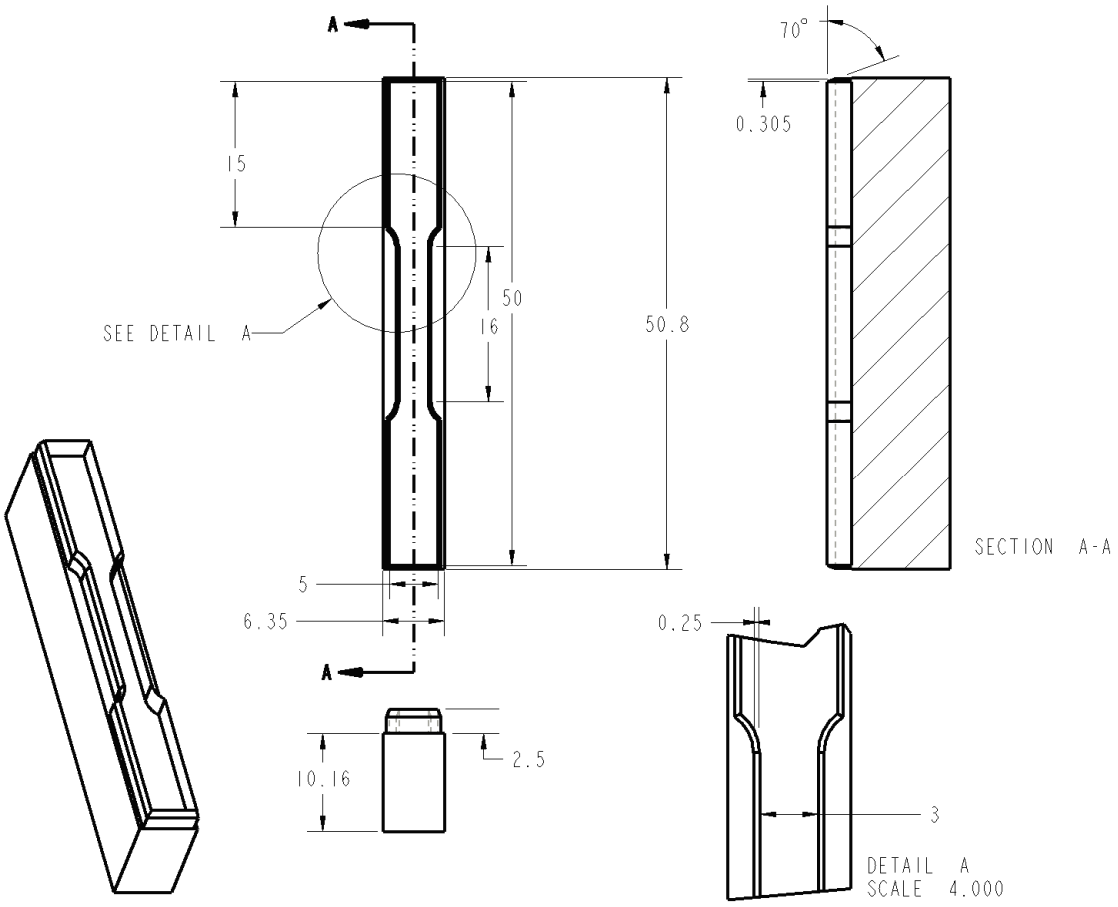


Figure 43: Dog Bone Punch

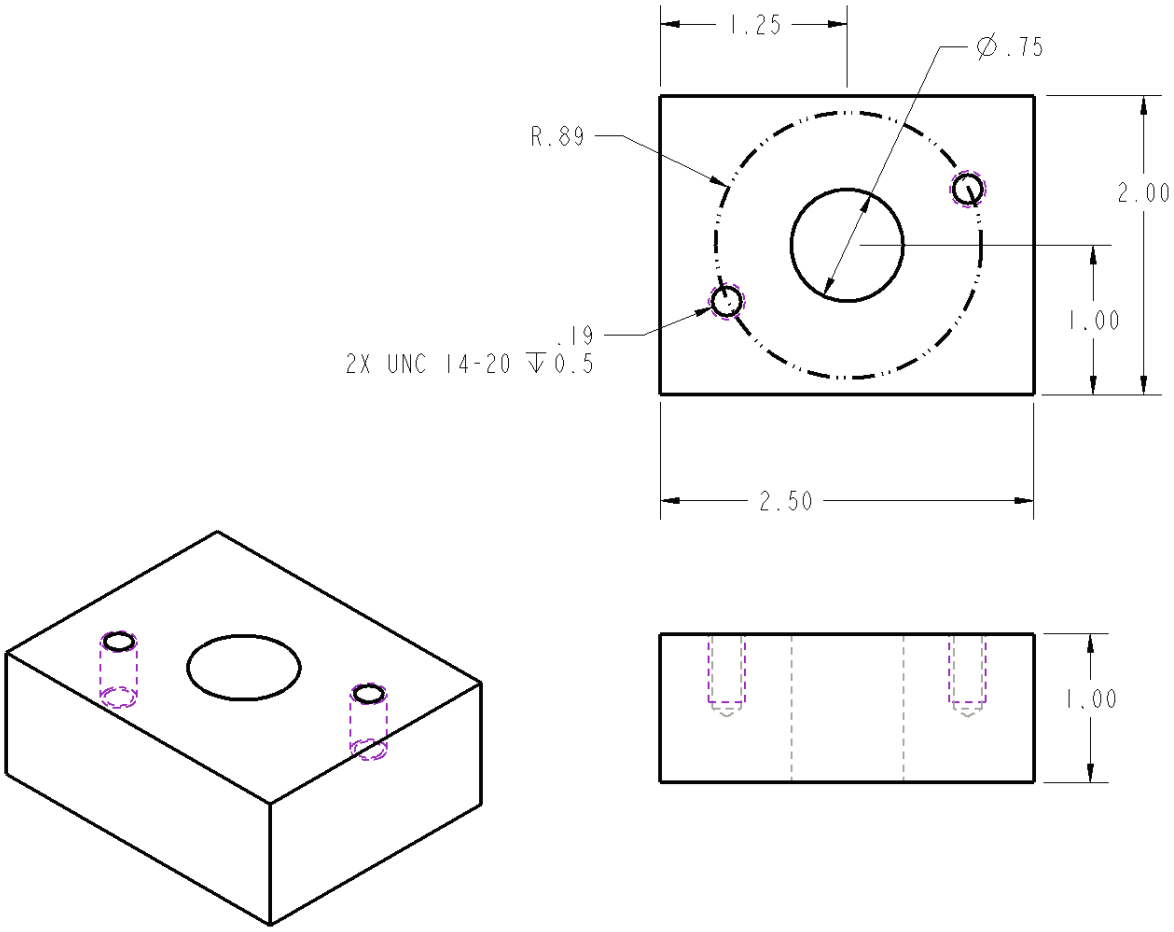


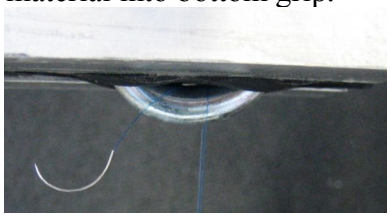
Figure 44: Suturability Base

Appendix K: ImageJ Strain Measurement

- Measure distance between markers
  - Open ImageJ – then open image
  - Convert the image to 8 bit format
    - In the Image menu select Type, then 8 bit
  - Set scale on image
    - Draw a line on a physical scale (e.g., 1 inch ruler) using the straight line sections tool from the button toolbar on and measure it (Ctrl+M)
    - In the Analyze menu, choose Set Scale – the number of pixels is given, type in the true length (e.g., 1.00 inch)
    - Re-measure (Ctrl+M) – this should now read the true length
    - Re-draw and re-measure the calibration line 5 to 10 times
    - Save: in the Results box, choose Save As from the file menu, this will save the measurements as a text file
    - Copy measurements to a spread sheet to determine the calibration error.
  - Measure marker positions
    - Make the image binary:
      - Threshold image by pressing Ctrl+Shift+T
      - In the Threshold box move both sliders around until the dots are red
      - Press Apply then Ok
    - In the Analyze menu choose Set measurements
      - Check the boxes next to centroid, area, invert y coordinates, and limit to threshold, press Ok
    - Use the wand tool on the button menu to select one marker, then measure it (Ctrl+M), repeat for other markers
    - Save: In the Results box, choose Save As from the file menu, this will save the measurements as a text file
  - Calculate distance
    - Copy x and y centroid positions to a spreadsheet and calculate distance. $d^2 = (x_2-x_1)^2 + (y_2-y_1)^2$ and strain.

Appendix L: Suture Retention Testing Protocols

	Suture Retention-Preliminary Testing
Objectives and Specifications	Sutures should not pull out of scaffold under a force of 2.74 N/suture (279.4 g/suture)
Materials and Equipment	• Instron In-Spec 2200 • 6-0 Prolene sutures • Camera with tripod • Sample size n = 2
Procedure	• Setup Instron according to 'Setup and Test Basics for Instron' • Set crosshead speed to about 100 mm/min (approx 2 mm/s) according to 'Setting Crosshead Speed' • Samples should be cut into 2cm by 1cm rectangles and hydrated in PBS for 30 minutes before testing • Put suture through material to form a loop 2 mm in from the edge of the material. • Secure material first into bottom grip then grip both ends of the looped suture in the top grip • Apply tare load by adjust position of upper grip so the material and suture are taught between the grips • Zero the displacement and tare according to 'Setup and Test Basics for Instron' • Re-measure and record gage length • Start test, allow it to run until suture pulls free of the material
Expected Results and Methods of Analysis	**Results** • Force vs Displacement curve **Analysis** • Pass: maximum force is greater than 2.74 N (279.4 g) • Fail: maximum force is equal to or less than 2.74 N (279.4 g)
References	[113]

	Suture Retention-Characterization Testing
Objectives and Specifications	Sutures should not pull out of scaffold under a force of 2.74 N/suture (279.4 g/suture)
Materials and Equipment	• Instron In-Spec 2200 • 6-0 Prolene sutures • Camera with tripod • Sample size n = 2
Procedure	• Setup Instron according to 'Setup and Test Basics for Instron' • Set crosshead speed to about 100 mm/min (approx 2 mm/s) according to 'Setting Crosshead Speed' • Samples should be cut into 2cm by 1cm rectangles and hydrated in PBS for 30 minutes before testing • Put suture through material to form a loop 2 mm in from the edge of the material. • Knot ends of suture around metal ring using a square knot with seven total throws • Grip the ring in the top grip as seen in the following figure. Then secure material into bottom grip.  • Apply tare load by adjust position of upper grip so the material and suture are taught between the grips • Zero the displacement and tare according to 'Setup and Test Basics for Instron' • Re-measure and record gage length • Start test, allow it to run until suture pulls free of the material
Expected Results and Methods of Analysis	**Results** • Force vs Displacement curve**:** force at failure **Analysis** • ANOVA: quantitative comparison of different materials to each other • Visual inspection of material/suture at specification and failure
References	[113]

