IN VITRO ANALYSIS OF AUTO-LOOM WOVEN TEST PATCHES FOR HERNIA REPAIR

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IN VITRO ANALYSIS OF AUTO-LOOM WOVEN TEST PATCHES FOR HERNIA REPAIR

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ABSTRACT

Hernias are defects in the layers of the abdominal wall that can cause discomfort or pain and lead to serious health problems if left untreated. A significant portion of the world’s population is afflicted by hernia formation, and the cost of treating those affected is in the billions of dollars in the US alone. The current best practices for repairing hernias involve the surgical implantation of polymeric meshes over and around the defect site. The mesh, acting as a synthetic replacement for the damaged abdominal wall layers, provides a barrier to further visceral protrusions through the defect, a support framework for the surrounding tissue, and, depending on the design of the mesh, a lattice for permanent incorporation into the body.

Current repair meshes provide excellent reinforcement of the abdominal wall and closure of existing hernias, but recurrence rates are still high for some hernia types, and other complications are not uncommon. Historically, research on the treatment of hernias has focused on reducing these complications through refinement of surgical technique or mesh composition. The ultimate goal of the research presented in this thesis is to advance the body of knowledge on mesh composition by providing a new avenue for investigating mesh properties.

While most hernia mesh researchers use animal models or patient studies to evaluate prostheses, we have developed an in vitro-centric mesh production and examination system to characterize mesh properties that affect the cellular affinity and treatment potential of repair mesh. These properties, known to impact implant success, are: material composition, surface architecture, pore configuration, and filament structure.
Feasibility studies conducted on auto-loom woven, degummed silk mesh seeded with murine-derived D1 multipotent mesenchymal stromal cells yielded promising results. The *in vitro* evaluation procedure and auto-loom mesh production described in this work are a first step toward developing a simple, inexpensive polymer post processing method to facilitate cell response-directed mesh design. It is hoped that by providing the means to evaluate mesh properties, a more successful hernia mesh prosthesis can be developed.
ACKNOWLEDGEMENTS

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PREFACE

Over 1 million hernia repairs are performed in the United States yearly, at a total cost of $5 to $5.5 billion (1-3). Furthermore, hernia repair is the most common type of elective surgery performed in most countries (4). Data on the overall prevalence of hernias is limited, but a 1941 study on the incidence of herniation estimates that 4.6% of the population suffers from hernia development (5). A hernia is a protrusion through a weakened area in the abdominal wall caused by some combination of evolutionary or acquired factors (6).

The current preferred method of repair is implantation of permanent polymeric meshes across the defect site to buttress the damaged region (7-10). Despite recent significant advances in both surgical technique and mesh design, hernia repairs still suffer from both hernia recurrence and complications following surgery. Common complications include pain, infection, and fistula, adhesion, and seroma formation (8). These complications represent challenges that must be minimized in order to create the ideal repair prosthesis.

The ultimate design goal for hernia repair mesh is the creation of a durable yet compliant patch that responds in vivo like autologous tissue (11). That is, the mesh should allow good tissue incorporation and resist scar tissue formation, all while buttressing the defect space in the short term and providing lasting support for the repaired region in the long term.

Chapter I outlines background information on hernia development, treatment, and areas of mesh improvement. The chapter concludes with an overview of the research presented in Chapters II and III as well as justification and scientific relevance of the study. Chapter II details the development of a semi-automated loom, and Chapter III highlights a proof-of-principle study assessing cell growth on woven mesh fabricated using the new loom.
I. CURRENT THEORIES OF HERNIA FORMATION AND APPROACHES TO DEFECT REPAIR

Defect Anatomy and Formation

Anatomy of the Abdominal Region

An understanding of abdominal region anatomy is needed to appreciate the particulars of hernia formation and repair. Besides an overview of the layers that make up the abdominal wall, this section focuses on the inguinal region because of the higher relative proportion of hernias that occur there compared to other parts of the body. Comprehensive understanding of the anatomical breakdown that follows is not necessary to grasp the concepts presented in the rest of the thesis, but it is provided as a reference for readers interested in the details of hernia development. The most important information in this section is that the abdominal wall is comprised of varying numbers of heterogenic layers of tissue with dissimilar mechanical strengths and properties enclosing structures such as blood vessels, ligaments, and other tubular projections. While differences in abdominal region anatomy can be found in the overall population, a common set of wall weaknesses and structures susceptible to hernia development cause hernias to form most often in a few, identifiable areas of the body. By convention, hernias are categorized by the areas of the body they affect.

The abdominal wall consists of layers of skin, adipose tissue, muscle, fascia, and membrane. The surface layer is comprised of skin and vascularized fatty tissue known as Camper’s fascia. Below the Camper’s fascia lies the membranous Scarpa’s fascia that connects medially to the linea alba (12).

The next layer of the abdominal wall is the rectus sheath. The rectus sheath is an aponeurotic tendinous covering that encases the rectus abdominis muscle and divides it
horizontally with three or four tendinous intersections. The linea alba denotes the medial line of the rectus sheath that runs vertically down the anterior abdominal wall. Inferiorly from the arcuate line (bottom quarter of rectus muscle) the posterior rectus sheath is nonexistent and the rectus muscle contacts the transversalis fascia directly (12, 13).

Mediolaterally from the rectus abdominis the rectus sheath divides into three aponeurotic layers that connect to the three flat muscle layers. The superficial most flat muscle is the external oblique, followed by the internal oblique, and finally the transversus abdominis. These muscle groups have laterally spanning fibers that begin posterolaterally and pass anteriorly (12, 13).

Below the anterolateral muscle groups is a continuous layer of aponeurotic fascia called the transversalis fascia that covers the entire abdominal cavity, attaches to the iliac crest in the pelvic region, and descends into the testes to become the internal spermatic fascia. The next deep layer is the highly vascularized extraperitoneal fascia (otherwise known as the preperitoneal space) which is of varying thickness depending on its position relative to internal viscera (12).

The final layer of the abdominal wall is a closed sac of thin, serous membrane that covers all abdominal viscera. The membrane that lines the abdominal wall is termed the parietal peritoneum, while the membrane that folds inward to directly cover organs is called the visceral peritoneum (12).

_Anatomy of the Inguinal Region_

The inguinal region refers to the lower, lateral portions of the anterior abdominal wall, also known as the left and right groin regions. There are two structures in the inguinal region
that are commonly associated with herniation. These structures are the processus vaginalis and the inguinal triangle.

The processus vaginalis is a tubular projection of the peritoneal cavity that forms the inguinal canal during fetal development by extending along the anterior side of the gubernaculum into the labioscrotal swellings. Following the descent of the testes through the inguinal canal, the portion of the processus vaginalis inside the inguinal canal collapses to form a ligamentous remnant that attaches to the tunica vaginalis, a closed sac of peritoneum wrapped around the anterior and lateral portions of the testis. If the processus vaginalis fails to fully collapse, the possibility of indirect hernia formation through the inguinal canal increases dramatically (12). This defect is known as a patent (open) processus vaginalis (PPV).

One area of the abdominal wall especially vulnerable to weakening is the inguinal triangle, also known as Hesselbach’s triangle (14). The inguinal triangle is a small patch of abdominal wall located directly superior to the lacunar ligament that lacks major musculature support. It is bordered medially by the rectus abdominis muscle, laterally by the inferior epigastric vessels, and inferiorly by the inguinal ligament. The inguinal triangle is actually only the upper portion of a larger structurally weak area known as the myopectineal orifice (15). This orifice is divided into two levels by the inguinal ligament. The inguinal triangle comprises the superior level, and the femoral sheath makes up a large portion of the inferior level (14). A protrusion medial to the inferior epigastric vessels in the inguinal triangle is considered a direct inguinal hernia, a protrusion lateral to the inferior epigastric vessels is considered an indirect inguinal hernia, and a protrusion into the femoral sheath (specifically the femoral canal) is considered a femoral hernia (12).
Anatomy of the Inguinal Canal

The inguinal canal is a narrow passage contained within the groin region of the abdominal wall that extends from the deep inguinal ring located at the midpoint of the inguinal ligament to the superficial inguinal ring located superior to the pubic tubercle. The deep inguinal ring is a tubular invagination of the transversalis fascia, located superior to the inguinal ligament and lateral to the inferior epigastric vessels. From here, the inguinal canal extends parallel to the inguinal ligament towards the pubic symphysis. The canal runs through the transversus abdominis muscle, is covered by the internal oblique muscle, and ends at the tubular invagination of the external oblique aponeurosis at the superficial inguinal ring (12).

The anterior wall of the canal is formed by the internal oblique muscle and external oblique aponeurosis. The posterior wall is formed by the transversalis fascia, supported by the conjoint tendon. The roof of the canal is formed by the arching fibers of the transversus abdominis muscle, and the floor is formed by the inguinal ligament, supported by the lacunar ligament (12).

The inguinal canal houses the spermatic cord in men, the round ligament in women, and the ilio-inguinal nerve in both sexes. The spermatic cord is a collection of structures including the ductus deferens, remnants of the processus vaginalis, as well as several nerves, blood vessels, and lymphatics embedded within a small amount of extraperitoneal fascia. These structures are protected by coverings from three of the abdominal wall layers through which the inguinal canal passes. These layers include the transversalis fascia (termed the internal spermatic fascia), beginning at the deep inguinal ring, the internal oblique muscle (cremasteric muscle and fascia), and the external oblique aponeurosis (external spermatic fascia), beginning
at the superficial inguinal ring. The spermatic cord exits the inguinal canal at the superficial inguinal ring and continues into the scrotum (12).

**Common Hernia Types**

There are four major types of hernias: inguinal, umbilical, incisional, and femoral. Approximately 66% of hernias are classified as inguinal, 16% as umbilical, 9% as incisional, 2% as femoral, and 7% as other types (4). Based on a review of the literature, the two most discussed types of hernias are inguinal and incisional. The large number of papers related to inguinal hernias is not surprising, given the prevalence of inguinal hernia formation. Inguinal hernia is the most common type encountered, and inguinal hernia repair is the most common general surgery procedure performed in the United States (2). Inguinal hernias affect a sizable portion of the population, presenting in 6% to 27% of all men (16, 17). The disproportionate number of publications addressing incisional hernias may be due to the high (50%) rate of recurrence following repair and the association with surgical incision sites (6, 18). In fact, an incisional hernia will develop in up to one in five patients who undergo laparotomies (1).

**Risk Factors and Formation**

The exact nature of hernia formation is not fully understood, due in part to the lack of consensus as to the cause of herniation, as well as the variety of hernia types (1). The basic premise of hernia formation is that a supporting wall or opening is weakened by some combination of factors. The affected region then slowly buckles due to tissue degradation and regularly occurring mechanical strain. This distention of the abdominal wall allows intra-
abdominal viscera to shift position and potentially migrate into the newly formed hernia sac, further aggravating the damaged area.

Historically, it was thought that hernias developed due to abrupt strain or trauma, and that defects were solely mechanical in nature (19). However, it is now widely accepted that while hernias are often revealed by strenuous physical activity, they usually take years to fully mature and are rarely caused by isolated, high stress events (2, 20, 21). Three often cited causes of hernia development include increased intra-abdominal pressure, congenital structural malformations (such as PPV), and poor closure technique of surgical incisions (6). Furthermore, many hernia types are now considered by several prominent researchers to be primarily caused by biochemical decomposition or malformation of collagen in the body (19, 21-23). As such, it may be more appropriate to classify hernia formation as a symptom of a chronic metabolic disorder rather than as an isolated mechanical defect (22). This also suggests that future treatment for herniation may include both implantation of a reinforcing patch to restore mechanical stability to the defect area and drug therapy to treat the underlying systemic cause of reduced tissue strength (21).

Hernia Repair and Treatment

Methods of Repair

Due to the constant stresses applied to the abdominal wall from intra-abdominal pressure and the structural malformation caused by herniation, the body is incapable of repairing a herniated region once the defect has begun to form. Currently, the only proven method of hernia repair is surgical reduction, closure or plugging, and, typically, reinforcement of the defect site. Nonoperative treatment of hernias is limited mainly to management of
symptoms rather than actual eradication of the defect. Because of the effectiveness and relative low risk associated with hernia repair, surgery is generally indicated in the presence of herniation unless comorbid conditions preclude treatment (1). In cases where surgical reduction of the hernia is elected, the defect is corrected by a procedure called a hernioplasty or herniorrhaphy. Because hernias can develop in a multitude of locations, progress due to a wide variety of causes, and present with varying severity, a myriad of different hernioplasty procedures have been developed to decrease the likelihood of post-surgical complications. Some of the most common hernioplasty procedures are shown in Table 1.1.

Table 1.1: Methods of Hernia Repair.

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<tr>
<th>Repair Type</th>
<th>Open</th>
<th>Laparoscopic</th>
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<td>Prosthesis Type</td>
<td>Suture (primary)</td>
<td>Mesh (tension free)</td>
</tr>
<tr>
<td>Prosthesis Setup</td>
<td>Interrupted</td>
<td>Continuous</td>
</tr>
<tr>
<td>Repair Name</td>
<td>Bassini, McVay</td>
<td>Shouldice</td>
</tr>
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*Hernioplasty techniques that lack specific names are displayed in quotations.

Hernia repairs can be divided into two basic categories: those that are performed using an open technique and those that are performed using a laparoscopic technique. The majority of inguinal repairs are performed using the more traditional open method, but the relative number of laparoscopic hernia repairs is on the rise. Even so, 86% of inguinal hernias still were repaired using an open technique in 2003, with only 14% of repairs being performed laparoscopically (24). While most hernias can be repaired using either method, open repairs
require less specialized training, generally have less risk of severe complications (such as undetected bowel laceration), and, at least in the case of inguinal hernia repair, can be performed under local instead of general anesthetic, meaning reduced cost and quicker operation time (25). The benefit of laparoscopic repair is that persistent pain is less common and patients can typically return to normal activity levels faster than compared to open repair (26). The outcomes of laparoscopic repairs also tend to be more dependent on surgeon experience and skill than the typically less technical, open repairs.

Hernia repairs can also be classified by the method of repair. Herniorrhaphies can be performed as primary/tissue repairs or as tension-free mesh repairs. Mesh repairs offer much lower recurrence rates than primary repairs, and are used in all but the smallest of hernias. In 2003, only 7% of inguinal hernias were corrected using a tissue-to-tissue primary repair (24). The generally accepted reason for high recurrence rates in primary suture repairs is the presence of tension at the repair site, which hinders the healing process. When a primary repair is elected, the application of relaxing incisions to spread muscle fibers in the area surrounding the repair can partially alleviate tension at the repair site and improve results.

There are several prosthesis configurations available for use in hernioplasties. In primary closures such as Bassini and McVay repairs, interrupted suture patterns are used to correct the defect (27). In contrast, the less recurrence prone Shouldice repair includes two running sutures that overlap the abdominal wall layers over the floor of the inguinal canal to recreate the internal inguinal ring (2, 27). In plug repairs, a flexible polymer funnel is introduced into the preperitoneal space to prevent hernia sacs from penetrating the relatively weak myopectineal orifice of Fruchaud and entering the inguinal canal. Plug repairs are easy to learn and have low recurrence rates, but patients may feel some discomfort in the region, depending
on the position of the plug. In onlay mesh repairs, such as the Lichtenstein procedure, a polymer mesh is attached to the floor of the inguinal triangle to reinforce the weakened area. Instead of overlapping the defect area as in onlay repairs, in an inlay repair the mesh is sutured to the edges of the damaged transversalis fascia. Inlay repairs suffer from high recurrence rates and mesh displacement due to the fixation sutures being placed in the healing region. Underlay repairs place mesh either preperitoneally or intraperitoneally. Underlay repairs are typically repaired laparoscopically, but specially designed Kugel patches can be inserted using an open technique. A plug and patch repair combines an overlay or underlay mesh with a plug, which both reinforces the inguinal region and prevents hernia sac encroachment into the inguinal canal. The Prolene Hernia System combines all three prosthesis placement locations to further buttress the susceptible inguinal region.

Complications

The most common complication of hernia surgery is chronic pain (28). Other complications include bacterial infection, seroma and adhesion formation, prosthesis migration, and damage to cord or visceral structures. Recurrence is generally treated as a separate occurrence from complications, although in many cases a causal relationship exists between the two. The most current hernia repair methods performed by experienced surgeons reduce the risk of recurrence to, at worst, a few percentage points, although the systemic nature of some hernia types and difference in length of follow-up studies can cause the true rate of recurrence to vary significantly from medical center to medical center. As recurrence becomes less of a problem, surgeons have begun to reassess how they characterize “successful” hernia repair.
This activity has led surgeons and researchers to focus more on reducing complications such as pain, discomfort, and swelling, and has led to the reduction of post-surgery recovery times (2).

Pain following herniorrhaphy has been estimated to occur in up to 60% of patients one year after inguinal hernia repair, although dedicated hernia repair centers report complications from pain in only 5% to 15% of patients (29, 30). Due to the difficulties involved with quantifying pain across patient groups and the differences in repair techniques the overall risk of post-operative pain is likely in the range of 6% to 11% (2). One reason why incidence of post-herniorrhaphy pain is especially concerning is that hernias often do not present with pain, meaning it is possible that some patients may be disserved by electing for surgical repair of their hernias if pain persists (2). Pain following herniorrhaphy is divided into three categories: somatic, neuropathic, and visceral. Somatic pain generally occurs during movement of the abdominal wall and can be related to tension in the repair or adhesion/scar formation around the implanted prosthesis. Neuropathic pain presents as sharp, jabbing pain related to nerve damage. Neuropathic pain can be controlled using neuropathy medication or neurectomy procedure. Visceral pain tends to present during ejaculation, due to cord damage to the sacral or sympathetic nerves (2). Regardless of the location or severity of the pain associated with hernia repair, if the possibility of post-herniorrhaphy pain and other complications is higher than the risk of hernia recurrence, the time has come to shift focus from preventing recurrence to eliminating complications.

Areas of Mesh Improvement

If tension-free mesh repairs are accepted as the pinnacle of hernia repair in terms of capacity to treat existing hernias and prevent the occurrence of future defects, hernia mesh
must be optimized to reduce complications such as pain, infection, and discomfort. Four aspects of hernia mesh morphology have been identified as having the most potential to influence the outcome of herniorrhaphy surgery. These four morphological components are material composition, surface architecture, pore configuration, and filament structure.

**Material Composition**

Besides forming the basis for the overall strength of a mesh construct, material choice also has a profound effect on tissue integration. Early mesh designers focused disproportionately on material strength when selecting materials for hernia mesh. Not surprisingly, the first implantable meshes were made from steel and silver filigrees. While metal meshes provided more than adequate defect support, patient discomfort and abdominal protrusions caused surgeons to seek out more biologically compliant materials. Since the 1940s, hernia mesh material development has largely followed the same progression as sutures, for which synthetic polymers dominate the market. Today, the most common materials used in mesh construction are polypropylene and expanded polytetrafluoroethylene (ePTFE).

A brief comparison of polypropylene and polytetrafluoroethylene provides an excellent example of how material choice can affect the success of a hernia repair. Both polypropylene and PTFE meshes have low rejection rates and minimal long-term adverse effects from implantation (31). However, while polypropylene elicits a moderate initial inflammatory response and a persistent low-level presence of multinucleate giant-cells, PTFE meshes stimulate a much less aggressive immune response. At first glance, such a dramatic difference in immune response would suggest that PTFE is the better mesh material. However, polypropylene’s strong inflammatory response promotes tissue growth, which secures the mesh
in place relatively quickly compared to PTFE meshes (32). This makes polypropylene an excellent material choice for interabdominal repairs, but severely limits its usefulness as an intraabdominal repair material, where the risk of bowel adhesion is a serious concern. Other significant differences between the materials include shrinkage rates and infection management. Polypropylene meshes typically shrink more than the more hydrophobic ePTFE meshes, but they are less susceptible to bacterial proliferation (33). In addition, once infected, ePTFE meshes typically require removal, whereas polypropylene mesh infections can sometimes be treated internally (34, 35).

While at first glance, the materials used in mesh construction may appear to be similar in function, in reality, each of the polymers or biodegradable substances used in mesh construction have unique traits open to exploitation. This high degree of processability in materials suitable for mesh construction allows mesh design to be adapted with advances in surgical technique and improved understanding of herniation. Just as a wide range of hernia types exist, so too should a wide range of repair materials tailored to each hernia environment.

Surface Architecture

Surface architecture refers to material texture or coatings applied to meshes to influence the biological response. Typically surface architecture is modulated for infection prevention and immune reaction control. One of the most widely researched areas of mesh surface manipulation is the application of antimicrobial coatings to meshes for infection resistance. Examples of such coatings include silver/chlorhexidine and titanium. These coatings are designed to kill bacteria before they can produce protective biofilms that prevent the body’s immune system from managing the infection. The most common bacteria seen in mesh
Infections are *Staphylococcus aureus* and its derivatives. The investigation of bacteria-resistant coatings is one of the few areas of mesh development that has produced a significant amount of published *in vitro* research, and is thus an especially fitting experimental avenue for the *in vitro* evaluation system described in this thesis (33, 36, 37).

Absorbable mesh coatings can be used to increase mesh rigidity during implantation without compromising the long-term compliance or changing the reactivity of the mesh materials. For example, Ethicon Proceed and Ultrapro meshes include absorbable polydioxanone coatings that stiffen the polypropylene filaments during the mesh implantation process. Once implanted, this coating then slowly absorbs, which softens the mesh to improve patient comfort. Polyglactin has also been investigated as an absorbable stiffening material by Klinge and coworkers (38). Emans and coworkers have also embraced surface architecture modification by developing a permanent coating designed to reduce adhesions to polypropylene mesh (39).

Polypropylene and PTFE have different surface characteristics, which in turn alter their respective tissue responses. The difference between regular PTFE meshes and their expanded PTFE counterparts are the micropores created by the expansion process. It is suggested that these micro pores ultimately give ePTFE meshes their stability by allowing tissue to incorporate directly into the material instead of just through macropores. The negative aspect of these micropores is that bacteria are small enough to infiltrate the pores, but larger leukocytes are unable to enter the pores to destroy the infection. This is one of the main reasons why ePTFE meshes must be removed from the body if infected with bacteria.

*Pore Configuration*
The prevailing theory of early mesh designers was that hernias were caused by mechanical strain, and thus, a strong lattice covering was necessary to hold abdominal contents in place. This belief caused early mesh designers to err on the side of caution by creating meshes that were unnecessarily bulky, to reduce the perceived risk of mesh rupture (40, 41). Recent increases in the understanding of hernia formation, combined with a better understanding of the biological response to mesh implantation, have now led mesh designers to reduce the amount of material used to construct mesh and increase the size of pores to allow for more tissue ingrowth. This shift has led to the categorization of mesh based on weight per area, where under 35 g/m² is considered “lightweight”, above 90g/m² is considered “heavyweight”, and any meshes in between “mid-weight” (42). Heavyweight meshes possess burst strengths close to five times that of the abdominal wall, which only increases with scar tissue formation; lightweight meshes can withstand twice the force needed to rupture abdominal tissue (42-44).

The concept behind using lightweight mesh with large pores is that, for a specific mesh chemistry and configuration, severity of immunological response to an implant is directly proportional to the amount of material implanted (45, 46). Less material implanted means the hernia site experiences a less pronounced and shorter inflammatory period, which reduces the amount of unorganized scar tissue that will form around the defect (42). Large, rounded pores also reduce the risk of bridging fibrosis and allow good blood flow around the repair site. Bridging fibrosis and encapsulation occur when scar tissue formation around each mesh filament forms a solid collagen barrier around the mesh (47, 48). Large quantities of scar tissue can lead to complications such as discomfort or paraesthesias in patients (42).
Little has been discussed in the literature regarding pore shape, and the only recommendations for pore size dimensions are that pores under one millimeter in diameter will lead to bridging fibrosis (42). While it is acknowledged that most evaluations of adhesion formation are qualitative in nature, a more rigorous and qualitative evaluation of pore size could lead to insight into hernia formation and mesh design. The auto-loom described in Chapter Two of this thesis can facilitate the production of mesh appropriate for such an investigation.

Filament Structure

Filament structure refers to the manner in which meshes are constructed. Most meshes are open lattices constructed from interlocking filaments. Sheet-like, molded meshes also exist, in which case, filament structure refers to the dimensions and composition of layers used to form the mesh. Increasingly, meshes are being developed that are made from combinations of materials or structure styles. These composite meshes attempt to solve many of the same problems addressed by surface coatings, but do so by introducing extra filaments or layers instead of by modifying existing fibers.