Appendix M: Suturability Testing Protocol

	Suturability-Characterization Testing
Objectives and Specifications	Force required to penetrate scaffold with a needle should be less than 5N (816.5 g)
Materials and Equipment	• Instron In-Spec 2200 • Base material holder* • 6-0 Prolene suture needles • Sample size n = 3
Procedure	• Setup Instron according to 'Setup and Test Basics for Instron' • Set crosshead speed to about 100mm/min (about 2 mm/s) according to 'Setting Crosshead Speed' • Samples should be cut into 2cm by 2cm squares and hydrated in PBS 30 minutes before testing • Place material between washers of base holder so face of material is perpendicular to the top grip. • Anchor suturing needle into the top grip pointing down at the base. • Move top grip to position so that needle head is 1cm from material • Zero displacement and tare according to 'Setup and Test Basics for Instron' • Start the test, allow it to run until needle has punctured through the opposite side of the material
Expected Results and Methods of Analysis	**Results** • Force vs. Displacement curve: maximum force **Analysis** • ANOVA: quantitative comparison of different materials to each other
References	[58]

Appendix N: Manufacturing of Fibrin Gel

Protocol	**Preparation of Fibrin Gel, revised 12/14/2006 00:18**
Objectives and Specifications	Resuspend fibrinogen in H_2O for stock solution. Prepare other components to make fibrin adhesive.
Materials and Equipment	**Materials** • Bovine fibrinogen • Bovine thrombin • Sterile phosphate buffered saline (PBS) • Sterile calcium chloride, anhydrous • Deionized (DI) water **Equipment** • 0.22 μm filters • Sterile 1 ml tubes • -20ºC freezer • Micromedics FibriJect applicator
Procedure	**Resuspend fibrinogen in H_2O [63]** 1. Add bovine fibrinogen to DI H_2O at a concentration of 30 mg/ml (refer to fibrinogen content on bottle to calculate quantities). 2. Warm solution to 37ºC and mix every 10 minutes until all fibrinogen has dissolved (could take approx. 3-10 hours). 3. Filter through 0.22 μm cell culture filter and aliquot into sterile 1 ml tubes. 4. Store at -20ºC or colder. **Resuspend thrombin in H_2O** 1. Add bovine thrombin to a clean tube. 2. Add DI H_2O to bring thrombin to a concentration of 2 U/ml [64]. 3. Procedure? (should be easier than fibrinogen). 4. Filter through 0.22 μm cell culture filter and aliquot into sterile 1 ml tubes. 5. Store at -20ºC. **Application of adhesive** 1. Thaw concentrated fibrinogen and thrombin at RT or 37ºC. 2. Dilute fibrinogen to the desired concentration (refer to Table 29 for basis of concentrations and references). a. Dilute fibrinogen solutions to 6 mg/ml in PBS for seeded fibrin gels and add cell suspension (10^6 cells/ml, see 'Migration' protocol) 1:10 to fibrinogen solution. b. Dilute fibrinogen solutions to 6, 10, or 18 mg/ml for migration substrate.

Table 29: Fibrinogen concentrations

Fibrinogen sol. conc.	Final fibrin conc.	Reference
0.6 mg/ml	0.3 mg/ml	Migration was the same as at physiological 3 mg/ml (data wasn't shown)[71]
3-6 mg/ml	1.5-3 mg/ml	Migration was no affected by fibrinogen concentration in this range (abstract only) [114]
6 mg/ml	**3 mg/ml**	**Physiological fibrinogen concentration is 2.64±0.807 in healthy volunteers and 3.43±2.040 in patients with angina pectoris [67]. Fibroblasts migrated optimally at 3 mg/ml (abstract only) [68]**
7-11 mg/ml	3.5-5.5 mg/ml	Induced site directed angiogenesis through delivery of α-endothelial cell growth factor [115]
10 mg/ml	**5 mg/ml**	**Mesenchymal stem cell proliferation within a 3D clot was maximum (lowest concentration tested) [52].**
18 mg/ml	**9 mg/ml**	**Optimized for proliferation of seeded mesenchymal stem cells [69].**

3. Add $CaCl_2$ (111 g/mol) to thrombin solution to give 20 mM concentration (basis: 5-20 mM [64], or 35 mM [116]).
4. Draw the desired volumes of the fibrinogen and thrombin solution into the two separate syringes of the Micromedics FibriJect fibrin adhesive applicator.
5. Attach the blending connector applicator tip.
6. Dispense equal parts concentrated fibrinogen and thrombin solution [64].
7. Allow to polymerize in situ.

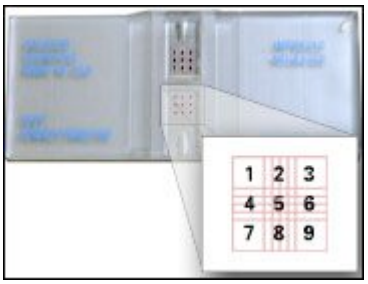
Appendix O: Migration Testing Protocols

Protocol Name	Thawing hMSCs
Objectives and Specifications	Thaw human mesenchymal stem cells (hMSCs) so that they can be used for experiments
Materials and Equipment	1. DMEM (Dulbecco's Modified Eagle's Medium), 10% FBS, 1% P/S with all supplements (serum = FBS, L-glutamine, antibiotics = P/S), 37C. Stored at -20C. 2. Pipets: 25mL, 10mL, 5mL Serological Pipets. 3. Miscellaneous items: Sterile culture flasks, 15mL conical tubes, 70% Isopropyl Alcohol (IPA), Pipet Aid, Lab marker for labeling.
Procedure	1. Spray inside surface of hood with 70% IPA. Spray all exterior surfaces of containers to be brought into hood with 70% IPA. Set up all necessary items in hood. 2. Remove stored cryovial(s) containing cells from Liquid Nitrogen cryotank/Dry ice. *Optional:* Wipe cryovial(s) with 70% IPA and in sterile field, brefly twist cap a quarter turn to relieve pressure, then retighten. 3. Thaw cells rapidly by immediately immersing vial(s) into 37° C water bath. Do not submerge them completely and watch them closely. Gently agitate for approx. 2 min (no longer than 3 min). *Note*: Most cell death occurs between –50° C and 0° C when thawing. 4. When fully thawed (all ice crystals melted), remove vial(s) immediately, wipe dry then spray outside of vial(s) thoroughly with 70% IPA before bringing cells into the hood. Transfer thawed cell suspension into 15mL tube containing 5mL pre-warmed media to dilute. 5. Centrifuge cells at 1000rpm for 5 minutes to remove any residual DMSO. While cells are being spun down, set up new T75 flasks and add 13mL of DMEM to each. Allow temperature to equilibrate to 37° C. 6. Decant supernatant; Resuspend cell pellet in minimum volume of fresh pre-warmed media. Perform CELL COUNTING. Seed cells by transferring the appropriate amount of cell suspension into new culture flask(s) with fresh medium. *Note*: Amount of suspension transferred will depend on the density at which cells were frozen and desired cell density for new seed. 7. Place cells in incubator, and replace with equal volume of fresh medium after 24 hrs to remove any (floating) dead cells. Observe cells daily for growth (confluency reached by ~1 week) and freedom from contamination. Media to be changed every 3-4 days.

	8. Clean up hood and spray down surface with 70% IPA. Close it and turn on UV light.
References	Protocol obtained from Gaudette laboratory

Protocol Name	Subculturing hMSCs
Objectives and Specifications	Culture hMSCs so that they can be used for experiments
Materials and Equipment	1. DMEM (Dulbecco's Modified Eagle's Medium), 10% FBS, 1% P/S with all supplements (serum = FBS, L-glutamine, antibiotics = P/S), 37C. Stored at 4C. 2. 0.25% Trypsin, 37C. Stored at -20C. (Not to be left in water bath for extended period of time.) 3. Pipets: 25mL, 10mL, 5mL Serological Pipets, 5mL aspirating Pasteur Pipets. 4. Miscellaneous items: Sterile culture flasks for seeding, 70% Isopropyl Alcohol (IPA), Pipet Aid, Lab marker for labeling.
Procedure	1. Spray inside surface of hood with 70% IPA. Spray all exterior surfaces of containers to be brought into hood with 70% IPA. Set up all necessary items in hood. 2. Remove all media from T75 flasks with hMSCs (P# donor #) previously cultured at *high density*. *Remember: Handle flasks vertically as to not allow media to enter neck of dish, specially designed for CO2 exchange.* 3. Add 5mL Trypsin gently to bottom edge/corner of flask (as to not shock/dislodge cells). Rock flask gently to ensure full coating of bottom surface. Check cells under microscope to make sure they are detaching from flask and have "rounded-up" morphology and are "flying around". (Trypsin is a protease that acts to degrade protein.) 4. When all cells appear round, add 5 mL fresh media (DMEM, 10%FBS), and thoroughly wash flask to gather up all cells from the bottom of the flask by gently triturating up and down while tilting the flask. *Note: No need to aspirate trypsin since DMEM will inactivate its proteolytic action.* 5. Centrifuge cells at 1000rpm for 5 minutes. 6. Decant supernatant and resuspend cell pellet in minimum volume of fresh pre-warmed media. Determine number cells according to CELL COUNTING protocol. 7. Add appropriate volume (depending on % confluency) of cell suspension to fresh flasks. Gently rock/swirl flask to spread out cells. 8. Place cells in incubator and observe daily for growth (toward confluency) and freedom from contamination. Media to be changed every 3-4 days. 9. Clean up hood and spray down surface with 70% IPA. Close it and turn on UV light.

References	Protocol obtained from Gaudette laboratory

Protocol Name	Cell Counting
Objectives and Specifications	Determine concentration of cells in suspension
Materials and Equipment	1. Hemacytometer 2. Trypan Blue 3. PBS 4. Cell counter; 10µL pipettes
Procedure	1. Prepare a 1:10 dilution (thus dilution factor = 10) of cell suspension to be counted as follows: Place 50 µL Trypan Blue + 40µL non-sterile PBS + 10µL cell suspension in a small Eppendorf tube. Triturate gently as to increase accuracy of count. *Note: Trypan Blue is toxic and a potential carcinogen so extra care should be taken with its use.* 2. Prepare the hemacytometer by placing a clean coverslip onto its center grid section (mirror-like polished surface with wells). Both should be cleaned with ethanol prior to use. 3. Carefully load a small amount (~10µL) of cell suspension into the wells underneath and on each end of the coverslip. *Note: A hemacytometer is a specialized glass slide with a 3x3 grid pattern etched upon it whose volume is known. When covered by a coverslip, cells spread out due to capillary action.* 4. Using a microscope, cells are counted within each of squares of the hemacytometer grid to obtain a measure of cell concentration as follows:  Count all viable cells in each of the 4 corner fields adjacent to the center square (i.e. squares that lie along a diagonal, here 1, 3, 7 & 9) for each side of hemacytometer for a total of 8 fields. Adopt a rule for counting cells that fall on grid lines to eliminate duplicate counts (i.e. count cells on left or top lines of a square, but not those on bottom or right lines). *Note: Dead cells appear blue as stained by Trypan Blue and should be excluded from the count, while viable cells appear bright and do not take up the dye unless exposed to it for an extended period of time after which they may absorb it and*

	appear non-viable. 5. Use the following equations with numbers attained from count to calculate cell concentrations. Final count or actual cell density in cells/mL → Eqn: $C_1V_1 = C_2V_2$ average count per field **# viable cells / mL** = [# viable cells / total # fields] X dilution factor (10) X 10^4 **Total # viable cells** = # viable cells/mL X original vol from which sample removed = C_1 X V_1 **Final resuspension volume** *(# mL of cells to add)* = total # cells/target cell density $V_2 = C_1V_1$ / C_2 **% viability** = total # viable cells / total # cells X 100 *Note:* Must perform dead count for total # cells.
References	Protocol obtained from Gaudette laboratory

Protocol Name	MTS Assay
Objectives and Specifications	Determine concentration of cells in suspension
Materials and Equipment	1. CellTiter 96® AQueous One Solution 2. 96 well plates 3. Pipettes; pipette tips; lab marker;
Procedure	1. Thaw the CellTiter 96® AQueous One Solution Reagent. It should take approximately 90 minutes at room temperature on the bench top, or 10 minutes in a water bath at 37°C, to completely thaw the 20ml size. 2. Pipet 20µl of CellTiter 96® AQueous One Solution Reagent into each well of the 96-well assay plate containing the samples in 100µl of culture medium. If culture volume is larger than 100µl, add solution to obtain total ratio of 1:5. 3. Incubate the plate for 2 hours at 37°C in a humidified, 5% CO2 atmosphere. 4. Record the absorbance at 490nm using a 96-well plate reader.
References	Protocol modified from manufacturers instructions.