Both suture and hernia mesh researchers have debated the benefits of monofilament versus multifilament designs. The primary concern with multifilament mesh styles is that, in the event of an infection, the increased surface area and niche distribution causes a corresponding increase in the amount of bacterial colonization and biofilm production (33). The niches created by the intersection of adjoining fibers in multifilaments are attractive hiding places for bacteria, and such microorganisms have been characterized as “more infectious” in the presence of highly microporous material (37, 49). For this reason, the popularity of monofilament and smooth surface sheets has risen in recent years.
Relevance of Thesis Research

The goal of the thesis research was to develop a system to quickly and cost-effectively evaluate different types of hernia mesh, in an *in vitro* environment, through investigation of material composition, surface architecture, pore configuration, and filament structure. In order to independently control mesh structural arrangement and composition, an automated loom was created to produce mesh for use in an *in vitro* experimental model. By having the ability to modulate each element of mesh design, it is hoped that the contribution from each critical aspect of hernia mesh architecture can be more precisely evaluated than if only commercially available meshes are investigated. This thesis research is unique as compared to other investigatory methods reported in the hernia repair literature because of the customizability of both the *in vitro* approach to evaluation and the woven auto-loom mesh. It is important to note that while surgeons, the principle authors in the hernia repair literature, largely focus on reducing the recurrence rate of hernias, the focus of the *in vitro* evaluation procedure described in this thesis is the biochemical and cellular response to implanted meshes. The long-term objective of this research is to provide a tool that will facilitate a reduction in the likelihood and severity of hernia repair complications, rather than the rate of hernia recurrence, following herniorrhaphy.
References


II. DESIGN AND OPERATION OF SEMI-AUTOMATED COMPUTER
CONTROLLED LOOM FOR THE PRODUCTION OF SILK MESH

Introduction

This chapter describes the design and operation of the auto-loom used to produce woven silk mesh. The mesh produced by the auto-loom was used to investigate mesh characteristics in an in vitro experiment described in chapter three of this manuscript. Investigating mesh characteristics requires a reliable supply of test samples with consistent parameters. Commercially available hernia patches have a wide variety of features and designs that make it difficult to isolate and compare how each individual attribute affects compliancy. By creating custom patches, it is possible to precisely control test sample parameters and thus more definitively characterize the biological response elicited by each mesh feature when implanted.

Initially, a commercially available loom was considered for producing custom meshes, but power looms proved too large, expensive, and rough on fabrics to be considered as a viable solution. Manually operated hand looms were not considered adequate because an impractical number of modifications were required to automate the weaving process in order to guarantee precise and repeatable mesh production. Thus, a custom loom was created. The finished custom auto-loom can be seen in Figure 2.1.

The auto-loom was designed to fabricate mesh with controllable pore sizes, pore shapes, thread materials, and weave styles. Each of these four parameters can be independently modified, which enables the auto-loom to produce numerous unique mesh designs. Auto-loom mesh production has been largely automated to reduce user error in the
weaving process and, at least theoretically, to increase the speed of mesh fabrication. The weaving process is controlled by a computer program that stores and implements weave patterns and mesh parameters. The auto-loom was designed to be much smaller than commercial power floor looms to conserve thread supplies and minimize the amount of material needed to string the loom. Several experimental thread types have been proposed to be tested in mesh form, some of which have limited availability.

![Auto-loom front view.](image)

**Figure 2.1.** Auto-loom front view.

**Overview of Looms and Weaving**

Weaving is the process of interlacing threads or yarn on a weaving machine or loom to create cloth [1]. The vast majority of woven fabrics are made using perpendicular intersections
of warp and weft threads, which are held together by friction. This is unlike knitted fabrics, where threads are held in place by physical loops. There are several varieties of standard weaves including plain (tabby), twill, and satin. More complicated styles include herring bone, honeycomb (waffle), and leno weaves [1]. These weaves are illustrated in Figure 2.2.

![Weave variations](image)

**Figure 2.2.** Weave variations. Black lines represent warp strings, and gray lines represent weft strings. Leno weaves require an additional mechanism attached to the harnesses to twist warp strings.

A loom is a device used to make woven cloth out of spools of spun thread or yarn. There are several varieties of looms available on the market. Traditional hand looms are typically crafted from wood and have no mechanized components. Hand looms require an operator to manufacture cloth and can weave 10-20 threads per minute depending on the skill
of the user. Automated looms are computer controlled machines capable of weaving hundreds of threads per minute. The auto-loom created over the course of this project is semi-automated, meaning much of the weaving process is computer controlled. However, an operator is still necessary to facilitate some aspects of loom operation.

A loom weaves cloth by inserting threads through an opening (shed) of strings tightened over the length of the loom between two “beams”. The tightened strings are called warp threads or “ends”, and the inserted threads are called weft threads or “picks”. Unwoven warp threads are wrapped around the warp beam on one side of the loom, and completed cloth is wound around the fabric beam on the opposite side. Each warp thread is inserted through the eye of a metal or textile heddle. By raising a group of heddles, a shed can be opened in the warp strings and a weft thread inserted into the cloth. Heddles are collected into groups and strung across a harness (also called a shaft) that raises the entire group of heddles simultaneously. A pattern is generated by raising the harnesses in specified sequences. For example, a plain weave uses two harnesses, with even numbered warp threads in one harness and odd in the other. When the harnesses are raised alternately for every weft thread inserted, the result is a plain weave pattern.

Looms that use a harness system to weave are called dobby looms. More advanced looms use a Jacquard instead of a dobby system to raise threads. A Jacquard system uses long synthetic strings attached to every warp thread to open sheds. Since each warp thread is individually controlled, intricate patterns can be woven into the produced cloth.

The auto-loom is a computer assisted dobby loom, meaning a computer is used to individually control the harnesses. This is an improvement over hand looms, which use a system of foot pedals (treadles) connected to multiple harnesses to produce a weave pattern. The
number of patterns the weaver can produce is limited by the number of treadles available. Complex weaves require multiple harnesses to be raised simultaneously, which can difficult for a single weaver to accomplish with only two feet with which to depress treadles. For this reason several harnesses are attached to each treadle, with each corresponding to a particular shed. Treadles must be prepared prior to beginning to weave, and adjustments can be time consuming.

Instead of relying on treadles to produce unique sheds, dobbi looms have independently controlled harnesses. A dobbi loom with \( n \) harnesses can produce \( 2^n \) different possible sheds. Therefore, the four harness auto-loom is capable of producing 16 different sheds for a given warp string arrangement. More advanced looms use jacquard attachments to increase the number of possible sheds to \( 2^k \), where \( k \) is the number of warp threads. Jacquards control the state of each warp thread individually, instead of bundling threads into harnesses. Jacquards dramatically increase the amount of time it takes to string a loom, but maximize the number of patterns that can be created.

There are several ways to insert a weft thread through an open shed. Delivery methods in power looms include flying shuttle, projectile, air stream, fluid jet, and rapier systems [2]. A flying shuttle system uses a weaving tool called a shuttle to pass weft threads through open sheds. Thread is stored on a bobbin inside the shuttle and unraveled as the shuttle is passed from one side of the loom to the other. In manual weaving, the shuttle is moved through the shed by hand. The word “flying” is used to describe a system in which the shuttle is punched from side-to-side of the loom by a machine. Projectile looms weave by firing projectiles attached to weft threads across the fabric. Air and fluid stream systems use pressurized gas/liquid to propel weft threads through the open shed. Rapier systems use a metal arm to
pull weft threads into place. Both single and dual rapier systems exist. A single rapier system uses one rapier to place threads. In a dual rapier system, weft threads are exchanged between two rapiers in the middle of the shed.

![Figure 2.3. Hand shuttle. The ergonomic design and solid wood construction of the pictured shuttle is characteristic of hand loom shuttles. The ends of flying shuttles are typically capped with metal to improve their durability and resistance to splintering.](image)

Weft threads are stored on a pirn (spool) attached to the side of the loom and are cut to size when the delivery system is ready to insert the next thread. Once a weft thread is inserted, the shed is closed and a beater (also called a reed) is used to pack the thread into place. This process is then repeated using different combinations of harnesses to interlace weft and warp threads and create cloth.
**Figure 2.4.** Modern looms. (left) Nuovo Pignone Rapier Loom with attached Staubli jacquard. It is capable of inserting up to 650 weft threads (picks) per minute. (right) Leclerc Nilus Hand Loom.

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warp thread, End</td>
<td>Thread tightened across the length of a loom. These threads are tied to the located beams at either end of a loom.</td>
</tr>
<tr>
<td>Weft thread, Pick</td>
<td>Thread inserted perpendicular to warp threads through an open shed. These threads are typically stored on pirns to the side of a loom.</td>
</tr>
<tr>
<td>Reed, Beater</td>
<td>A comb-like device used to push weft threads into position. Technically, the term “reed” refers only to the comb component, while “beater” refers to the overall loom mechanism used to pack weft threads.</td>
</tr>
<tr>
<td>Harness, Shaft</td>
<td>A frame used in a dobby loom to hold a collection of heddles. Moving some harnesses up while holding others in place creates a shed.</td>
</tr>
<tr>
<td>Pirn, Spool, Bobbin</td>
<td>A cylinder that thread is wrapped around for storage. The different terms are sometimes used to convey information about the diameter of the cylinder. Pirns are typically long and thin, and bobbins are typically short and wide. Spool is the most general term for such a device.</td>
</tr>
<tr>
<td>Heddle</td>
<td>A long, thin piece of either steel or textile that is used to raise and lower warp threads. Each warp thread is inserted through the eye of a corresponding heddle.</td>
</tr>
<tr>
<td>Shed</td>
<td>A gap created by raising warp threads that weft threads are inserted into. Each shed represents a different state of the loom, and can be expressed in binary notation. The number of unique sheds a loom is able to produce is dependent on the method employed to raise warp threads.</td>
</tr>
<tr>
<td>Beam (warp &amp; fabric)</td>
<td>A cylindrical drum located at each lengthwise end of a loom. The warp beam stores unwoven warp threads, and the fabric beam stores completed cloth.</td>
</tr>
</tbody>
</table>

**Auto-Loom Design**

The auto-loom is designed as a collection of subsystems attached to an aluminum frame. This includes the harness system, the beater system, the rapier system, the
beam and ratchet system, and the computer and control system. The loom weaves by coordinating the movements of each of these systems. The overall loom dimensions are 32 inches long, 29 inches wide (40 inches including rapier tower), and 24 inches tall. Additionally, the loom requires an extra 24 inches of space on its rapier tower side to accommodate the moving rapier. This does not include the space occupied by the computer that controls the loom. Some of the important materials and parts used on the auto-loom are listed in Table 2.2.

The auto-loom was designed to be small enough to conserve potentially scarce experimental thread materials and styles, yet large enough to retain the functionality of larger power looms. The length of the auto-loom was influenced by the number of harnesses needed to create intricate weave patterns as well as the force needed to lift the harnesses. The width of the auto-loom was influenced by the desire to be able to weave mesh large enough for future in vivo animal studies. Sterilization was a final contributing factor to auto-loom size. While the current auto-loom dimensions make the device too large to fit in a standard laboratory fume hood, it is hoped that a future incarnation of the auto-loom will be able to produce mesh in a sterile environment in order to minimize the potential for mesh contamination.

Many of the peripheral loom sections are made from clear, cast acrylic pieces (McMaster-Carr, Santa Fe Springs, CA) sealed together with Weld-On® 4, a chlorohydrocarbon-based plastic adhesive (IPS Corp., Compton, CA). Acrylic pieces were designed using AutoCAD 2007 software (Autodesk, San Francisco, CA) and cut using a computer controlled CO₂ laser. Acrylic pieces on the loom are either 0.236 or 0.118 inches thick.

Various nuts, bolts, rods, and tubing hold together each of the loom’s sections. The rods and tubes present on the loom are made of aluminum, brass, or stainless steel because of the materials’ high strength and excellent corrosion resistance. A wide variety of steel screws and
nuts bolt the auto-loom subsystems to the aluminum frame. Threaded bolts were chosen over permanent adhesives in the vast majority of sections to allow for the loom to be easily modified or upgraded in the future.

Figure 2.5. Auto-loom bird’s eye view. Important parts are labeled.

Table 2.2. List of major auto-loom components and their manufacturers.