Protocol	Cellular Migration Plate Handling, revised 3/1/2007 11:17 AM
Objectives and Specifications	Measure the migration of cells into a fibrin gel.
Materials and Equipment	**Materials** • Unseeded and seeded fibrinogen solution and thrombin/$CaCl_2$ solution from fibrin gel preparation protocol • 70% ethanol solution • Sterile deionized H_2O (0.22 µm filtered) **Equipment** • Sterile 12-well tissue culture plates (16 mm well diameter) • Sterile scoring tool (autoclaved) • Sterile score-marking guide (8 mm diameter, autoclaved) • Sterile Micromedics FibriJect applicator (soaked in 70% ethanol, rinsed with sterile H_2O), 3 ml syringes, and needles (22 gauge, 1”). • Nikon Eclipse TS100 inverted microscope • RT Color Spot camera system and Spot imaging software

	(Diagnostic Instruments, Inc.)
Procedure	**Scoring the plate** Under sterile conditions: 1. Place the score-marking guide into the well. 2. Use the scoring tool to score the bottom of the well around the inside of the marking guide. 3. Ensure that the score mark goes all the way around the circumference. 4. Wash each well with 70% ethanol to ensure sterility and that all plastic particles are removed. 5. Wash thoroughly with sterile H_2O. **Application of fibrin gels** 6. Flush the blending applicator tip with sterile H_2O. 7. Fill 3 ml syringes with an equal amount of unseeded fibrinogen solution and thrombin solution. 8. Remove as much air as possible from syringes. 9. Screw the syringe containing fibrinogen onto the side of the blending applicator tip marked with an O. 10. Screw the syringe containing thrombin onto the othr side of the blending applicator. 11. Snap syringes into the FibriJect housing and attach the connecting plunger cap. 12. Screw a needle onto the tip of the blending applicator. 13. Uncap the needle and place the tip between the score mark and the outer edge of the plate. 14. Depressed the plungers simultaneously via the connecting plunger cap to dispense 800 µl of unseeded fibrin gel into the outer ring. 15. Flush the blending tip thoroughly with 70% ethanol and leave to soak. 16. Allow ~10 min for the unseeded gel to polymerize. 17. Repeat steps 1-7 for the seeded fibrinogen solution. 18. Uncap the needle and place the tip in the center of the inner ring at the bottom of the well. 19. Depressed the plungers simultaneously via the connecting plunger cap to dispense 300 µl of seeded fibrin gel into the inner ring. 20. Allow ~10 min for seeded gel to polymerize. 21. Add media before taking initial images of the gels. 22. Store plate in incubator at 37ºC. **Imaging of gels** 1. Place plate on microscope, ensure that 10X objective is in place and turn light on to full brightness. 2. Power camera on and start Spot imaging software.

	3. Focus microscope using the bottom of the score mark for reference. 4. Focus 184 μm (300 tic marks or 1.5 revolutions on the microscope dial) up into the gel. 5. Acquire images at 4 locations surrounding the score mark using the 'Cardiac Patch MQP Bright Field' image setup (auto brightness). 6. Save images in a different folder for each day of the experiment with appropriate filenames. 7. Repeat steps 3-6 for each well.

Calibration of Focal Depth for Cellular Migration Plate Handling

The thickness of a standard glass microscope slide was measured using digital calipers. The number of tic marks was that this corresponded to was then measured by focusing first on a fingerprint on the bottom of the slide, then focusing up to a fingerprint on the top surface of the slide. The calculated conversion factor is given below:

$$645 \text{ tic marks} = 1.05 \text{ mm} = 1050 \ \mu\text{m}$$

$$\text{Conversion factor} = \frac{645 \text{ tic marks}}{1050 \ \mu\text{m}} = 0.614 \text{ tic marks}/\mu\text{m}$$

Appendix P: Calculations for Migration Assay

How thick should the gels be?

Greiling et al: 0.6ml of final solution poured into 24-well plates, d=16mm (Fisher Scientific Costar Cell Culture Plates)

$$V = At = \pi\left(\frac{d}{2}\right)^2 t \therefore t = \frac{V}{\pi\left(\frac{d}{2}\right)^2}$$

$$t = \frac{V}{\pi\left(\frac{d}{2}\right)^2} = \frac{0.6ml}{\pi\left(\frac{16mm}{2}\right)^2} = \frac{0.6cm^3}{\pi\left(\frac{1.6cm}{2}\right)^2} = 0.298cm = 2.98mm \sim \mathbf{3mm}$$

How big should the seeded and unseeded gels be?

The cell seeded gels used by Greiling et al. were made by pouring collagen gels that covered the well and allowing them to contract before pouring the outer gel. Liu et al. observed the contraction of collagen gels seeded in 24-well plates (16mm diameter) with corneal fibroblasts at the following concentrations:

- Collagen: 1.8 mg/ml
- Cells: $2x10^5$ cells/ml

The average gel contraction was ~2.5mm, therefore the average final diameter was: 16mm – 2.5 mm = 13mm → 13/18*100 = 81.25% ~ 80% of original diameter

The average diameters of the cell seeded collagen gels used by Greiling et al. were probably about 80% of the well diameter (i.e. ~13mm). This was sufficient for observing migration after 24 hours. Since we are interested in observing cell spreading over a longer time period we should have a larger unseeded region for them to migrate into → 50/50 ratio between diameters is a reasonable start.

D_{seeded} = 50% well diameter → R_{seeded} = 25% well diameter
$D_{unseeded}$ = 50% well diameter → $R_{unseeded}$ = 25% well diameter

12-well plates: $A=3.8cm^2 = \pi r^2 \rightarrow r = \sqrt{\frac{A}{\pi}} = \sqrt{\frac{3.8cm}{\pi}} = 1.1cm \therefore d = 2.2cm$

Total Volume (d=22mm; r=11mm):
V=At where $A=\pi r^2=\pi(11mm)^2(1cm/10mm)^2=3.80cm^2$
$V=(3.80cm^2)(0.298cm)=1.13cm^3 \sim$ **1.10ml of total fibrin gel**

Volume of seeded fibrin gel (d=11m; r=5.5mm):
V=At where $A=\pi r^2=\pi(5.5mm)^2(1cm/10mm)^2 = 0.95cm^2$
$V=(0.95cm^2)(0.298cm) = 0.283cm^3 \sim$ **0.280ml of cell seeded fibrin gel**

Volume of unseeded fibrin gel=1.10ml-0.28ml = **0.820ml of unseeded fibrin gel**

What proportion of the final solution do other people use as cell suspension?

- Pins Labratory: **1/6 cell suspension**, 1/6 thrombin solution, 2/3 fibrinogen solution
- Jockenhoevel et al.: "The Fibrinogen solution was serial Filtered and sterilized. 10% 50 mMCaCl2, 20% Thrombin (20 units/ml) and 70% resuspended cells in Tris buffered saline were mixed gently. The Fibrinogen was added in a ratio 1:1 and mixed by gently shaking." ½ Thrombin/cell solution (~1/3 thrombin solution, ~2/3 cell suspension), ½ fibrinogen solution to give final ratios of 1/6 thrombin solution, **1/3 cell suspension**, ½ fibrinogen solution

What concentrations of cell suspensions are required for the following ratios of cell suspension in the fibrin gel for the migration tests? (Note: Greiling et al. used 60,000 cells/600µl gel = **100,000 cells/ml**).

- 12-well tissue culture plates: We want 28,000 cells/280µL gel (final concentration = 100,000 cells/mL) = 28,000 cells/140µL fibrinogen solution. Any of these concentrations would work well.
    - 1/2: 70µl of cell suspension (28,000 cells/0.070ml = 400,000 cells/ml)
    - 1/3: **47µl** of cell suspension (28,000 cells/0.047ml = 595,744 cells/ml ~ **600,000 cells/mL**)
    - 1/4: 35µl of cell suspension (28,000 cells/0.035ml = 800,000 cells/ml)

REFERENCES: [71, 117, 118]

Appendix Q: Transformation of Strength/Strain Preliminary Data

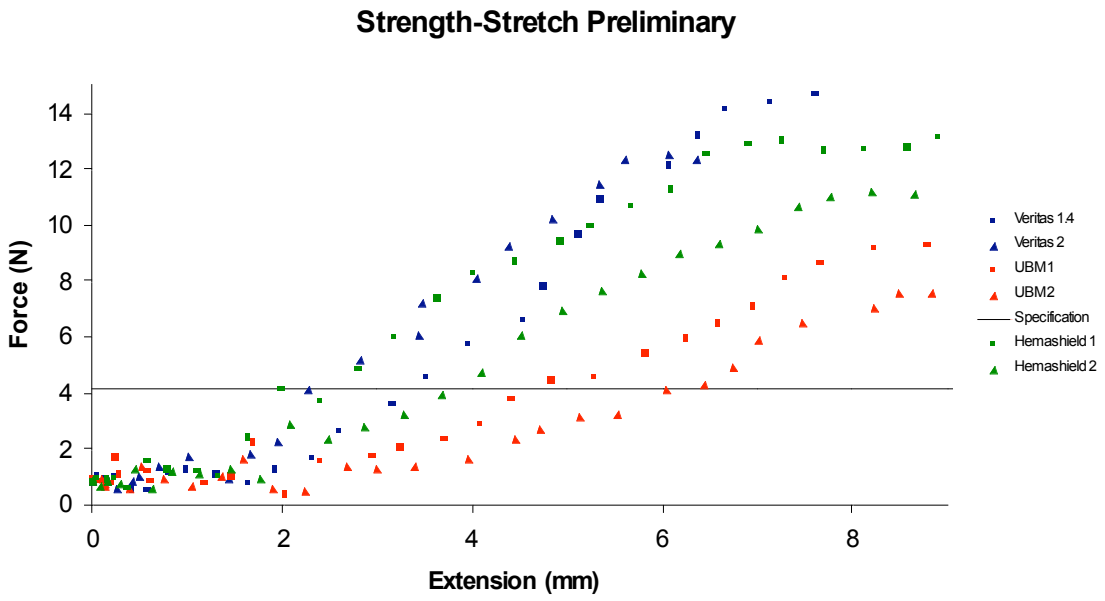


Figure 45: Raw Strength/Strain Preliminary Data

- Initial toe region, need to zero out this portion so that strain and modulus can be calculated
    - Find point in bounce of force where the values stop fluctuating and begin rising
    - Use this point as zero load
    - Use corresponding extension at this point to zero the extension