<table>
<thead>
<tr>
<th>Part</th>
<th>Manufacturer &amp; Supplier (if different)</th>
<th>Model Name (code)</th>
<th>Auto-loom Subsystem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extruded aluminum</td>
<td>Bosch Rexroth Corp. (Buchanan, MI)</td>
<td>Rexroth 45 series (8981004773)</td>
<td>Frame</td>
</tr>
<tr>
<td>framing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheet acrylic</td>
<td>McMaster-Carr (Santa Fe Springs, CA)</td>
<td>Clear, cast with dimensions 12x12x0.118 in or 12x12x0.236 in (8560K191 or 8560K221)</td>
<td>All systems</td>
</tr>
<tr>
<td>Acrylic Glue</td>
<td>IPS Corp. (Compton, CA) &amp; Ridout Plastics Company Inc. (San Diego, CA)</td>
<td>Weld-On #4 pint (IPS4-PT)</td>
<td>All systems</td>
</tr>
<tr>
<td>Item Description</td>
<td>Supplier/Manufacturer</td>
<td>Details</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Large RC servo motor</td>
<td>GWS Tech Co. (City of Industry, CA) &amp; Pololu Corporation (Las Vegas, NV)</td>
<td>S04 BBM large servo (#517)</td>
<td></td>
</tr>
<tr>
<td>Heddle</td>
<td>Norwood Looms (Northampton, MA) &amp; Earth Guild (Asheville, NC)</td>
<td>10 inch, flat, stainless steel (weNHFL)</td>
<td></td>
</tr>
<tr>
<td>Cable</td>
<td>Pure Fishing, Inc. (Spirit Lake, IA)</td>
<td>Stren® Original Lo-Vis Green 8 lb. test monofilament (SNMK-00080)</td>
<td></td>
</tr>
<tr>
<td>Reed</td>
<td>Schacht Spindle Co. Inc. (Boulder, CO) &amp; Earth Guild (Asheville, NC)</td>
<td>27 inch stainless steel reed with 12 dents per inch (ws2712)</td>
<td></td>
</tr>
<tr>
<td>Ball Bearing Slide</td>
<td>Hickory Hardware (Grandville, MI) &amp; Lowe’s Home Improvement store (North Wilkesboro, NC)</td>
<td>P1050/16-2C 16 in steel drawer slide with 35 lb capacity (#89226)</td>
<td></td>
</tr>
<tr>
<td>Endoscopic micro-dissecting forceps</td>
<td>SOLOS Endoscopy (Boston, MA)</td>
<td>Model GS1100</td>
<td></td>
</tr>
<tr>
<td>Small RC servo motor</td>
<td>GWS Tech Co. (City of Industry, CA) &amp; Pololu Corporation (Las Vegas, NV)</td>
<td>Park L mini servo (#505)</td>
<td></td>
</tr>
<tr>
<td>Polyurethane strip</td>
<td>McMaster-Carr (Santa Fe Springs, CA)</td>
<td>2x36x¼ in Ultra-Soft, smooth finish (3462K16)</td>
<td></td>
</tr>
<tr>
<td>Box wrench</td>
<td>Ace Hardware Corp. (Oak Brook, IL)</td>
<td>½ in x 9/16 in Ace Ratcheting Box wrench (20137)</td>
<td></td>
</tr>
</tbody>
</table>

### Aluminum Frame

The frame of the loom is comprised of Bosch Rexroth 45 series extruded aluminum framing (Bosch Rexroth Corp., Buchanan, MI). The tubing has a profile in the shape of an ‘X’ with channels to allow the fixture of adjacent aluminum tubing or peripheral devices. The tubing has a height and width of 45 mm (1.772 in) and 10 mm T-slots. Pieces were cut to size using a hand saw with metal cutting blade. Cross section and profile views of the aluminum framing can be seen in Figure 2.6.
Figure 2.6. Extruded aluminum framing. (left) Cross section. (right) Side view. The recessed grooves allow acrylic pieces to be attached using modified carriage bolts.

Harness System

The harness system controls the vertical position of the heddle filled harnesses on the auto-loom. Each harness can exist in either an “up” or “down” state, and the combination of the states of all harnesses creates a shed. The harness system is composed of four sets of heddles and supporting frames, four servo motors, a set of acrylic pulleys on top of each tower, and polymer lines with 300 gram counterweights to connect each servo to a heddle frame. The harnesses operate as cable drive systems, with each harness’s vertical position controlled by a corresponding large RC servo motor (GWS Tech Co., City of Industry, CA). Each harness can hold up to 100 heddles, giving the loom a maximum warp thread capacity of 400 threads. Heddles on the loom are 10 inches long, flat, and made of stainless steel (Norwood Looms, Northampton, MA). A close-up view of some of the auto-loom’s heddles can be seen in Figure 2.7. The harnesses are comprised of two acrylic side pieces bound together by three aluminum slats. Two of the three aluminum slats on each harness are removable to allow heddles to be easily added and removed.
Figure 2.7. Heddles. Close-up view of eyes of steel heddles used on the auto-loom with inserted silk warp threads.

Although the harness system supports up to four harnesses, images in this text display only two active harnesses on the loom. Each harness is limited to vertical motion by u-channel aluminum rails attached to either side of the auto-loom frame. The heavy duty RC servo motors are housed on top of each of the loom’s towers and fixed with circular acrylic wheels wrapped with polymer cable (Pure Fishing Inc., Spirit Lake, IA). Cables are wrapped three times around each acrylic wheel to increase friction and prevent slippage. Mechanical power from the servo is transferred through the cables and converted into vertical harness motion by pulleys. The harness system can be seen in Figure 2.8.
Figure 2.8. Harness system. (left) Two harnesses with heddles held in place by u-channel wells on each side. High strength fishing line can be seen attached to each harness to control vertical movement. (right) Harness servos and acrylic pulley mechanism attached to the top of the loom.

Beater System

The purpose of the beater system is to push weft threads into position in the woven mesh. The beater system is composed of the reed, its frame, and the attached timing belt. The device that contacts the weft threads is the reed (Schacht Spindle Co. Inc., Boulder, CO). The reed used on the auto-loom is 12 inches long and made of stainless steel. Its steel slats are bound together with epoxy and protected on the top and bottom by a soft plastic coating. The reed was cut from a larger 27 inch reed produced by Schacht using a reciprocating saw. Each dent (space) on the reed is roughly 1/18 in wide and each slat is roughly 1/36 in. This gives the reed 12 dents per inch. The reed is pictured in Figure 2.9.
Figure 2.9. Reed. Front-side view of threaded reed. The two acrylic clamps used to lock the reed in place can be seen in the foreground and background of the picture.

The reed is controlled using a belt drive system. The timing belt used in the system was originally part of a Hewlett-Packard Deskjet printer and is 30 inches long. The belt is driven by a heavy duty servo motor connected to an acrylic gear. Teeth in the belt lock with those in the gear to create a belt drive. This converts rotational movement of the servo arm into linear motion of the reed. The belt is connected to a frame (beater) that rides on a set of ball bearing linear slides (Hickory Hardware, Grandville, MI). The range of motion of the reed is defined by small acrylic pulleys fastened along the side of the loom’s aluminum frame. These pulleys are pictured in Figure 2.10 and are used to stretch the drive belt taut. The reed is locked into its frame by screws connected to hex nuts embedded in the acrylic. The drive gear has a 5 inch diameter and the pulley wheels have 1 inch diameters.
Figure 2.1. Beater system. Side view of the auto-loom showing the beater system. The beater system moves the reed along the length of the warp strings, pushing weft strings into place on the fabric beam side of the loom. Precision movement of the reed is crucial to creating equally spaced weft threads.

Rapier System

The auto-loom uses a single rapier system to insert weft threads into open sheds. A rapier system was chosen for its simple architecture and perceived ease of automation. The steel rapier is attached to an acrylic tower that houses a heavy duty servo motor. The rapier’s gripper is opened and closed by a small RC servo motor (GWS Tech Co., City of Industry, CA). The servo is attached to the back end of the steel rapier and controls the grasper by pushing and pulling a shaft, made from a small diameter steel tube, connected to the opening mechanism of the grasper. The shaft is encased in a large diameter steel tube.
The rapier is made from a modified version of an endoscopic instrument manufactured by SOLOS Endoscopy. The instrument is a micro-dissecting forceps with fenestrated jaw (SOLOS Endoscopy, Boston, MA). It is tipped by 5 mm grooved graspers with double-action gripping motion. The handle of the instrument was removed, and the insulated shaft sheathed in a steel tube. A small piece of red foam inserted between the grasper and steel tube holds the forceps in place. The rapier maintains a strong connection to the rest of the rapier system through contact with a thin sheet of ¼ in ultra-soft polyurethane (McMaster-Carr, Santa Fe Springs, CA) wrapped around the drive wheel.

Figure 2.11. Rapier grasper. Close-up view of grasper end of the auto-loom rapier. The interlocking teeth of the grasper improves its ability to firmly hold weft threads.

The rapier tower is an 11x11 inch acrylic structure, located adjacent to the main loom body, which uses a series of external spur gears connected to a friction based rack and pinion system to drive the rapier. The diameters of the gears used to drive the rapier are 5, 2, and 4 inches, facilitating 0.0873 inches of linear movement per degree of servo rotation. A thin strip
of ultra-soft polyurethane around the acrylic wheel (pinion) increases friction between the rapier (rack) and wheel. The tower is designed to have adjustable parts so that modifications can be made during weaving to account for changes in shed size and location. The rapier is 38 inches long and has a 15 inch range of motion.

![Figure 2.12. Rapier tower and grasper actuator. (left) Rapier tower. The rapier tower uses a series of gears and wheels to move the rapier horizontally. (right) Grasper actuator clamped to the back end of the rapier. The servo motor’s arm is attached to an internal metal rod that opens and closes the grasper on the opposite end of the rapier.](image)

**Beam and Ratchet System**

The purpose of the beam and ratchet system is to hold warp threads taut and store completed fabric. This system is comprised of two acrylic and wood cylinders (beams) and four reversible box wrenches (Ace Hardware Corp., Oak Brook, IL). The auto-loom uses two sets of
two ratchet wrenches to permit beam motion in one rotational direction while restricting it in the other. The beams of the loom are made of half inch dowel rods bound on each end by acrylic plates. The beam and ratchet system is not automated and relies on the user to advance the warp strings. Beam motion can be reversed by changing the position of the pawl on each of the two fixed-position wrenches. The ratchets are connected to the beam by a threaded rod held in place by brass tubing inserted into the loom’s aluminum frame. All connections are held in place solely by the compression friction of hex and lock nuts.

Figure 2.13. Beam and ratchet system. (left) Auto-loom warp beam. The aluminum rods to the left of the beam hold the warp threads at a fixed height even with the relaxed heddle eye level. (right) Close-up view of the ratchet mechanism.

Computer and Control System

The computer and control system operates the auto-loom’s servo motors and controls each of the looms subsystems. The control system is comprised of a laptop computer with installed MATLAB software (Mathworks Inc, Natick, MA) and available USB port, the Pololu RC servo controller board, and the wires used to power and send instructions to each of the servos.
The board’s dimensions are 1 inch by 1.9 inches, and it can support up to 16 servo motors. The USB servo controller board is pictured in Figure 2.14. The board’s circuitry is powered by the USB computer connection (silver connector), while the servos are powered by a dedicated 6V power adapter connected to a 120V wall outlet (blue connector). The servo controller interfaces with the computer using a mini-B-to-A USB cable. To the computer, the servo controller appears as a standard RS-232 serial controller, i.e. COM port. The electrical wire used to connect the controller board to the servo motors is three conductor, sheathed, 24 AWG wire with 2.54 mm Molex housings and terminals (Molex Electronics Ltd., Lisle, IL).

![USB servo controller board.](image)

Figure 2.14. USB servo controller board.

Loom movement is facilitated by up to seven RC servo motors. The number of active servos varies depending on the number of harnesses in use, with one servo used per harness. The auto-loom in its current configuration can weave with a minimum of two harnesses and can support up to four. The other RC servos on the loom are a part of the beater and rapier systems. The two types of servos used on the loom are heavy duty S04 BB and mini Park L RC servos. The servos are controlled through a Pololu USB 16-Servo Controller.
Figure 2.15. Servo motor. Two harness servo motors in their acrylic housings.

Auto-Loom Control Software

The auto-loom is controlled by a MATLAB script running on a computer connected to the loom through a microcontroller board. The board relays the computer’s commands to each of the loom’s servo motors, causing the motors to rotate to the specified angle, providing precise control of the loom’s movements. The act of weaving can be broken down into four simple steps. These steps are (1) open shed, (2) insert thread, (3) close shed, and (4) pack thread. The MATLAB code is designed to convert these four simple steps into actions assigned to appropriate loom subsystems. These actions are then executed by the computer in the correct order to produce mesh.

Two important weaving actions are not controlled by the computer. The ratchet system and weft thread hand-off are handled by the user. The ratchet system requires the application of a significant amount of force to advance the warp strings. Servos with the power necessary to advance the warp strings were not available, and automated warp string advancement was not considered essential because the fabric only needs to be advanced once for every three inches of mesh woven. The hand-off of the weft string to the rapier and thread cutting is a
complicated process. It was decided to test the overall function of the rest of the loom before investing time in automating weft pick-up.

**Figure 2.16.** Manual loom systems. (left) Side view of a beam advancement ratchet. (right) Bobbin used to store the weft thread. The bobbin is located on the opposite side of the loom from the rapier tower.

**Function Hierarchy**

In MATLAB each function corresponds to an m-file, i.e. a file with “.m” extension. For the loom control code the functions are divided into three nested groups containing loom, servo, and pololu functions. The top tier files have the designation “loom” in their names and control loom initialization and shutdown and maintain user defined variables. Nested within the loom tier are the servo control functions, which have the designation “servo” in their names. Servo functions control servo initialization, power status, and movement. The lowest tier contains Pololu functions, which facilitate communication between the controller board and the computer and alter servo parameters such as speed and status. These functions have a variety
of titles including “pololu”, “set”, and “open.” The loom is controlled by calling higher order
loom and servo functions from the main code, which in turn call Pololu functions to make
changes to the loom’s operational status.

Figure 2.17. Loom file structure. The hierarchical file structure means each tier of files,
corresponding to MATLAB functions, is independent of the tiers above it. This allows loom code
to evolve separately from servo code, without fear of compromising loom function.

The auto-loom main code relies on five functions to operate: (1) the load command,
(2) loomInitialize, (3) loomWeave, (4) servoMove, and (5) loomShutdown. The
weaving process begins with the load command to upload variables and settings stored in a data
file, which is described in the next section. The `loomInitialize` function then opens communication to the controller board, activates the servo motors, and sets the loom to its starting position. The weaving process is handled by the `loomWeave` function, which is comprised of an ordered series of `servoMove` commands that communicate with servo motors. The first and last points of the reed’s position array and a vector containing the movement pattern of the harnesses are passed to the `loomWeave` function. After weaving is complete, a `servoMove` command in the main code is used to place the rapier in its storage position. Finally, the `loomShutdown` function turns off the servo motors and severs communication between the computer and controller board.

![Loom main code diagram](image)

**Figure 2.18.** Loom main code diagram. The five functions used in the main code are shown in their call order with passed variables.

*Settings and Variables*
Settings for the loom are stored in data files generated by a MATLAB script separate from the main loom code. Running the script loomData.m creates a .dat file containing all the variables the loom needs to operate. Variables are stored in external data files to prevent accidental overwriting of values by the user. The ability to load premade variable lists also simplifies changing the loom configuration.

Variables are stored in a scalar array structure called “LOOM”, which is subdivided into fields by loom system. The variables are assigned values using MATLAB’s definition by extension nomenclature to make it easier for programming novices to modify settings. Settings exist for each servo’s speed of rotation, position, and placement on the controller board. All servos are assigned two absolute position values. The posInit position refers to the starting position of the motor, and the posFinal position refers to the final or active position for the motor. In addition to initial and final positions, the servo assigned to the beater system (reed) has an extra array of position values corresponding to the packing distance between each thread. This allows the reed to position each thread a specified distance from adjoining weft strings. The posStart and posEnd variables in the main loom code specify from which index of the reed position array to begin and end weaving. The cardOrder variable in the main code is a vector that stores the repeatable pattern to be woven in terms of harness raises.

Auto-Loom Discussion

The auto-loom was built for the express purpose of producing mesh for in vitro cell culture experimentation. To this end, the mesh produced by the auto-loom is of sufficient quality to provide statistically relevant data in mesh characteristic studies. The details of such a study are presented chapter three of this manuscript. While mesh quality is sufficient for basic
in vitro research, refinements to the mesh production process are needed to raise mesh to in situ experimentation standards.

There is one major limitation of the auto-loom that negatively affects mesh quality. The problem is the tendency of mesh threads to drift during mesh removal from the loom and subsequent transport and storage. While this problem is not caused solely by the auto-loom, it is influenced by the design of the beam and ratchet system (Figure 2.13) and spacing bar (Figure 2.20), as well as mesh removal protocol. The underlying cause of thread drift is the open structure of gauze weave. Individual threads do not make enough perpendicular contact with other threads for friction to hold threads in place, and parallel threads are too far away to provide a physical barrier to movement. There are three areas related to the auto-loom weaving process that could reduce the occurrence of thread drift: (1) weave style, (2) mesh border control, and (3) use of mesh templates.

Weave style refers to the pattern of the constructed mesh. Instead of using a plain gauze weave, a more complicated open waffle or leno weave may provide more structural support, and decrease thread drift. Mesh border control refers to the how the outside warp threads are stabilized during weaving. The edges of woven fabrics tend to fray due to harness motion if not stabilized using one of more of the following techniques: textured beams, border rollers/stretchers, or false throw-away edges. Currently, the auto-loom does not employ any of these techniques to control fraying and prevent the cascading drift caused by the frayed threads. Mesh templates refers to the use of custom templates or bracing devices during the mesh removal and storage process to maintain pore dimensions.
The auto-loom can weave between five and ten picks per minute depending on user skill. This is not appreciably (if at all) faster than weaving on a hand loom, but the auto-loom has the potential to be able to weave up to thirty picks per minute with some modifications, discussed below. Most of these modifications involve eliminating user participation in the weaving process, thereby turning the auto-loom into a true power loom.

The auto-loom has two major drawbacks that negatively affect weaving speed. These problems are manual weft thread handoff and manual warp string advancement. The weft thread handoff is by far the most influential limiting factor of loom weaving speed. In the loom’s current form, the weaving script must be paused each time a weft thread needs to be inserted through the shed to allow time for the user to manually cut and place the thread into the jaws of the rapier’s grasper. This process is slow, labor intensive, and requires a significant amount of thread handling. Prolonged handling of degummed silk threads is not recommended because of the possibility of fraying. A single rapier was chosen for weft thread delivery for simplicity, but weaving time could be further reduced by adding an additional rapier to reduce
the distance needed to be traveled by each rapier to deliver each thread. A shuttle system
could possibly improve mesh quality by eliminating weft thread fraying, but this would require a
major redesign of the auto-loom.

Manual warp string advancement allows for fine control of warp string tension, but
limits the loom to weaving only small patches of material at a time. Adoption of a computer
controlled beam advancement system would allow the loom to weave long swaths of cloths,
limited in length only by the amount of material loaded onto the warp beam. The limiting factor
in creating such a system is the amount of force needed to mechanically tighten warp strings.
The amount of force needed is directly related to the number of warp strings used on the loom.
This necessitates the use of a high-torque motor for advancing more than a few warp strings.

A final, minor problem with the auto-loom is that the version of MATLAB software
currently used to operate the auto-loom, version R2006a, has a known serial port timeout bug
that causes the script to end prematurely. This can strand the loom in the middle of complex
weaves and damage servo motors. Dozens of weft threads had to be rewoven due to this
software error during the production of silk mesh for the in vitro experiments described in
chapter three of this manuscript. When the weave program terminates without calling the
loomShutdown function, servo motors are often left powered up and under load. This can
strip internal servo motor gears and overheat circuitry. This software glitch has been corrected
in newer versions of MATLAB. Aside from this correctable software bug, the auto-loom code
should be forward compatible, meaning MATLAB version updates will not adversely affect loom
performance or require additional programming corrections.

The auto-loom requires 28 inches of silk per warp thread to meet its minimum stringing
requirement. This does not include the length of thread needed to tie each warp string at each
end. It is recommended to calculate minimum thread amounts based on three feet of silk per warp string. The auto-loom as shown in Figures 2.1 and 2.5 is strung with 108 warp threads spaced 1 mm apart and tied in bundles of four. The warp threads are tied in bundles to reduce the chance of threads breaking when tightened due to uneven loading. Weft threads are typically about 12 inches long. The loom has an effective weaving area 9 inches wide and 4 inches long, meaning a 9x4 inch patch of mesh can be woven without advancing the beams. If precise pore sizes are desired, the spacing bar, shown in Figure 2.20, should be used to maintain constant separation between the warp threads.

![Figure 2.20. Spacing bar. Wide and close-up views of auto-loom spacing bar. The spacing teeth are laser cut into interchangeable strips of acrylic so that pore sizes can be easily adjusted.](image)

The minimum precision warp thread spacing currently available on the auto-loom is 1 mm. This is limited only by the ability to precisely laser cut acrylic teeth in the spacing bar strips. Residual heat from the cutting process causes the teeth to deform if smaller sizes are attempted. Finer spacing would be possible if the spacing bar were fabricated using other methods. Weft thread spacing is limited by the precision of the servo motor that controls the
reed. The minimum theoretical spacing between weft threads is 0.04 mm, but this spacing is not consistently attainable in practice due to variation in servo motion and backlash in the mechanical coupling used to drive the beater system. To prevent fraying, the three most outer warp threads are tightly packed together. This locks the weft thread into position, and reduces weft thread displacement caused by changing the shed configuration. There is no maximum distance between threads, but loose thread packing can allow weft threads to drift from their set positions.

**Conclusion**

The auto-loom is a semi-automated, tabletop weaving apparatus used to produce mesh utilized in cell culture studies for the purpose of optimizing implantable hernia patches. The auto-loom has successfully produced silk mesh with 1 mm and 2 mm thread spacing, and is capable of creating up to 16 unique shed patterns, which are used to produce advanced weave styles. The auto-loom’s ability to produce mesh with precisely controlled mesh dimensions makes it a valuable tool in the endeavor to understand and manipulate the biological reaction to implanted repair mesh. Future improvements to the auto-loom have the potential to drastically improve its weaving speed and reduce its overall size without compromising mesh quality.
References


III. *IN VITRO ANALYSIS AND EVALUATION OF AUTO-LOOM WOVEN SILK MESH FOR USE IN HERNIA REPAIR*

**Introduction**

The current method of hernia repair calls for the implantation of permanent, synthetic, polymer mesh at the defect site [1]. The purpose of this implanted mesh is to buttress the damaged area until new tissue growth can seal the herniated region. Two of the most widely used polymers for defect repair, polypropylene (PP) and expanded polytetrafluoroethylene (ePTFE), are non-resorbable, meaning they remain in place for the life of the repair [2]. While permanent polymer meshes provide excellent defect support and low rejection rates, hernioplasty patients are still plagued by hernia recurrences, mesh infections, and chronic pain [1, 3]. There is also growing concern over fibrous encapsulation of permanent implants and erosion of surrounding tissue [4, 5]. In an effort to prevent long-term fibrous tissue formation, meshes are now being developed that can be absorbed or degraded by the body over time. Without a permanent, stable construct to harbor bacteria and irritate surrounding tissues, the probability of development of complications such as persistent infection and chronic pain is decreased. However, absorbable meshes are not without problems of their own. Results following implantation of xenograft meshes, i.e. degradable meshes, are mixed, with approximately 40% of recipients suffering from complications ranging from hernia recurrence to fistula formation [6]. Of greatest concern with degradable or absorbable meshes is loss of strength prior to defect closure, which results in high rates of hernia recurrence [7].

There are many methods geared toward advancing the understanding of the biological response to the physiochemical properties of implanted materials: animal studies,
preclinical/clinical studies, retrieval studies, and *in vitro* studies [8]. There is an abundance of human and animal studies for hernia mesh evaluation, but comparatively far fewer *in vitro* studies. While it is recognized that cell culture experimentation can only crudely approximate *in vivo* conditions, *in vitro* research can serve as a valuable testing environment for investigating the biological response towards implantable materials or devices [8-11]. Furthermore, *in vitro* studies offer investigators a way to iteratively differentiate between the active and passive participants in the interfacial response of biological tissues to implants by investigating the morphology, biochemical reaction, and molecular genetics involved in the interaction [12]. An *in vitro* approach was chosen for this study specifically because of the low relative cost, reproducibility, and configurability afforded by cell cultures compared to other procedures [13].