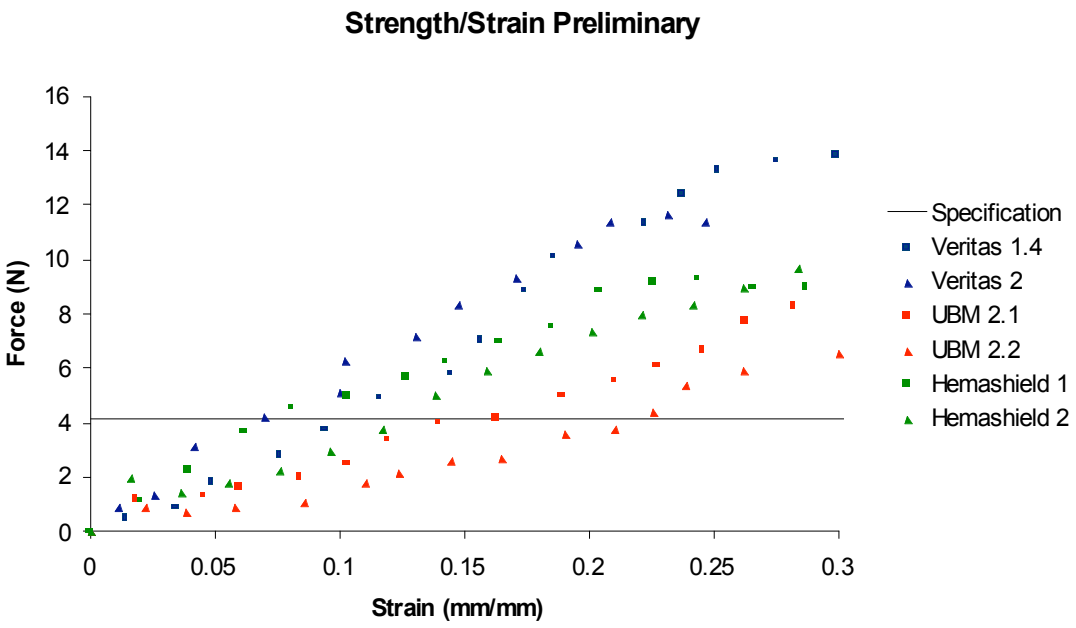


Figure 46: Zeroed Strength/Strain Preliminary Data

- Figure 46 shows corrected data, eliminates discrepancies in beginning region
- Use corrected data to validate strain rate, see Figure 47, strain rate validation

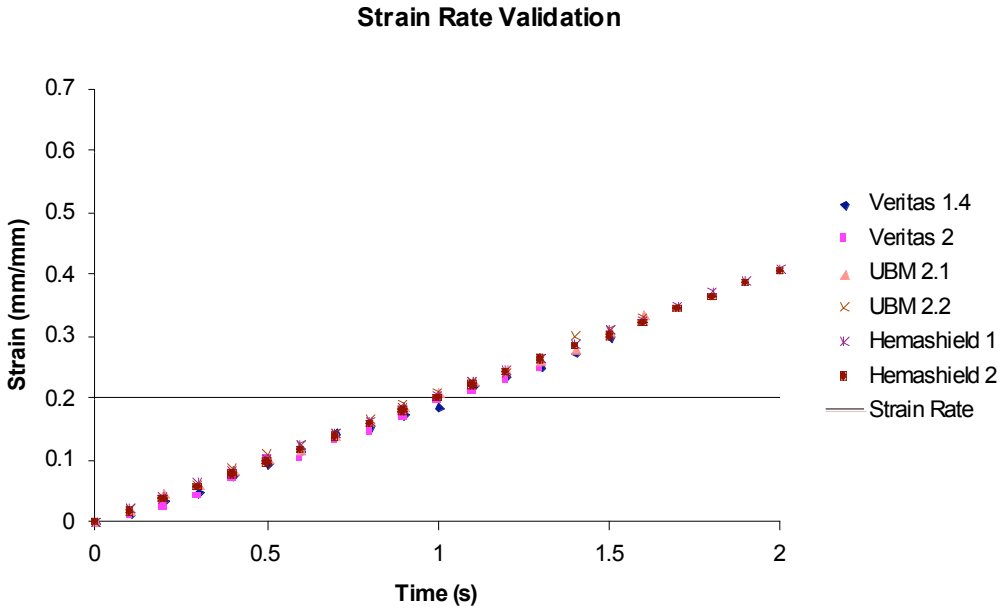


Figure 47: Strength/Strain Strain Rate Validation

- 4mm/s crosshead speed = 20mm gage length x strain rate
- Strain rate = 0.2/s
- Data intersects at 1s and 20% strain: VALIDATED

- With the procedure validated: can now examine the difference between crosshead strain and local strain measured through image analysis
  - Graph crosshead strain and calculated local strain against each other, find relationship

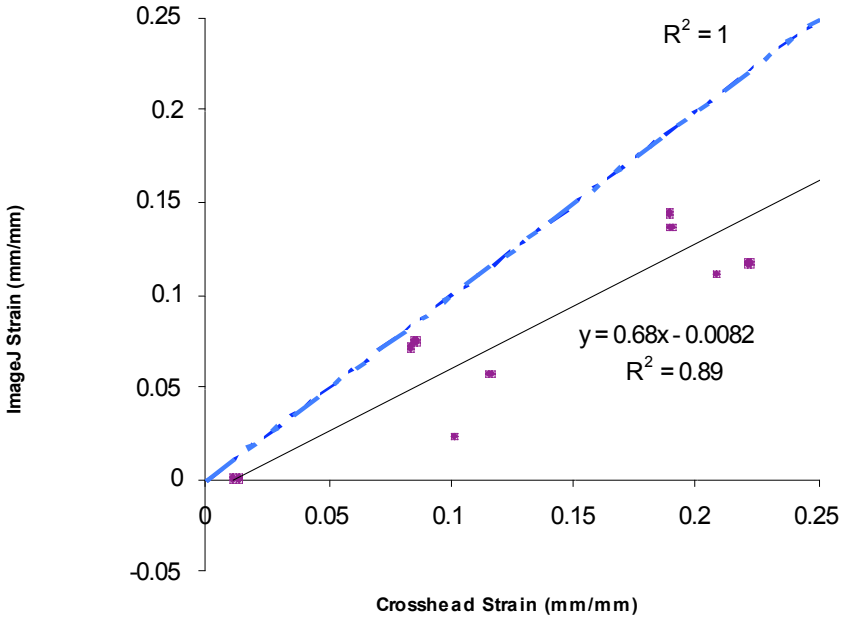


Figure 48: Crosshead Strain vs Local Strain

- R squared value fairly high (close to 1 indicates high linear correlation between data sets)
- Use relationship (slope/intercept equation) to predict ImageJ strain from the crosshead strain, plot the predicted strain vs the actual ImageJ strain and compare to perfect relationship (points are equal $R^2 = 1$)

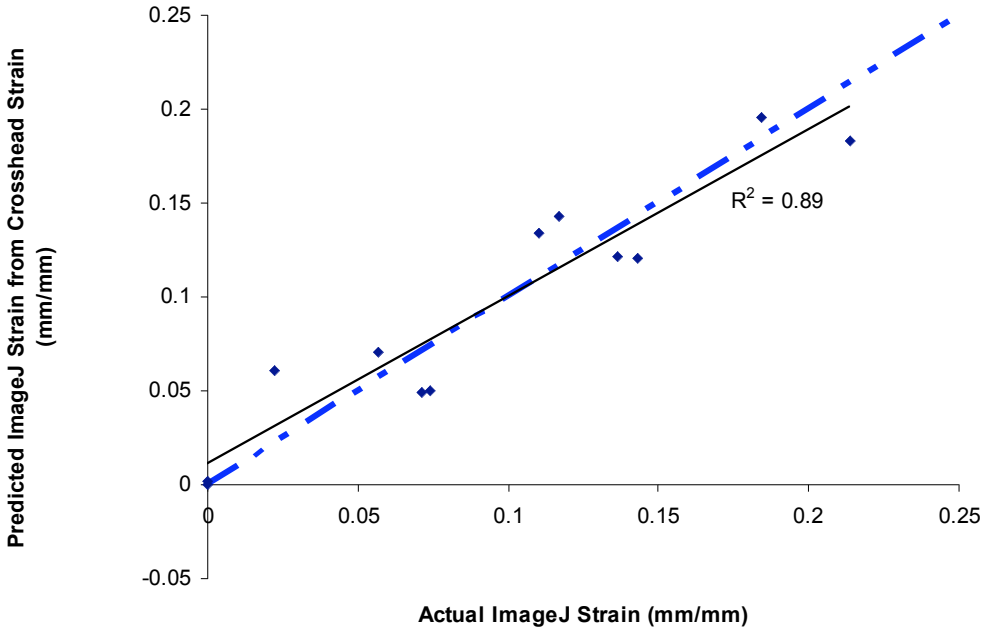


Figure 49: Predicted vs. Actual Image J Strain

- Use equation: Local Strain = 0.68 (Crosshead Strain) -0.0082 to convert crosshead strain to local strain

- With the data now graphed against corrected local strain, the strain at the specification can be calculated
  - Isolate three or four points around the specification line for each material
    - Draw a line of best fit through these points and display equation

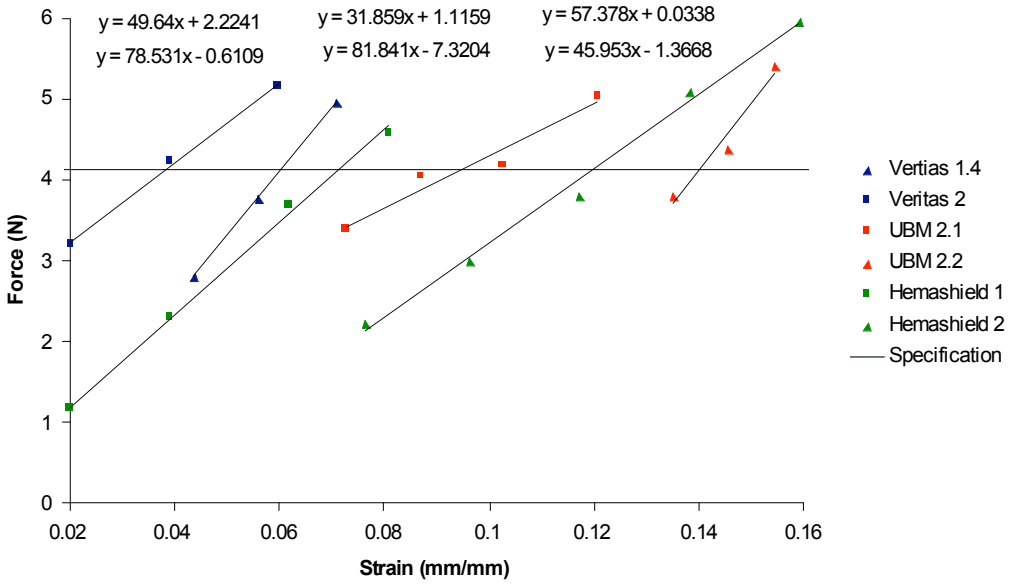


Figure 50: Calculation of Strain at Specification – Line of Best Fit and Equation

- Equations are grouped, from right to left, as Veritas, UBM, then Hemashield
- Take these equations and plug in 4.11N for y, solve for x
- X= strain at specification

Appendix R: Suture Retention Statistical Analysis

Oneway

[DataSet1] C:\Documents and Settings\megmurph\Desktop\Strength Strain.sav

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.280	2	2.140	1.804	.244
Within Groups	7.118	6	1.186		
Total	11.397	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VAR00002

Bonferroni

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
UBM	Veritas	1.34441799	.88930695	.544	-1.5791387	4.2679747
	Hemashield	-.21337077	.88930695	1.000	-3.1369275	2.7101860
Veritas	UBM	-1.3444180	.88930695	.544	-4.2679747	1.5791387
	Hemashield	-1.5577888	.88930695	.391	-4.4813455	1.3657680
Hemashield	UBM	.21337077	.88930695	1.000	-2.7101860	3.1369275
	Veritas	1.55778876	.88930695	.391	-1.3657680	4.4813455