Mesenchymal stem cells (MSCs) have been considered an ideal choice for many implantation and regenerative medicines studies [14]. However, they have not been used before in any known hernia mesh study. *In vitro* mesh studies conducted by other authors have used or suggested using cultures of endothelial cells, mesothelial cells, fibroblasts, or muscle tissue to investigate mesh characteristics [8, 9, 15, 16]. While each of these cell types is present in the abdominal wall and is, therefore, a logical choice for *in vitro* mesh experimentation, mesenchymal stem cells were chosen for this study because of their regenerative and therapeutic capacity, differentiation potential, availability, and relative ease of handling [14, 17-19]. Of great interest is the assertion of Caplan, who posits that MSCs are capable of inhibiting cell apoptosis, preventing scarring, and stimulating angiogenesis at injury sites [17]. Interest in the medical applications of stem cells has steadily increased in recent years, and it is hoped that by examining stem cell reaction to hernia repair mesh a unique perspective can be added to the body of hernia patch research [20].
While commercial companies focus on synthetic polymers for use in hernia repair, current research suggests that degradable silk patches may be a viable alternative to permanent mesh implantation [5]. Silk fibroin has been shown to have good biocompatibility, controllable rates of degradation in vivo, and mechanical properties analogous to other natural polymers [21-24]. Besides its potential as a material for hernia mesh construction, silk was chosen for this study because of its long time use as surgical suture and the well documented biological response to its implantation [25, 26]. Several studies have investigated the in vitro response of MSCs to silk scaffolds, with results showing significant proliferation and differentiation of stem cells after seeding [27, 28]. Furthermore, biological implants made from chemically modified silk fibroin show promise for their ability to spur regeneration of damaged tissue, an attractive prospect for the field of hernia repair [29].

Absorbable or degradable materials may provide a means to overcome the limitations of current hernia patches by affording a repair mesh with both excellent biocompatibility and the ability to strengthen the afflicted area during the defect healing process, yet absorb/degrade once the mended area is able to provide lasting support [1]. There are several properties related to mesh construction that can influence the overall success of the implant. These include characteristics such as material composition, pore configuration, surface architecture, and filament structure [11, 30-33]. By understanding and ultimately manipulating these mesh properties, it is hoped that more successful hernia repair meshes can be developed. Unfortunately, few studies have investigated individual mesh characteristics, with most preferring to focus on overall repair outcomes [34]. The overarching goal of this study is to begin to fill this void in mesh property research by providing scientific background for choices in mesh design.
In order to investigate the effect mesh characteristics has on cellular response, an in vitro evaluation procedure was developed, in conjunction with a semi-automated loom created for the express purpose of producing woven meshes acceptable for mesh property assessment studies. A feasibility study was conducted to demonstrate the mesh production capabilities of the newly designed auto-loom, and to test a new method of evaluating mesh characteristics in vitro. This exploratory work measured the proliferation, distribution, and viability of murine D1 mesenchymal stem cells on silk mesh samples of varying pore size.

Materials and Methods

This study was broken into two phases. Part A of the study focused on refining and improving the method of evaluating silk meshes in vitro. This work investigated the cellular response to adhesives to be used in the creation of miniature silk patches. Silk degumming and cell seeding procedures were also refined during Part A of the study. Part B of the study used the in vitro mesh characterization method developed in Part A to evaluate the proliferation, distribution, and viability of D1 mouse mesenchymal stem cells seeded onto silk patches.

Part A: Adhesive Selection and Method Refinement Experiments

Adhesion Selection Experiment - Cell Culture

D1 murine mesenchymal stem cells (ATCC, Manassas, VA) were cultured according to the manufacturer’s suggested protocol. Cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM). Every 500 ml of DMEM medium was supplemented with 50 ml fetal bovine serum (FBS), 5 ml antibiotic/antimycotic, and 1 ml fungizone. The culture medium was replaced every 24-72 hours as required, and cells were stored in a humidified incubator maintained at
37°C and 5% CO₂. Cells were grown to confluence in two 500 ml flasks and cultured from passage 27 to passage 29.

Adhesive Selection Experiment - Application of Adhesives

Cyanoacrylate glue (Hobby Lobby Stores, Inc., Oklahoma City, OK), Elmer’s Glue-All (Elmer’s Products, Inc., Columbus, OH), Liquid Stitch (Blair Adhesives, Santa Fe Springs, CA), Acid-Free glue (Helmar, New South Wales, Australia), Ad-Tech 2030 Hot Melt glue (Adhesive Technologies, Inc., Hampton, NH), and Polyfin® paraffin wax (Polysciences, Inc., Warrington, PA) were applied to the bottom, outer half of four Corning® Costar® 6 well culture plates (Sigma-Aldrich Co, St. Louis, MO). A description of each adhesive is provided in Table 3.1. Three wells were used for each adhesive. The well diameter for the 6-well plate was 11/8 in. Adhesives were applied as evenly as possible by hand to the outer half of the well bottom as shown in Figure 3.1. Adhesives were allowed to dry/set for 12 hours before being seeded with cells.

![Figure 3.1. Adhesive distribution in 6-well plate. Gray areas represent well areas covered by adhesive. White areas are free of adhesive.](image)

Table 3.1. Summary of adhesives used in adhesive selection experiment.
<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Composition</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanoacrylate Glue</td>
<td>Ethyl or methyl cyanoacrylate polymer (MCA or ECA)</td>
<td>Clear, watery liquid</td>
</tr>
<tr>
<td>Elmer’s Glue-All®</td>
<td>Polyvinyl acetate (PVAc) based</td>
<td>Milky white liquid</td>
</tr>
<tr>
<td>Liquid Stitch</td>
<td>Ethylene vinyl acetate (EVA) copolymer based with trace amounts of ethylene, vinyl acetate, polyesters, and water</td>
<td>Milky white liquid</td>
</tr>
<tr>
<td>Acid-Free glue</td>
<td>Polyvinyl acetate (PVAc) resin emulsion with Phthalate plasticizer and trace amounts of vinyl acetate monomer and water</td>
<td>White, medium viscosity liquid</td>
</tr>
<tr>
<td>Ad-Tech 2030 Hot Melt glue</td>
<td>Ethylene vinyl acetate (EVA) copolymer based with unknown copolymer additives</td>
<td>Colorless, translucent solid</td>
</tr>
<tr>
<td>Polyfin® paraffin wax</td>
<td>Hydrotreated, petroleum-based, paraffin waxes with alpha-Methyl styrene/vinyl toluene copolymer</td>
<td>Opaque, waxy pellets with flat side</td>
</tr>
</tbody>
</table>

**Adhesive Selection Experiment - Seeding of Adhesive Wells**

Plates were sterilized with ethylene oxide (Anprolene; Anderson Products, Haw River, NC) and degassed for 24 hours under house vacuum. Well plates were seeded with $5.0 \times 10^4$ mouse D1 mesenchymal stem cells suspended in 2 ml of culture media. Immediately following seeding, well plates were placed on an orbital shaker for 10 seconds at 60 rpm to evenly distribute cells, then placed in a 37°C, 5% CO$_2$ incubator for the duration of the experiment. Medium was changed every three days. A total of 21 wells were used in the study, with three replicates per adhesive (total six adhesives) plus three wells for adhesive-free controls.
Adhesive Selection Experiment – Cellular Response

Adhesive samples were evaluated for cellular response at Days 6, 7, and 11. Cellular activity was evaluated by measuring cellular metabolism, viability/cytotoxicity, and cell number.

Cellular metabolism was evaluated on Day 6 using alamarBlue® mix-and read solution (DAL1025; BioSource International Inc., Camarillo, CA). A medium change was performed prior to the addition of the alamarBlue® solution. A volume of 200 μl (100 μl per 1 ml medium) of solution was added to two wells of each adhesive and control. The samples were then returned to the incubator and stored for a period of 6 hours. Medium volumes of 200 μL were then aliquoted from each well into a 96 well, black, clear bottom plate and the absorbance read at both 570 nm and 630 nm on a 96-well MRX microplate reader with Revelation software (Dynex Technologies, Chantilly, VA). Since this assay was not performed as an end point procedure, the adhesive test samples were returned to the incubator following a rinse with Dulbecco’s Phosphate Buffered Saline (PBS) (Mediatech, VA) and a medium change.

A CytoTox 96 Non-Radioactive Cytotoxicity Assay (G1780; Promega, Madison, WI) was used to determine the relative number of viable cells in each sample group by measuring the amount of lactate dehydrogenase (LDH) released from lysed cells. This test was performed as an end point assay at Day 6 on one well from each adhesive group. Cells were lysed by adding 300 μl of supplied Lysis Solution (10X) to each sample well. Samples were incubated for 60 minutes at room temperature to allow time for LDH to be released from the lysed cells. A 50 μl sample was then taken from each well and pipetted into a fresh, clear 96-well plate. A volume of 50 μl of reconstituted Substrate Mix was then added to each supernatant sample. The sample plate was covered to prevent light exposure and allowed to incubate at room
temperature for 30 minutes. A volume of 50 μl of Stop Solution was then added to each well, and the absorbance read at 490 nm on a 96-well MRX microplate reader.

A LIVE/DEAD® Viability/Cytotoxicity Kit (L-3234; Molecular Probes, Eugene, OR) was used to identify viable cells. The assay was performed on control, Hot Melt glue, and paraffin samples at Day 7 as an end-point assay. The LIVE/DEAD® reagent was prepared by combining PBS with the chemicals supplied by the LIVE/DEAD® kit in accordance with assay protocol. A volume of 2 ml of LIVE/DEAD® reagent was added to each sample well. The well-plates were covered in aluminum foil to prevent light exposure, placed on an orbital shaker set at 60 RPM for 30 seconds, then allowed to rest in a stationary position for 45 minutes at room temperature. The wells with adhesives were photographed using a Zeiss Axiovert 40 CFL microscope (Carl Zeiss AG; Oberkochen, Germany) equipped with a 50 W Xenon lamp. The images were captured using an AxioCam MRC 5, processed with Zeiss AxioVision LE 4.6, and combined and post-processed using the open-source GNU Image Manipulation Program (GIMP). Images were captured from the center of wells and at adhesive edges. Images of live cells (green) were rotated and aligned overtop their respective dead cell images (red) using screen mode. Brightness and contrast of the combined images was adjusted, and an unsharp mask filter set at radius 5, amount 0.50, and threshold 0 was applied for visual clarity.
Figure 3.2. Adhesive selection experiment plate layout. C = Control, HG = Hot Melt glue, P = Paraffin, CA = Cyanoacrylate, ELM = Elmer’s Glue-All®, LS = Liquid Stitch, and AF = Acid Free glue. The gray areas in used wells approximate cell coverage area. Time points (in days) are displayed on the left side of the figure, along with the assays performed at each time point.

Method Refinement Experiment - Mesh Characterization

The mesh used in the study was commercially available nylon fabric used in costume design purchased from a local Walmart store (Anderson, SC) (Wal-Mart Stores, Inc.; Bentonville, Arkansas). Two fabrics of different pore size were chosen for their resemblance to the silk meshes to be used in later studies. The meshes were made from knit multifilament nylon. The
small pore mesh had a pore diameter of roughly 1.5 mm and the large pore mesh had a pore diameter of roughly 3.5 mm.

Figure 3.3. Nylon knit mesh. Small pore mesh (left) has an average pore diameter of 1.5 mm. Large pore mesh (right) has an average pore diameter of 3.5 mm. One pore in each photo is labeled with a red arrow. The small pore mesh has tetragonal pores, and the large pore mesh has hexagonal pores. The difference in pore shape is caused by different knitting styles.

Method Refinement Experiment - Adhesive Characterization

From the previously conducted Adhesive Selection Experiment (described above) it was determined that hot-melt glue was the best choice of adhesive for future mesh studies because it was structurally stable in an aqueous environment, non-toxic to D1 mouse stem cells, and easy to prepare and apply. In this Method Refinement Experiment, the ease of application and cellular response to two types of hot-melt glue were compared. The first adhesive used was Ad-Tech 962 Hot Melt glue (Adhesive Technologies, Inc., Hampton, NH), which was yellow in color and advertised as having a high melting temperature (98°C). The second adhesive investigated was Ad-Tech 2030 Hot Melt glue (Adhesive Technologies, Inc., Hampton, NH), which was clear in
color and advertised as having a low melting temperature (92°C). Both fixatives were marketed as Ace® brand adhesives (Ace Hardware Corp.; Oak Brook, IL), and were purchased at a local Ace Hardware Store (Clemson, SC).

**Method Refinement Experiment - Sample Production**

Nylon mesh patches were produced by applying circular patterns of hot-melt glue to the mesh sheets. Nylon mesh sheets were placed over glass microscope slides during the glue application process. The glue/mesh pieces were removed from the glass slides using a single-edged straight razorblade. Glue bordered mesh patches were then cut using scissors from the surrounding fabric to produce fray-resistant, circular test patches roughly 0.75 inches in diameter.

**Method Refinement Experiment - Cell Culture**

D1 murine mesenchymal stem cells (ATCC, Manassas, VA) were cultured according to the manufacturer’s suggested protocol. Cells were maintained in DMEM solution. Every 500 ml of DMEM medium was supplemented with 50 ml FBS, 5 ml antibiotic/antimycotic, and 1 ml fungizone. The culture medium was replaced every 24-72 hours as required, and cells were stored in a humidified incubator maintained at 37°C and 5% CO₂. Cells were grown to confluence in two 500 mL flasks and cultured from passage 27 to passage 29.

**Method Refinement Experiment - Seeding of Mesh Patches**

Two 12-well plates were coated with 1 ml of Sigmacote® (Sigma-Aldrich; St. Louis, MO) to prevent cell attachment to the plate itself, and maintained for 5 minutes at room
temperature. The Sigmacote® was then aspirated and the wells rinsed with sterile PBS. Using autoclaved tweezers, the mesh test patches were inserted into the wells. A volume of 1 ml of cell-rich media was then added to each well, with either $5 \times 10^4$, $1 \times 10^5$, $5 \times 10^5$, or $1 \times 10^6$ cells (Figure 3.4). Well plates were placed on orbital shaker plates set at 60 RPM in a 37°C, 5% CO$_2$ incubator for 24 hours. An additional 1 ml of cell-free media was then added to each well, and the plates returned to a stationary position in the incubator for the duration of the experiment.

**Method Refinement Experiment - Cellular Response**

Cellular metabolism was evaluated on Day 7 using alamarBlue® mix-and read solution. A medium change was performed prior to the addition of the alamarBlue® solution. A volume of 200 μl (100 μl per 1 ml medium) of solution was added to each test sample. The samples were then returned to the incubator and stored for a period of 6 hours. Individual volumes of 200 μL test samples of media were then aliquoted into corresponding wells of a 96 well, black, clear bottom plate and the absorbance read at both 570 nm and 630 nm on a 96-well MRX microplate reader with Revelation software. This procedure was performed as an end point assay.

A LIVE/DEAD® Viability/Cytotoxicity Kit was used to identify viable cells. The assay was performed at Day 7 as an end point assay. The LIVE/DEAD® reagent was prepared by combining PBS with the chemicals supplied by the LIVE/DEAD® kit in accordance with assay protocol. A volume of 2 ml of LIVE/DEAD® reagent was added to each sample well. The well plates were covered in aluminum foil to prevent light exposure, placed on an orbital shaker set at 60 RPM for 30 seconds, then allowed to rest in a stationary position for 45 minutes at room temperature. The adhesives were photographed using a Zeiss Axiovert 40 CFL microscope.
equipped with a 50 W Xenon lamp. The images were captured using an AxioCam MRC 5, processed with Zeiss AxioVision LE 4.6, and combined and post-processed using GIMP. Images of live cells (green) were rotated and aligned overtop their respective dead cell images (red) using screen mode. Brightness and contrast of the combined images was adjusted, and an unsharp mask filter set at radius 5, amount 0.50, and threshold 0 was applied for visual clarity.

Figure 3.4. Method refinement experiment plate layout. Samples were evaluated at only one time point (Day 7). The glue model number assigned to each well is preceded by “AT” for Ad-Tech, the manufacturer of the hot-melt glue. The number of cells seeded is reported in scientific notation. Control groups (“Ctrl”) contained meshless glue rings, and were seeded with \(5 \times 10^4\) cells.

Part B: Silk Mesh Experiment

Silk Characterization

Raw, untreated silk was obtained from Poly-Med, Inc (Anderson, SC). The sericin-coated silk thread was 8-ply with 50 twists per yard. The length weight of the thread was 22 deniers
per foot (dpf). Silk fibers were photographed using a Zeiss Axiovert 40 CFL microscope equipped with a 50 W Xenon lamp. The images were captured using an AxioCam MRC 5, processed with Zeiss AxioVision LE 4.6, and post-processed using GIMP. A tensile strength test was performed on 6 inch samples of both raw and degummed silk on an Instron® 4500 series testing frame (Instron; Norwood, MA) using Instron® Series IX Materials Testing Software. Data was sampled at 10 hertz, and the silk extended at a crosshead speed of 25 mm/min.

Silk Degumming

The raw silk was degummed using a process similar to one described by Freddi, Mossotti, and Innocenti [35]. Silk was degummed in batches ranging from 35 to 90 yards. For each batch of silk to be degummed, silk thread was spooled in a single layer around a 2.75 in diameter bobbin made from acrylic end pieces supported by 0.25 in brass rods (Figure 3.5). A 500 ml glass beaker containing a degumming solution of 5.0 g Savon de Marseille soap (Touch of Europe; Marietta, GA) and 0.50 g sodium carbonate (Arm & Hammer Super Washing Soda; Church & Dwight Co., Inc., Princeton, NJ) dissolved in 500 mL deionized (DI) water was heated on a hot plate to a solution temperature of 98°C. A 500 RPM magnetic stirrer was placed on the bottom of the beaker to agitate the solution. The fully loaded silk bobbin was inserted into the beaker, and positioned above the still rotating stir bar. Lastly, a thermometer was inserted into the beaker to monitor solution temperature during the degumming process. The beaker was then placed in a water bath-style sonicator filled with room temperature DI water for a 2 minute interval, then transferred back to the hot-plate until the temperature of the degumming solution returned to 98°C, a process that took roughly 5 minutes. The beaker was alternated between the sonicator and hot plate in this manner for a period of 90 minutes. At the end of
the degumming procedure, the bobbin was removed from the degumming solution, rinsed with DI water, placed in a clean, dry 500 ml beaker covered with a Kimwipe® (Kimberly-Clark Corp.; Dallas, TX) to protect against dust while allowing air flow, and dried for 24 hours at room temperature. At no point were weighting agents introduced to the silk thread to replace the removed sericin, meaning the degummed silk produced was pure silk.

![Degumming bobbin](image)

**Figure 3.5.** Degumming bobbin. Dried degummed silk thread is shown wrapped around a partially-filled degumming bobbin placed inside a 500 ml beaker.

**Mesh Production**

Silk mesh was produced using the auto-loom described in Chapter Two of this thesis. The auto-loom was strung with 108, 7 foot warp threads of degummed silk, and the weft thread holder loaded with a bobbin of degummed silk. Mesh was woven using a semi-automated process controlled by a computer connected to the auto-loom. Weft thread bobbins were replaced as needed. One 8.5x2 inch sheet of 2 mm pore mesh and two 4.5x2 inch sheets of 1mm pore mesh were woven. During production, Ad-Tech 972 Hot Melt glue was applied to the edges of the mesh sheets to prevent fraying. From these mesh sheets, twenty-four 1-mm and
twenty-four 2-mm test patches were produced by applying circular patterns of Ad-Tech 972 hot-melt glue to the mesh. Glue bordered test patches were then cut from the sheets using scissors to create fray-proof, circular test patches roughly 0.75 inches in diameter. From these 48 test samples, the 36 most uniform and high quality patches were selected to be used in the subsequent experiment. The selected test patches were rinsed in ACS grade petroleum ether (S93320; Thermo Fischer Scientific Inc., Waltham, MA) to remove residual fatty acids left over from the degumming process, and then rinsed in microfilter purified DI water to remove any other contaminants. The patches were placed in 12-well plates, covered by Kimwipes®, and dried for 24 hours at room temperature before being sterilized by gaseous ethylene oxide, as previously described, and degassed under house vacuum for 24 hours. Experiment-ready silk mesh test patches are displayed in Figure 3.6.

![Figure 3.6. Silk mesh test patches. Graphics at top are concept diagrams of test patches with black grid lines representing test material and gray areas representing edge-sealing adhesive.](image)
Photographs at bottom show silk mesh test patches with white, degummed silk threads bordered by yellow Ad-Tech 972 Hot Melt glue. Mesh on left is 1-mm pore size test patch. Mesh on right is 2-mm pore size test patch.

Silk Mesh Experiment Cell Culture

D1 murine mesenchymal stem cells (ATCC, Manassas, VA) were cultured according to the manufacturer’s suggested protocol. Cells were maintained in DMEM solution. Every 500 ml of DMEM medium was supplemented with 50 ml FBS, 5 ml antibiotic/antimycotic, and 1 ml fungizone. The culture medium was replaced every 24-72 hours as required, and cells were incubated at 37°C and 5% CO₂. Cells were grown to confluence in six 500 ml flasks and cultured from passage four to passage eleven. The high number of passages was necessary due to delays in mesh test patch production.

Seeding Mesh Patches

Six 12-well plates were coated with 1 ml of Sigmacote® to prevent cell attachment to the plate itself, and incubated for 5 minutes at room temperature. The Sigmacote® was then aspirated and the wells rinsed with sterile PBS. Using autoclaved tweezers, mesh test patches were inserted into wells, as previously described. A volume of 1 ml of medium containing 5x10⁵ cells was then added to each well, along with 1 mL of cell-free medium. Well plates were placed on orbital shaker plates set at 60 RPM in a 37°C, 5% CO₂ incubator for 24 hours. An additional 1 ml of cell-free medium was then added to each well, and the plates returned to a stationary position in the incubator for the duration of the experiment. Two-dimensional control sample
wells were not treated with Sigmacote®, but were otherwise treated in the same manner, without mesh test patches.

Cellular Response

Silk mesh samples were evaluated for cellular response at 7, 14, and 21 days. Cellular activity was evaluated by measuring cellular metabolism, viability/cytotoxicity, and cell number. A LIVE/DEAD® Viability/Cytotoxicity Kit was used to identify viable cells and assess cell morphology. The assay was performed as a non-end-point assay at Days 7, 14, and 21. The LIVE/DEAD® reagent was prepared by combining PBS with the chemicals supplied by the LIVE/DEAD® kit in accordance with the manufacturer’s protocol. A volume of 2 ml of LIVE/DEAD® reagent was added to each sample well. The well plates were covered in aluminum foil to prevent light exposure, placed on an orbital shaker set at 60 RPM for 30 seconds, then maintained statically for 45 minutes at room temperature. The samples were photographed using a Zeiss Axiovert 40 CFL microscope equipped with a 50 W Xenon lamp. The images were captured using an AxioCam MRC 5, processed with Zeiss AxioVision LE 4.6, and combined and post-processed using GIMP. Images of live cells (green) were rotated and aligned overtop their respective dead cell images (red) using screen mode. Brightness and contrast of the combined images was adjusted, and an unsharp mask filter set at radius 5, amount 0.50, and threshold 0 was applied for visual clarity. Images were captured with objective magnifications of 2.5x, 5x, and 10x. Since this assay was not end point, well plates were aspirated and rinsed three times with PBS, then filled with 3 ml of millipure water. The plate tops were taped closed and the cells lysed by performing three freeze/thaw cycles at -80°C and 37°C. The samples were then stored in a -80°C freezer until samples from all time points had been collected.
A CytoTox 96 Non-Radioactive Cytotoxicity Assay was used to determine the relative number of viable cells in each sample group by measuring the amount of lactate dehydrogenase (LDH) released from lysed cells. This test was performed as an end-point assay at Days 7, 14, and 21 on samples on which a LIVE/DEAD® Viability/Cytotoxicity assay had previously been performed. A 50 μl sample was taken from each sample well and pipetted into corresponding wells in a fresh, clear 96-well plate. A volume of 50 μl of reconstituted Substrate Mix was then added to each supernatant sample. The sample plate was covered to prevent light exposure and incubated at room temperature for 30 minutes. A volume of 50 μl of Stop Solution was then added to each well, and the absorbance read at 490 nm on a 96-well MRX microplate reader.

Cellular metabolism was evaluated on Days 7, 14, and 21 using alamarBlue® mix-and-read solution. A media change was performed prior to the addition of the alamarBlue® solution. A volume of 300 μl (100 μl per 1 ml medium) of solution was added to each test sample. The samples were then returned to the incubator and stored for a period of 6 hours. Volumes of 200 μL test samples of medium were then aliquoted into corresponding wells of a 96 well, black, clear bottom plate and the absorbance read at both 570 nm and 630 nm on a 96-well MRX microplate reader with Revelation software. Since this assay was not end point, well plates were aspirated and rinsed three times with PBS, then filled with 3 ml of millipure water. The plate tops were taped closed and the cells lysed by performing three freeze/thaw cycles at -80°C and 37°C. The samples were then stored in a -80°C freezer until samples from all time points had been collected.

The Pierce BCA Total Protein Assay Kit (23227; Thermo Fischer Scientific Inc., Waltham, MA) was used to measure protein concentration. This assay was end point and was conducted
on the same mesh patches and 2-D controls on which an alamarBlue® assay had previously been conducted. Roughly 12.5 ml of Working Reagent (WR) was prepared by mixing 12.5 ml of Reagent A with 250 μl of Reagent B. BCA standards were then prepared in a 24-well plate according to the manufacturer’s protocol, as described in Table 3.2. These standard solutions were used to derive protein concentrations from absorbance values by creating a standard curve.