Appendix S: Transformation of Strength/Strain Characterization Data

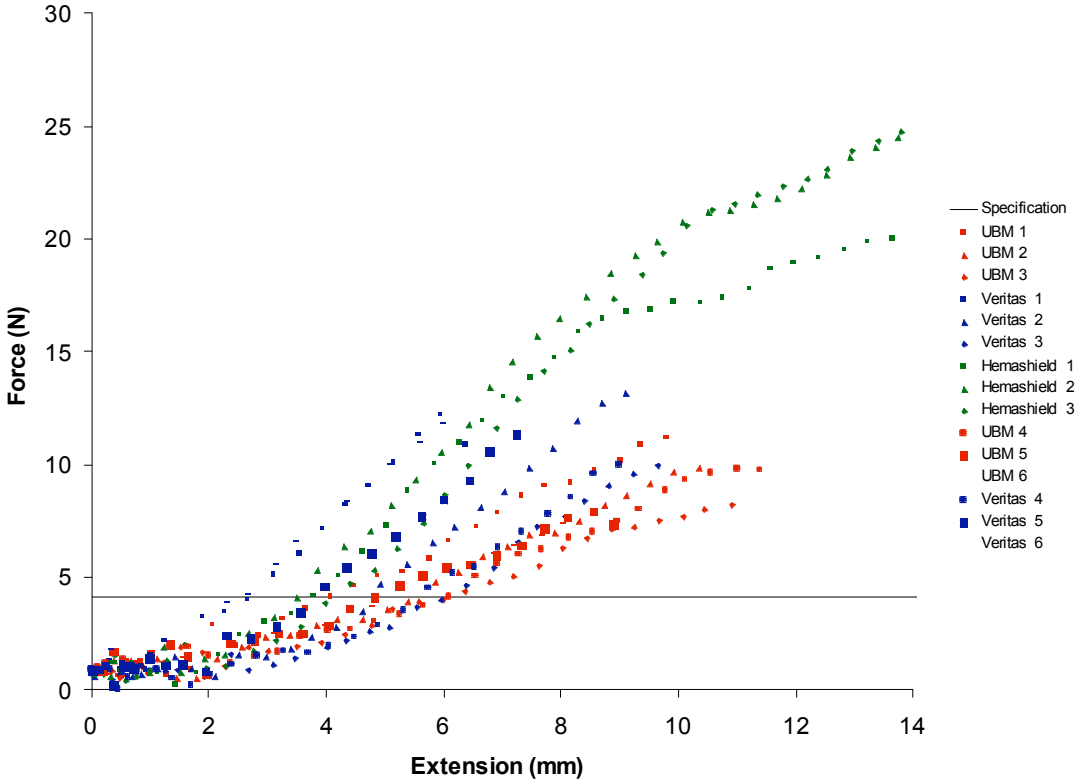


Figure 51: Raw Strength/Strain Preliminary Data

- Initial toe region, need to zero out this portion so that strain and modulus can be calculated
    - Find point in bounce of force where the values stop fluctuating and begin rising
    - Use this point as zero load
    - Use corresponding extension at this point to zero the extension

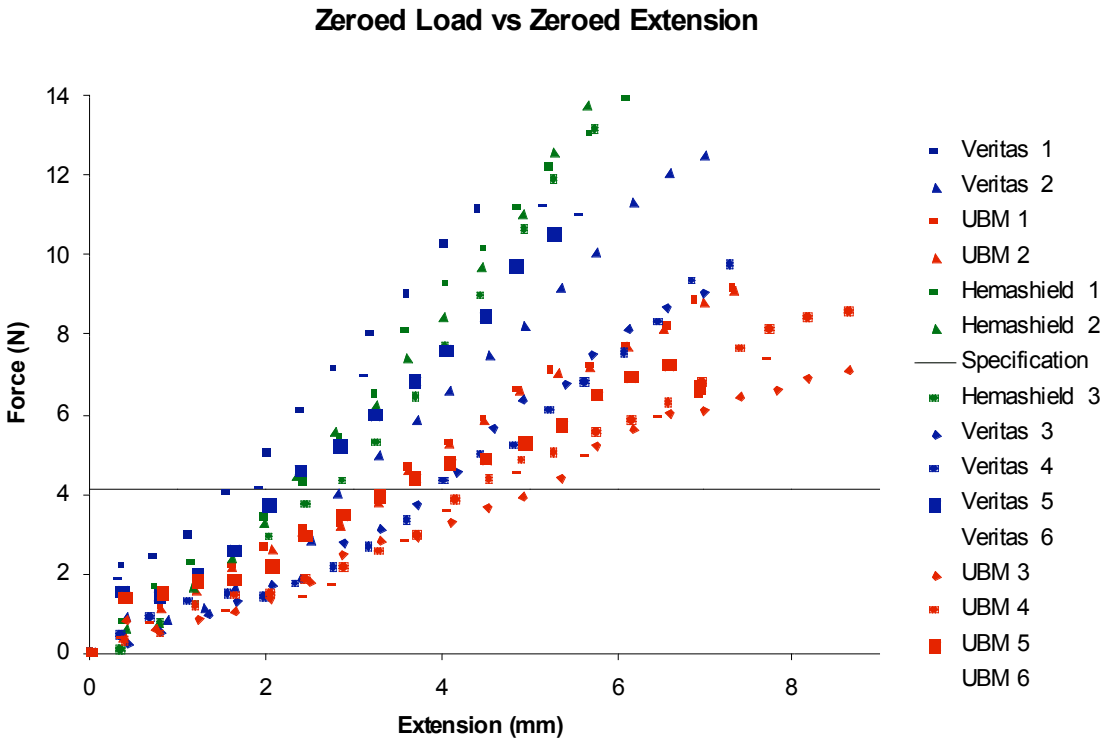


Figure 52: Zeroed Strength/Strain Preliminary Data

- Figure 52 shows corrected data, eliminates discrepancies in beginning region

- Use corrected data to validate strain rate, see Figure 53, strain rate validation

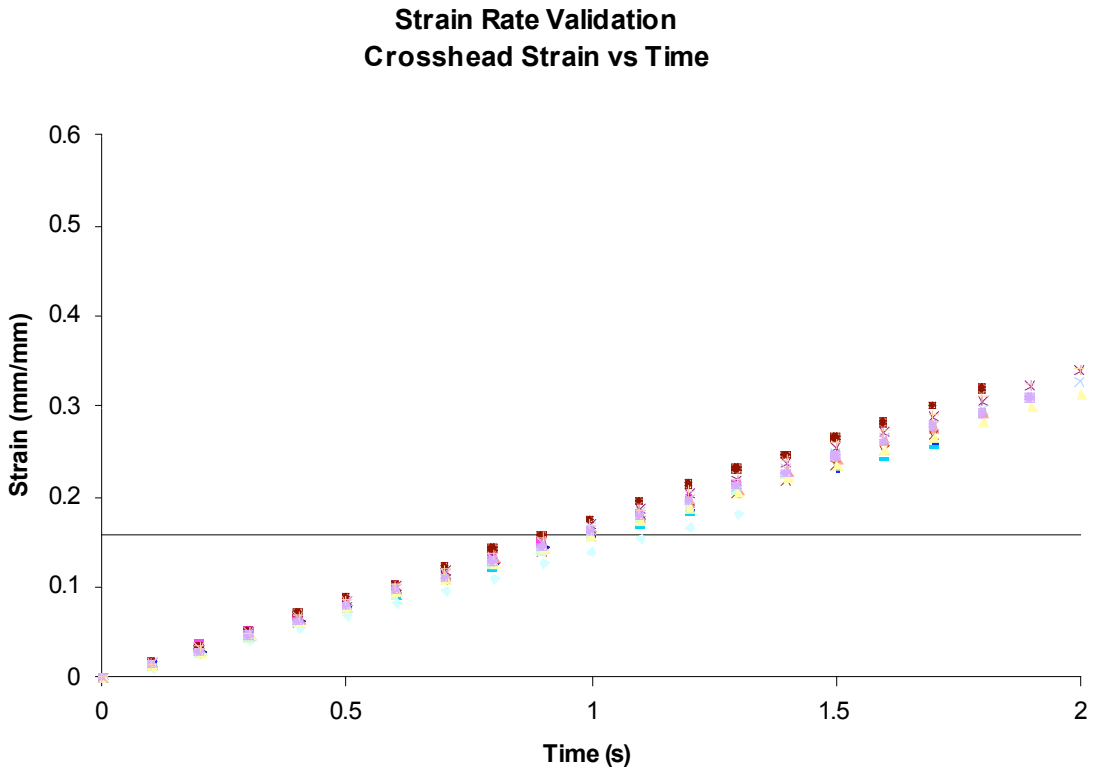


Figure 53: Strength/Strain Strain Rate Validation

  - 4mm/s crosshead speed = 25.4mm gage length (overall average) x strain rate
  - Strain rate = 0.16/s
  - Data intersects at 1s and 16% strain: VALIDATED

- With the procedure validated: can now examine the difference between crosshead strain and local strain measured through image analysis

- Graph crosshead strain and calculated local strain against each other, find relationship

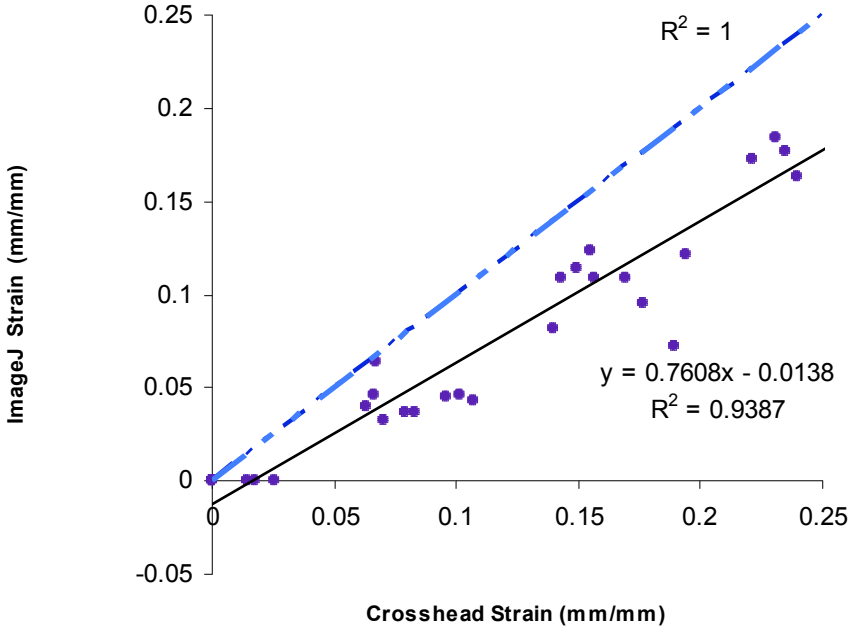


Figure 54: Crosshead Strain vs. Local Strain

- R squared value fairly high (close to 1 indicates high linear correlation between data sets)
- Use relationship (slope/intercept equation) to predict ImageJ strain from the crosshead strain, plot the predicted strain vs the actual ImageJ strain and compare to perfect relationship (points are equal $R^2 = 1$)

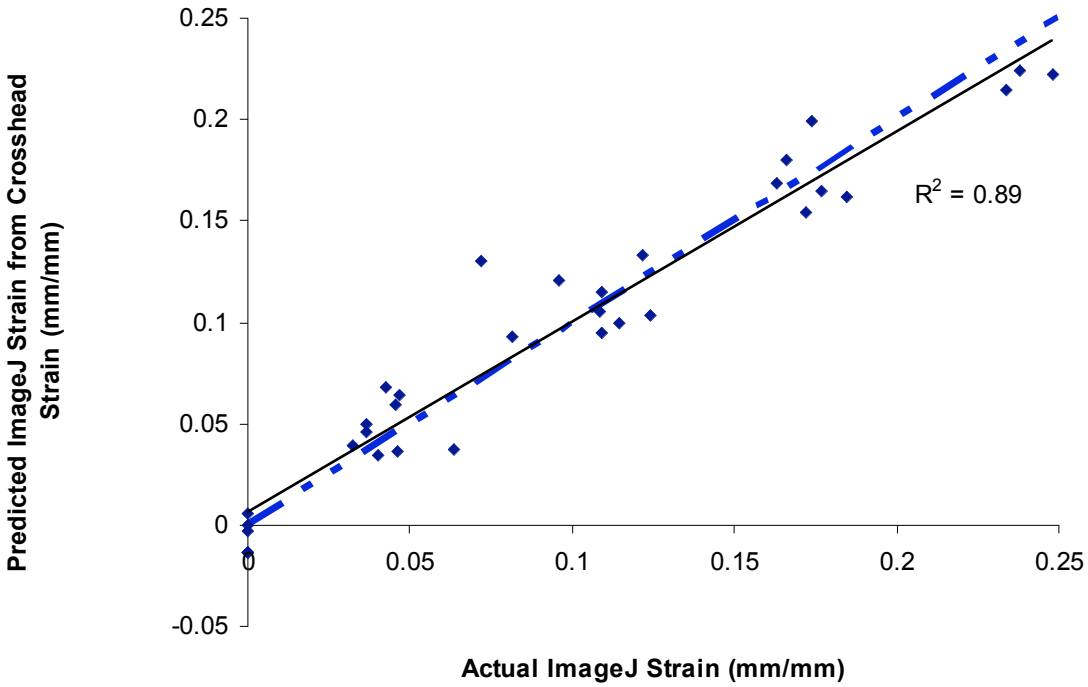


Figure 55: Predicted vs. Actual Image J Strain

  - Use equation: Local Strain = 0.76 (Crosshead Strain) -0.0138 to convert crosshead strain to local strain
- With the data now graphed against corrected local strain, the strain at the specification can be calculated
  - Isolate three or four points around the specification line for each material
    - Draw a line of best fit through these points and display equation

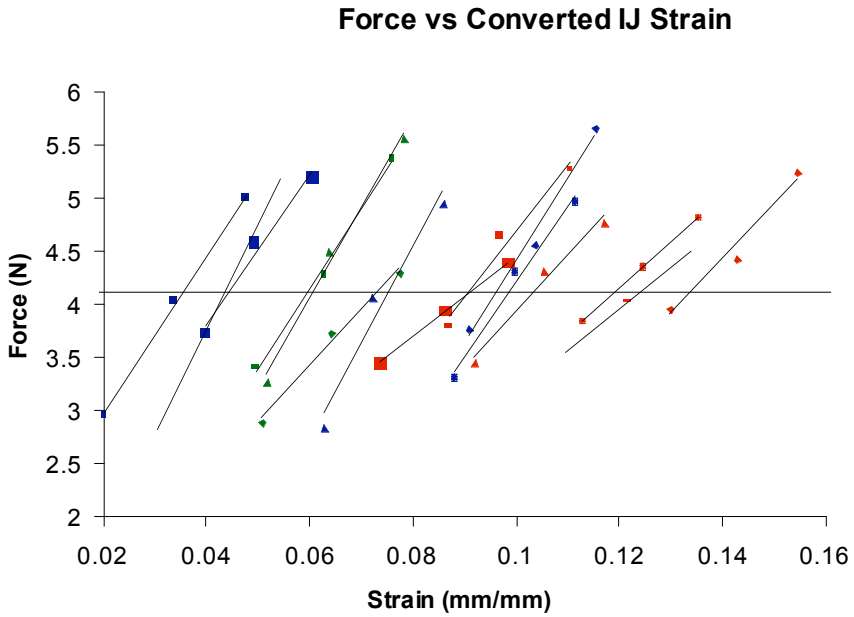


Figure 56: Calculation of Strain at Specification – Line of Best Fit

- Equations are shown in the following table:

Table 30: Calculation of Strain at Specification - Equations

Sample	Equation
Veritas 1	75.231x + 1.4568
Veritas 2	89.913x - 2.6398
Veritas 3	76.837x - 3.259
Veritas 4	70.537x - 2.8469
Veritas 5	69.64x + 1.0445
Veritas 6	97.159x - 0.1106
UBM 1	60.843x - 1.3975
UBM 2	53.093x - 1.3725
UBM 3	52.073x - 2.8554
UBM 4	43.507x - 1.0727
UBM 5	37.704x + 0.6841
UBM 6	39.47x - 0.7775
Hemashield 1	73.953x - 0.2834
Hemashield 2	86.003x - 1.0905
Hemashield 3	53.053x + 0.2536

- Take these equations and plug in 4.11N for y, solve for x
- X= strain at specification

Strain ANOVA (SPSS)

Oneway

[DataSet0]

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	2	.004	7.739	.007
Within Groups	.006	12	.000		
Total	.013	14			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VAR00002

Bonferroni

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
UBM	Veritas	.04483830*	.01259883	.012	.0098202	.0798564
	Hemashield	.04603768*	.01543035	.034	.0031494	.0889259
Veritas	UBM	-.04483830*	.01259883	.012	-.0798564	-.0098202
	Hemashield	.00119938	.01543035	1.000	-.0416889	.0440876
Hemashield	UBM	-.04603768*	.01543035	.034	-.0889259	-.0031494
	Veritas	-.00119938	.01543035	1.000	-.0440876	.0416889

*. The mean difference is significant at the .05 level.

Appendix T: Analysis of Suturability Data

Oneway

```
[DataSet1] C:\Documents and Settings\megmurph\Desktop\Strength Strain.sav
```

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.092	2	.046	1.708	.259
Within Groups	.161	6	.027		
Total	.253	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VAR00002

Bonferroni

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
UBM	Veritas	.23464212	.13377992	.390	-.2051534	.6744376
	Hemashield	.18487272	.13377992	.649	-.2549228	.6246682
Veritas	UBM	-.23464212	.13377992	.390	-.6744376	.2051534
	Hemashield	-.04976940	.13377992	1.000	-.4895649	.3900261
Hemashield	UBM	-.18487272	.13377992	.649	-.6246682	.2549228
	Veritas	.04976940	.13377992	1.000	-.3900261	.4895649

Appendix U: Statistical Analysis of MTS Viability

Table 31: Comparison between % increase at Day 1 Experiment 1

Dependent Variable	(I) Material2	(J) Material2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Day1	3mg/ml	5mg/ml	-33.66386	15.05910	.677	-88.6128	21.2851
		9mg/ml	23.31764	15.05910	1.000	-31.6313	78.2666
		Veritas	36.92166	15.05910	.457	-18.0273	91.8706
		UBM	-118.58052(*)	15.05910	.000	-173.5295	-63.6315
		Plastic	-87.68294(*)	15.05910	.001	-142.6319	-32.7340
	5mg/ml	3mg/ml	33.66386	15.05910	.677	-21.2851	88.6128
		9mg/ml	56.98150(*)	15.05910	.039	2.0325	111.9305
		Veritas	70.58552(*)	15.05910	.008	15.6365	125.5345
		UBM	-84.91667(*)	15.05910	.002	-139.8656	-29.9677
		Plastic	-54.01908	15.05910	.056	-108.9681	.9299
	9mg/ml	3mg/ml	-23.31764	15.05910	1.000	-78.2666	31.6313
		5mg/ml	-56.98150(*)	15.05910	.039	-111.9305	-2.0325
		Veritas	13.60402	15.05910	1.000	-41.3450	68.5530
		UBM	-141.89816(*)	15.05910	.000	-196.8471	-86.9492
		Plastic	-111.00058(*)	15.05910	.000	-165.9496	-56.0516
	Veritas	3mg/ml	-36.92166	15.05910	.457	-91.8706	18.0273
		5mg/ml	-70.58552(*)	15.05910	.008	-125.5345	-15.6365
		9mg/ml	-13.60402	15.05910	1.000	-68.5530	41.3450
		UBM	-155.50218(*)	15.05910	.000	-210.4512	-100.5532
		Plastic	-124.60460(*)	15.05910	.000	-179.5536	-69.6556
	UBM	3mg/ml	118.58052(*)	15.05910	.000	63.6315	173.5295
		5mg/ml	84.91667(*)	15.05910	.002	29.9677	139.8656
		9mg/ml	141.89816(*)	15.05910	.000	86.9492	196.8471
		Veritas	155.50218(*)	15.05910	.000	100.5532	210.4512
		Plastic	30.89759	15.05910	.940	-24.0514	85.8466
	Plastic	3mg/ml	87.68294(*)	15.05910	.001	32.7340	142.6319
		5mg/ml	54.01908	15.05910	.056	-.9299	108.9681
		9mg/ml	111.00058(*)	15.05910	.000	56.0516	165.9496
		Veritas	124.60460(*)	15.05910	.000	69.6556	179.5536
		UBM	-30.89759	15.05910	.940	-85.8466	24.0514

Table 32: Comparison between % increase at Day 4 Experiment 1

Dependent Variable	(I) Material2	(J) Material2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Day4	3mg/ml	5mg/ml	-24.87976	54.85163	1.000	-225.0273	175.2678
		9mg/ml	22.20931	54.85163	1.000	-177.9382	222.3569
		Veritas	-89.11757	54.85163	1.000	-289.2651	111.0300
		UBM	-349.28452(*)	54.85163	.001	-549.4321	-149.1370
		Plastic	-126.89704	54.85163	.588	-327.0446	73.2505
	5mg/ml	3mg/ml	24.87976	54.85163	1.000	-175.2678	225.0273
		9mg/ml	47.08907	54.85163	1.000	-153.0585	247.2366
		Veritas	-64.23781	54.85163	1.000	-264.3853	135.9097
		UBM	-324.40476(*)	54.85163	.001	-524.5523	-124.2572
		Plastic	-102.01729	54.85163	1.000	-302.1648	98.1303
	9mg/ml	3mg/ml	-22.20931	54.85163	1.000	-222.3569	177.9382
		5mg/ml	-47.08907	54.85163	1.000	-247.2366	153.0585
		Veritas	-111.32688	54.85163	.978	-311.4744	88.8207
		UBM	-371.49383(*)	54.85163	.000	-571.6414	-171.3463
		Plastic	-149.10636	54.85163	.280	-349.2539	51.0412
	Veritas	3mg/ml	89.11757	54.85163	1.000	-111.0300	289.2651
		5mg/ml	64.23781	54.85163	1.000	-135.9097	264.3853
		9mg/ml	111.32688	54.85163	.978	-88.8207	311.4744
		UBM	-260.16695(*)	54.85163	.007	-460.3145	-60.0194
		Plastic	-37.77948	54.85163	1.000	-237.9270	162.3681
	UBM	3mg/ml	349.28452(*)	54.85163	.001	149.1370	549.4321
		5mg/ml	324.40476(*)	54.85163	.001	124.2572	524.5523
		9mg/ml	371.49383(*)	54.85163	.000	171.3463	571.6414
		Veritas	260.16695(*)	54.85163	.007	60.0194	460.3145
		Plastic	222.38748(*)	54.85163	.024	22.2399	422.5350
	Plastic	3mg/ml	126.89704	54.85163	.588	-73.2505	327.0446
		5mg/ml	102.01729	54.85163	1.000	-98.1303	302.1648
		9mg/ml	149.10636	54.85163	.280	-51.0412	349.2539
		Veritas	37.77948	54.85163	1.000	-162.3681	237.9270
		UBM	-222.38748(*)	54.85163	.024	-422.5350	-22.2399

Table 33: Comparison between increases at both time points for each material (Exp 1)

		Sum of Squares	df	Mean Square	F	Sig.
Fibring3mg	Between Groups	668.165	1	668.165	.421	.552
	Within Groups	6346.978	4	1586.744		
	Total	7015.143	5			
Fibrin5mg	Between Groups	227.726	1	227.726	.547	.501
	Within Groups	1666.071	4	416.518		
	Total	1893.798	5			
Fibrin9mg	Between Groups	740.183	1	740.183	2.188	.213
	Within Groups	1352.972	4	338.243		
	Total	2093.155	5			
Veritas	Between Groups	32477.368	1	32477.368	4.661	.097
	Within Groups	27872.044	4	6968.011		
	Total	60349.412	5			
UBM	Between Groups	95112.054	1	95112.054	36.463	.004
	Within Groups	10433.741	4	2608.435		
	Total	105545.796	5			
Plastic	Between Groups	5457.687	1	5457.687	2.066	.224
	Within Groups	10566.798	4	2641.700		
	Total	16024.486	5			

Table 34: Comparison of % increase between materials at each time point (Exp 2)

Dependent Variable	(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Day1	3mg/mL	5mg/mL	-.14220	.10381	1.000	-.4695	.1851
		9mg/mL	.08116	.10381	1.000	-.2461	.4084
		Plastic	-.24907	.10381	.201	-.5763	.0782
	5mg/mL	3mg/mL	.14220	.10381	1.000	-.1851	.4695
		9mg/mL	.22335	.10381	.315	-.1039	.5506
		Plastic	-.10687	.10381	1.000	-.4341	.2204
	9mg/mL	3mg/mL	-.08116	.10381	1.000	-.4084	.2461
		5mg/mL	-.22335	.10381	.315	-.5506	.1039
		Plastic	-.33023(*)	.10381	.047	-.6575	-.0030
	Plastic	3mg/mL	.24907	.10381	.201	-.0782	.5763
		5mg/mL	.10687	.10381	1.000	-.2204	.4341
		9mg/mL	.33023(*)	.10381	.047	.0030	.6575
Day4	3mg/mL	5mg/mL	-.01592	.31255	1.000	-1.0013	.9694
		9mg/mL	-1.47163(*)	.31255	.003	-2.4570	-.4863
		Plastic	-.50024	.31255	.813	-1.4856	.4851
	5mg/mL	3mg/mL	.01592	.31255	1.000	-.9694	1.0013
		9mg/mL	-1.45570(*)	.31255	.003	-2.4411	-.4703
		Plastic	-.48432	.31255	.883	-1.4697	.5010
	9mg/mL	3mg/mL	1.47163(*)	.31255	.003	.4863	2.4570
		5mg/mL	1.45570(*)	.31255	.003	.4703	2.4411
		Plastic	.97138	.31255	.054	-.0140	1.9567
	Plastic	3mg/mL	.50024	.31255	.813	-.4851	1.4856
		5mg/mL	.48432	.31255	.883	-.5010	1.4697
		9mg/mL	-.97138	.31255	.054	-1.9567	.0140
Day7	3mg/mL	5mg/mL	-.47956	.44842	1.000	-1.8933	.9342
		9mg/mL	-3.56151(*)	.44842	.000	-4.9752	-2.1478
		Plastic	1.10601	.44842	.178	-.3077	2.5197
	5mg/mL	3mg/mL	.47956	.44842	1.000	-.9342	1.8933
		9mg/mL	-3.08195(*)	.44842	.000	-4.4957	-1.6682
		Plastic	1.58556(*)	.44842	.025	.1718	2.9993
	9mg/mL	3mg/mL	3.56151(*)	.44842	.000	2.1478	4.9752
		5mg/mL	3.08195(*)	.44842	.000	1.6682	4.4957
		Plastic	4.66751(*)	.44842	.000	3.2538	6.0812
	Plastic	3mg/mL	-1.10601	.44842	.178	-2.5197	.3077
		5mg/mL	-1.58556(*)	.44842	.025	-2.9993	-.1718
		9mg/mL	-4.66751(*)	.44842	.000	-6.0812	-3.2538
Day14	3mg/mL	5mg/mL	-.61164	.93019	1.000	-3.5442	2.3210
		9mg/mL	-2.50754	.93019	.117	-5.4402	.4251
		Plastic	2.27702	.93019	.184	-.6556	5.2096
	5mg/mL	3mg/mL	.61164	.93019	1.000	-2.3210	3.5442
		9mg/mL	-1.89590	.93019	.385	-4.8285	1.0367

		Plastic	2.88867	.93019	.055	-.0439	5.8213
	9mg/mL	3mg/mL	2.50754	.93019	.117	-.4251	5.4402
		5mg/mL	1.89590	.93019	.385	-1.0367	4.8285
		Plastic	4.78457(*)	.93019	.001	1.8520	7.7172
	Plastic	3mg/mL	-2.27702	.93019	.184	-5.2096	.6556
		5mg/mL	-2.88867	.93019	.055	-5.8213	.0439
		9mg/mL	-4.78457(*)	.93019	.001	-7.7172	-1.8520

Table 35: Comparison of % increase between time points for each material (Exp 2)

Dependent Variable	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Fibrin3mg	Day 1	Day 4	-.18829	.59561	1.000	-2.0661	1.6895
		Day 7	-2.52698(*)	.59561	.007	-4.4047	-.6492
		Day 14	-3.58094(*)	.59561	.000	-5.4587	-1.7032
	Day 4	Day 1	.18829	.59561	1.000	-1.6895	2.0661
		Day 7	-2.33869(*)	.59561	.012	-4.2165	-.4609
		Day 14	-3.39265(*)	.59561	.001	-5.2704	-1.5149
	Day 7	Day 1	2.52698(*)	.59561	.007	.6492	4.4047
		Day 4	2.33869(*)	.59561	.012	.4609	4.2165
		Day 14	-1.05396	.59561	.613	-2.9317	.8238
	Day 14	Day 1	3.58094(*)	.59561	.000	1.7032	5.4587
		Day 4	3.39265(*)	.59561	.001	1.5149	5.2704
		Day 7	1.05396	.59561	.613	-.8238	2.9317
Fibrin5mg	Day 1	Day 4	-.06202	.70816	1.000	-2.2946	2.1706
		Day 7	-2.86434(*)	.70816	.010	-5.0969	-.6317
		Day 14	-4.05039(*)	.70816	.001	-6.2830	-1.8178
	Day 4	Day 1	.06202	.70816	1.000	-2.1706	2.2946
		Day 7	-2.80233(*)	.70816	.011	-5.0349	-.5697
		Day 14	-3.98837(*)	.70816	.001	-6.2210	-1.7558
	Day 7	Day 1	2.86434(*)	.70816	.010	.6317	5.0969
		Day 4	2.80233(*)	.70816	.011	.5697	5.0349
		Day 14	-1.18605	.70816	.719	-3.4186	1.0465
	Day 14	Day 1	4.05039(*)	.70816	.001	1.8178	6.2830
		Day 4	3.98837(*)	.70816	.001	1.7558	6.2210
		Day 7	1.18605	.70816	.719	-1.0465	3.4186
Fibrin9mg	Day 1	Day 4	-1.74107(*)	.53047	.039	-3.4135	-.0687
		Day 7	-6.16964(*)	.53047	.000	-7.8420	-4.4972
		Day 14	-6.16964(*)	.53047	.000	-7.8420	-4.4972
	Day 4	Day 1	1.74107(*)	.53047	.039	.0687	3.4135
		Day 7	-4.42857(*)	.53047	.000	-6.1010	-2.7562
		Day 14	-4.42857(*)	.53047	.000	-6.1010	-2.7562
	Day 7	Day 1	6.16964(*)	.53047	.000	4.4972	7.8420
		Day 4	4.42857(*)	.53047	.000	2.7562	6.1010
		Day 14	.00000	.53047	1.000	-1.6724	1.6724
	Day 14	Day 1	6.16964(*)	.53047	.000	4.4972	7.8420
		Day 4	4.42857(*)	.53047	.000	2.7562	6.1010
		Day 7	.00000	.53047	1.000	-1.6724	1.6724
Plastic	Day 1	Day 4	-.43946	.19279	.250	-1.0473	.1684
		Day 7	-1.17191(*)	.19279	.000	-1.7797	-.5641
		Day 14	-1.05485(*)	.19279	.001	-1.6627	-.4470
	Day 4	Day 1	.43946	.19279	.250	-.1684	1.0473
		Day 7	-.73244(*)	.19279	.015	-1.3403	-.1246

		Day 14	-.61538(*)	.19279	.046	-1.2232	-.0076
	Day 7	Day 1	1.17191(*)	.19279	.000	.5641	1.7797
		Day 4	.73244(*)	.19279	.015	.1246	1.3403
		Day 14	.11706	.19279	1.000	-.4908	.7249
	Day 14	Day 1	1.05485(*)	.19279	.001	.4470	1.6627
		Day 4	.61538(*)	.19279	.046	.0076	1.2232
		Day 7	-.11706	.19279	1.000	-.7249	.4908

Appendix V: Information About Harvesting Bovine Blood

Phone interview with Maureen Brown, a technician at the University of Massachusetts Medical School Medical School blood bank.

12:46 pm, November 30, 2006
Notes:

- Surgeons sometimes like to use whole blood, especially for cardiac applications
- Whole blood can be store for 28 days under 1-6ºC refrigeration with EDTA for anticoagulation and CPD for nourishment. Should get colder than 1ºC or cells will be damaged, but should be under 6ºC to keep bacteria from growing.
- Anticoagulants vary, the one they use for collecting blood is Absol, it come in the blood collection bags. Absol comes in different mixture and must be added to blood within 8 hours.
- Seperated red cells can be stored for 42 days in 1-6ºC depending on anticoagulant (Absol)
- Plasma can be frozen at temperatures below -18ºC
- Plasma with platelets should probably be stored at 1-6ºC for 28 days
- ABB website will have specific information about anticoagulants

Phone conversation of Richard Blood of Blood Farm, Groton, Ma.

- Bovine whole blood is sold for $20/gallon.
- Beef is killed on Wednesday mornings.
- To collect blood a wide-mouth container must be brought to slaughter, along with the desired anticoagulant.
- A government release form must be signed. This form simply states that the blood will not be used as part of any food products and that Blood Farm takes no responsibility for the way in which it is used.

Appendix W: Power Analysis

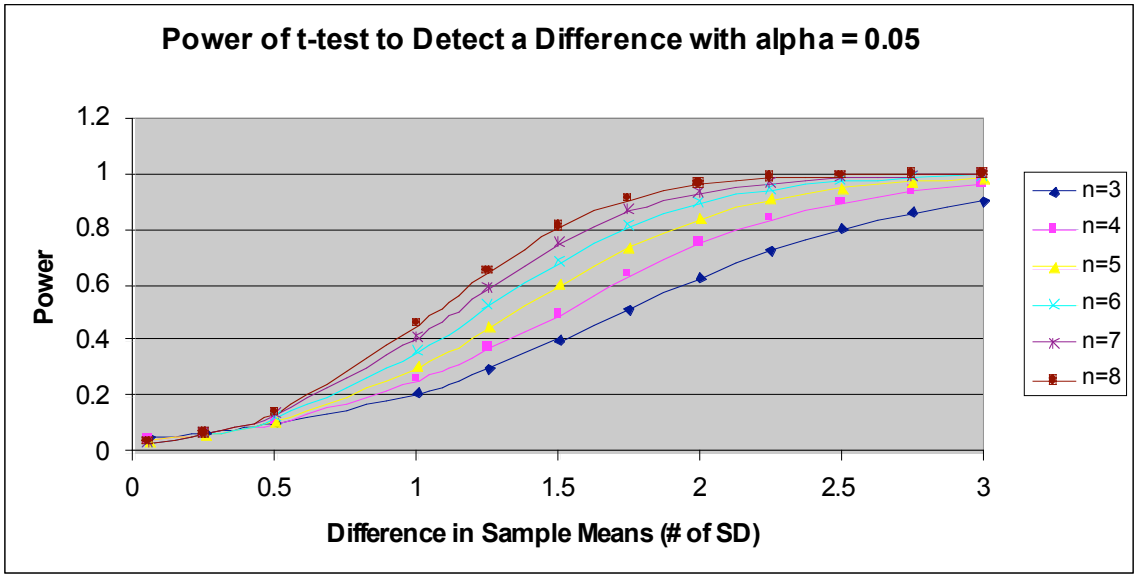


Table 36: Power of t-test to detect a difference between sample groups

Difference (# of SD)	Sample Size (n)					
	3	4	5	6	7	8
0.05	0.055	0.044	0.039	0.036	0.034	0.033
0.25	0.074	0.066	0.063	0.063	0.064	0.066
0.50	0.105	0.106	0.113	0.123	0.133	0.145
1.00	0.215	0.259	0.309	0.360	0.411	0.460
1.25	0.300	0.375	0.452	0.525	0.592	0.652
1.50	0.402	0.492	0.603	0.685	0.753	0.808
1.75	0.516	0.639	0.738	0.813	0.868	0.908
2.00	0.627	0.753	0.840	0.899	0.937	0.961
2.25	0.726	0.839	0.908	0.949	0.972	0.985
2.50	0.804	0.899	0.949	0.975	0.988	0.994
2.75	0.863	0.938	0.973	0.988	0.995	0.998
3.00	0.905	0.962	0.985	0.994	0.998	0.999

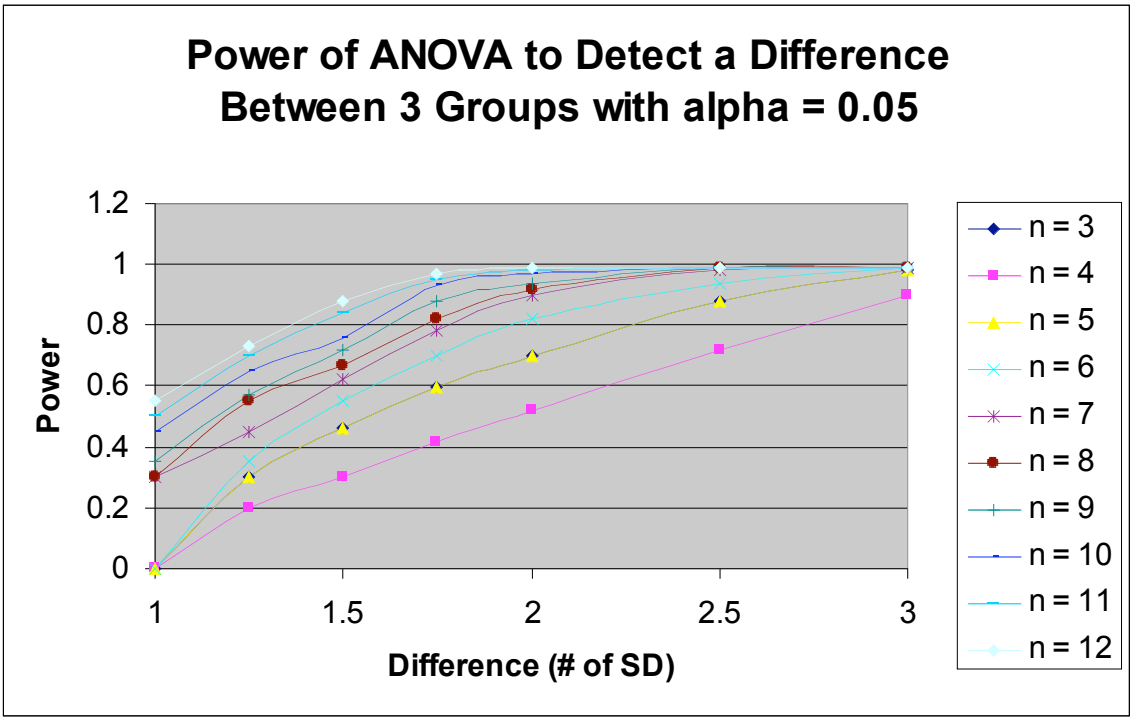


Table 37: Power of ANOVA to detect a difference between 3 groups

Difference (# of SD)	Sample Size (n)									
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1.00	0.00	0.00	0.00	0.00	0.30	0.30	0.35	0.45	0.50	0.55
1.25	0.00	0.20	0.30	0.35	0.45	0.55	0.57	0.65	0.70	0.73
1.50	0.20	0.30	0.46	0.55	0.62	0.67	0.72	0.76	0.84	0.88
1.75	0.25	0.42	0.60	0.70	0.78	0.82	0.88	0.93	0.95	0.97
2,00	0.50	0.52	0.70	0.82	0.90	0.92	0.94	0.97	0.98	0.99
2.50	0.48	0.72	0.88	0.94	0.98	0.99	0.99	0.99	0.99	0.99
3.00	0.70	0.90	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.99

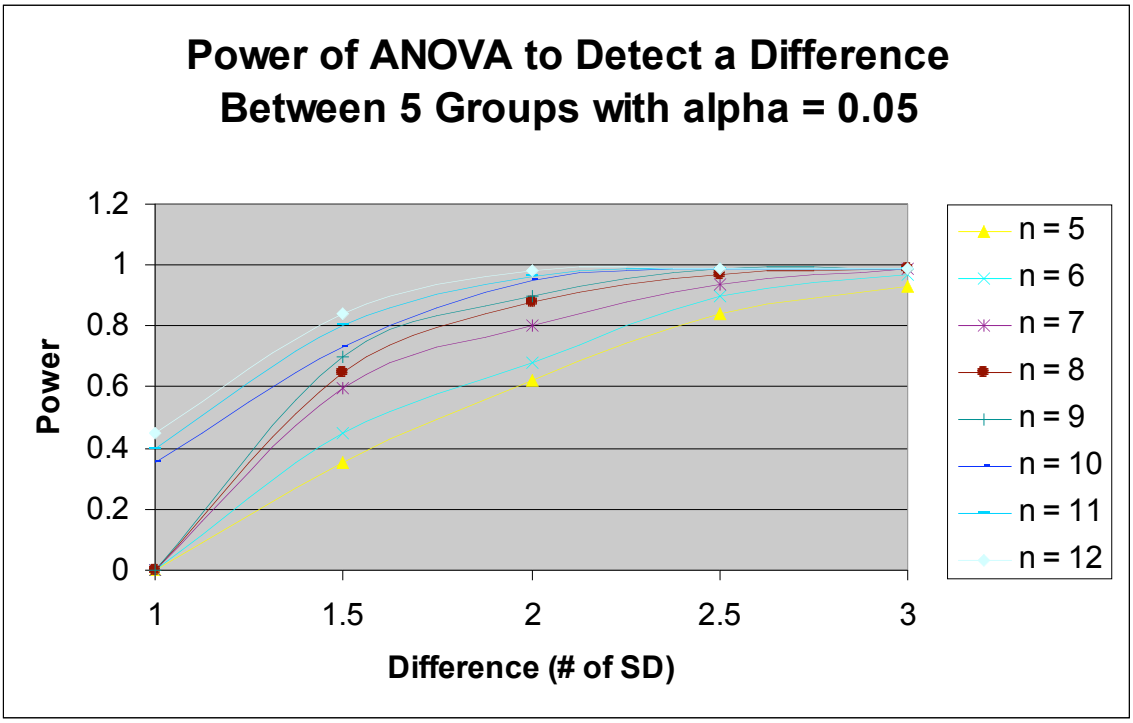


Table 38: Power of ANOVA to detect a difference between 5 groups

Difference (# of SD)	Sample Size (n)									
	3	4	5	6	7	8	9	10	11	12
1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.40	0.45
1.50	0.00	0.00	0.35	0.45	0.60	0.65	0.70	0.73	0.80	0.84
2.00	0.35	0.50	0.62	0.68	0.80	0.88	0.90	0.95	0.96	0.98
2.50	0.60	0.74	0.84	0.90	0.94	0.97	0.99	0.99	0.99	0.99
3.00	0.65	0.85	0.93	0.97	0.99	0.99	0.99	0.99	0.99	0.99

Calculating power for unpaired t-test [119]

DEFINITIONS
H_0 = there is no difference between the means of the two groups
H_A = there is a difference between the means of the two groups

ASSUMPTIONS
- $n_1 = n_2$
- $\sigma_1 = \sigma_2$
- i.e. variances are equal

STEPS
1. Compute t statistics for H_0 and H_A
2. Compute difference between t statistics for H_0 and H_A

3. Power is probability of difference in t statistics (determined from one-tailed critical values of t table)

$$t' = \frac{\mu_1 - \mu_2}{\sqrt{\frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2}}} = \frac{\delta}{\sigma}\sqrt{\frac{n}{2}}$$

Where:

- μ = population mean
- σ = population standard deviation
- n = sample size
- δ = difference in population means

Calculating power for ANOVA [119]

DEFINITIONS
H_0 = there is no difference between the migration in the different groups
H_A = migration is different for at least one of the groups

STEPS
1. Compute the non-centrality parameter (size of the treatment we wish to detect)

$$\phi = \frac{\delta}{\sigma}\sqrt{\frac{n}{2k}}$$

where δ = minimum difference between treatment groups
σ = standard deviation within the underlying population
k = number of treatment groups
n = sample size of each treatment group

2. Compute degrees of freedom
$\nu_n = k - 1$
$\nu_d = k(n - 1)$
3. Obtain power from power chart

REFERENCES
[119]

Mechanical Testing Sample Size:

$$\frac{average - specification}{stdev} = \#SD$$

Calculate #SD, go to chart, find sample size with 0.9 or better

Appendix X: Project Budget

Vendor	Contents	PO #	Total Cost	
Micromedics	FibriJect applicators		$8.16	Donation (shipping charge)
Boston Scientific	Hemashield Platinum		$0.00	Donation
Bio Stockroom	Filters, syringes		$20.00	(Est.)
Sigma-Aldrich	Fibrinogen, thrombin	BME7-165	$67.02	
Cambrex	Trypsin	BME7-185	$57.40	
Sigma-Aldrich	Fibrinogen	BME7-187	$103.62	
Sigma-Aldrich	Thrombin	BME7-223	$38.62	
Promega	MTS assay	BME7-228	$101.00	
Promega	MTS assay	BME7-239	$101.00	
		Total	**$496.82**	
		Budget	$624.00	
		Remaining	**$127.18**	