Table 3.2. BCA Total Protein assay standards.

<table>
<thead>
<tr>
<th>Plate well</th>
<th>DI water volume</th>
<th>BSA volume &amp; source</th>
<th>Final conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 μL</td>
<td>300 μL stock</td>
<td>2000 μg/mL</td>
</tr>
<tr>
<td>B</td>
<td>125 μL</td>
<td>375 μL stock</td>
<td>1500 μg/mL</td>
</tr>
<tr>
<td>C</td>
<td>325 μL</td>
<td>325 μL stock</td>
<td>1000 μg/mL</td>
</tr>
<tr>
<td>D</td>
<td>175 μL</td>
<td>175 μL stock from B</td>
<td>750 μg/mL</td>
</tr>
<tr>
<td>E</td>
<td>325 μL</td>
<td>325 μL stock from C</td>
<td>500 μg/mL</td>
</tr>
<tr>
<td>F</td>
<td>325 μL</td>
<td>325 μL stock from E</td>
<td>250 μg/mL</td>
</tr>
<tr>
<td>G</td>
<td>325 μL</td>
<td>325 μL stock from F</td>
<td>125 μg/mL</td>
</tr>
<tr>
<td>H</td>
<td>400 μL</td>
<td>100 μL stock from G</td>
<td>25 μg/mL</td>
</tr>
<tr>
<td>I</td>
<td>400 μL</td>
<td>0 μL</td>
<td>0 μg/mL = Blank</td>
</tr>
</tbody>
</table>

A 25 μl supernatant sample from each test patch and standard well was pipetted into a corresponding well of a fresh, black 96-well plate. A volume of 200 μl of Working Reagent was then added to each in-use well of the 96-well plate. The plate was then covered in aluminum foil to prevent light exposure, placed on an orbital shaker set at 60 RPM for 30 seconds, then maintained statically for 30 minutes in a humidified 37°C, 5% CO₂ incubator. The plate was then cooled to room temperature for 10 minutes and the absorbance read at 592 nm on a 96-well MRX microplate reader.
**Figure 3.7.** Silk mesh experiment plate layout. Assays were conducted at Days 7, 14, and 21. The alamarBlue and BCA Total Protein Assays were performed on the same set of samples. LIVE/DEAD and CytoTox 96 Assays were performed on the remaining set of samples. Wells labeled as “1 mm” indicate a mesh patch with a pore diameter of 1 mm. Wells labeled as “2 mm” indicate a mesh patch with a pore diameter of 2 mm. Wells labeled as “2-D C” indicate a 2-dimensional control containing no mesh patch. Cells in the control wells were seeded on the bottom of the wells.

**Statistics**

Statistical analyses were performed using MATLAB (Mathworks Inc, Natick, MA) and Microsoft Excel (Microsoft Corp., Redmond, WA). Graphs and tables were produced using MATLAB and Microsoft Excel. Significant differences between means were determined using a two sample unpooled one-sided t test with a significance level ($\alpha$) of 0.05. All error bars on charts and graphs represent standard error of the mean.
Results

Part A: Adhesive Selection and Method Refinement

Photographs taken during the adhesive selection experiment are displayed in Figures 3.8, 3.9, and 3.10. Only Hot Melt glue and paraffin adhesives were able to support cellular proliferation. This result is showcased by Figure 3.8, which shows stem cells growing directly adjacent to both the hot-melt glue and paraffin adhesives. Figure 3.9 shows the results of a LIVE/DEAD assay performed on hot-melt glue and paraffin well plates. The vast majority of cells in the figure are alive (green) and spreading across the bottom of the well plates. Dead cells (red) are spherical and detached from the bottom of the well plate.

Figure 3.8. Hot Melt glue and paraffin well plates. Photographs of Hot Melt glue (left) and paraffin (right) wells taken at Day 3 of adhesive selection experiment. Photos were taken with a 10x objective magnification. The black areas in each photograph are adhesive deposits. In both pictures, cells are shown growing directly adjacent to the edge of each adhesive.
Figure 3.9. Representative LIVE/DEAD photographs of Hot Melt glue and paraffin wells. Left photo is Hot Melt glue Day 7. Right photo is paraffin a Day 7. Photos were taken at 10x objective magnification. Live cells are colored green and dead cells are colored red.

Digitally enhanced photographs of the adhesives determined to be inappropriate are displayed in Figure 3.10. All color in the photographs was added for clarity during post-processing using freeware image manipulation program GIMP.

The liquid stitch glue broke apart in the media into spongy clumps of gelatin-like particulate, and was toxic to cells, killing all seeded cells Day 3 of the study. The clumps can be seen in the top left photograph in Figure 3.10.

The vast majority of cells in the acid-free glue well plates died by Day 5, but roughly one dozen cells were able to remain alive to the Day 5 time point. One of these cells can be seen in the top right picture in figure 3.10. The acid-free glue slowly decomposed in the well medium, turning the medium opaque.

The cyanoacrylate glue was unaffected by the aqueous environment of the medium, but was highly toxic to cells. No living cells were present in cyanoacrylate well plates by Day 3. A
photo of the cyanoacrylate wells with dead cells can be seen in the bottom left corner of Figure 3.10.

The Elmer’s glue rapidly decomposed into a fine cloud of particulate, clouding the medium to the point of impairing visual inspection of the cells by Day 3. The bottom right picture in Figure 3.10 shows a view of the particulate cloud created by the adhesive.

**Figure 3.10.** Cell viability negatively impacted by adhesives Liquid Stitch (top left), Acid-Free Glue (top right), Cyanoacrylate (bottom left), and Elmer’s Glue (bottom right). All photographs were taken at 20x objective magnification on Day 5 of the adhesive selection experiment. Live cells are green. Dead cells are red. Adhesive particulate is colored yellow. Original images were taken in grayscale. All color was added for clarity during post-processing using freeware
image manipulation program GIMP. The state (live or dead) of each cell was determined by inspection and the appropriate color was added. Particulate was identified based on object shape and translucency.

The results of the adhesive selection experiment alamarBlue® assay are presented in Figure 3.11. The figure indicates that all adhesives, except the Elmer’s glue, supported cell metabolic activity within 10% of the control sample’s activity.

![Percentage difference between fixative and control samples](image)

**Figure 3.11.** Adhesive selection experiment alamarBlue assay results.

The results of the adhesive selection experiment CytoTox 96 assay are presented in Figure 3.12. The Hot Melt glue and paraffin adhesive samples had cell counts within 5% of the control samples. The other adhesives displayed significantly lower cell counts than the control.
samples. This result is consistent with the photographs of adhesive well plates presented above in Figures 3.8, 3.9, and 3.10.

Figure 3.12. Adhesive selection experiment CytoTox 96 assay results.

The results of the adhesion selection experiment BCA Total Protein assay are presented in Figure 3.13. The paraffin adhesive samples contained protein concentrations significantly lower than both the control and Hot Melt glue samples.
Figure 3.13. Adhesive selection experiment BCA Total Protein assay results.

A LIVE/DEAD photograph from day 7 of the method refinement experiment is presented in Figure 3.14. Few live cells were found on the nylon meshes. Those that were found were located mainly in crevices and at thread intersections.

Figure 3.14. Method refinement experiment LIVE/DEAD assay photograph. Knit nylon mesh pictured at 10x objective magnification. Only a few live cells (bright green areas) are present.

The method refinement alamarBlue assay results are presented in Figure 3.15. The Ad-tech 972/small pore mesh combination showed evidence of greater cellular activity in all groups. Differences in percent reduction of alamarBlue were not significant (p<0.05) for both the Ad-tech 972 and Ad-tech 2030 groups. The 5x10^5 Ad-tech 972 group had a significantly lower percent reduction of alamarBlue compared to the other Ad-tech 972 groups.
Part B: Silk Mesh Experiment

Silk fibers seen in Figure 3.16 show a near total removal of sericin from the underlying fibroin filaments. The rough, scaly sericin coating seen on fibers in the left picture is removed by the degumming process described in the methods and materials section, leaving only the smooth fibroin brins seen in the right picture. Observed from a distance under fluorescent lights, the sericin coated silk fibers are musty yellow and opaque, while the degummed filaments are bright white and reflect light from their relatively flat surfaces.
Figure 3.16. Silk fibers, including raw silk on left and degummed silk on the right. Both photos taken at 20x objective magnification. The sericin coating on the raw silk gives the fiber a rough appearance. The degummed silk is smooth and detached into individual brins.

The results of the silk tensile strength test are shown in Figure 3.17. Degummed silk exhibited on average a 28% increase in ultimate strength and a 26% decrease in yield strength compared to raw silk. Both thread types display mostly linear elastic and plastic regions of deformation in all trials, with only roughly 5% of each thread’s elastic region occurring below each respective thread’s true elastic limit. In the trial reported in Figure 3.17, the raw silk fails in stages, whereas the degummed silk fails nearly instantaneously. Similar failure patterns occurred in most trials. In trials where raw silk failed abruptly, the failure occurred around 13 mm of displacement. This event corresponds to the final failure point in Figure 3.17. The
failure load of raw silk trials that failed abruptly was on average 5% greater than raw silk trials that failed in stages.

**Figure 3.17.** Representative load versus displacement curves for raw and degummed silk.

Cell number was determined using the CytoTox 96 Non-Radioactive Cytotoxicity assay. The results of the assay can be seen in Figure 3.18. The assay indicated that cell number peaked at Day 14 and decreased by Day 21. Cell number was determined to be higher at all time points in the 1 mm mesh compared to the 2 mm mesh, but this difference was only significant (p<0.05) at the Day 7 and Day 21 time points. The 2-D control wells contained much higher cell numbers than either mesh size samples at all time points.
Figure 3.18. Silk mesh experiment CytoTox 96 assay results. Y axis (top) spans large range in order to show mesh data series in relation to 2-D control. Y axis (bottom) spans smaller range in order to showcase the difference in mesh absorbencies.

Protein production was measured using the BCA Total Protein assay. The results of this assay are displayed in Figure 3.19. While the 2-D control samples and 2 mm mesh indicated increasing cell proliferation for each time point, the 1 mm mesh samples peaked at Day 14 and maintained the same level of protein concentration at Day 21. The 1 mm mesh samples had
higher than or equal protein production to the 2 mm mesh samples at each time point, but these observations are not statistically significant (p<0.05). Protein concentration for 2-D control samples was much higher than for either mesh samples at all time points.

Figure 3.19. Silk mesh experiment BCA Total Protein assay results. Y axis (top) spans large range to show mesh data series in relation to 2-D control. Y axis (bottom) spans smaller range showcasing the difference in mesh absorbencies.
Cell metabolic activity was evaluated using the alamarBlue assay. The results of this assay are displayed in Figure 3.20. While the 2-D control samples increased in metabolic activity for each time point, the activity of the mesh samples each peaked at Day 14. The 1 mm mesh samples had significantly higher metabolic activity than the 2 mm mesh samples at all time points ($\alpha<0.05$). Metabolic activity for 2-D control samples was statistically higher ($p<0.05$) than mesh samples at all time points.
**Figure 3.20.** Silk mesh experiment alamarBlue assay results. Y axis (top) spans large range to show mesh data series in relation to 2-D control. Y axis (bottom) spans smaller range showcasing the difference in mesh absorbencies.

Figure 3.21 displays digital photographs taken after each LIVE/DEAD assay. At each time point, a large number of live cells can be seen adhered to the silk fibers. While cells can be observed in all areas of each type of mesh, cells tended to be most concentrated around fiber intersections and in crevices. This phenomenon is most clearly seen in the Day 21 photograph of the 1 mm mesh. In all photographs cells can be observed adhering to all surfaces of silk fibers, not just surfaces facing the top of the well plate. Photographs from Day 7 clearly show cells burrowed within the interior of each silk thread, adhering to the smaller silk filaments that comprise each thread. Photographic evidence indicates significant cell proliferation from Day 7 to Day 14, with cell numbers appearing to stabilize between Day 14 and Day 21.
Figure 3.21. LIVE/DEAD photographs of silk meshes. All images taken at 5x objective magnification. Live cells are colored green. The silk mesh is colored red.
Figure 3.22. LIVE/DEAD photographs of silk meshes. All images taken at 10x objective magnification. Live cells are colored green. The silk mesh is colored red.
Figure 3.23. LIVE/DEAD photographs of silk meshes. All images taken at 2.5x objective magnification. Live cells are colored green. The silk mesh is colored red.
Figure 3.24. LIVE/DEAD photograph of silk mesh. Image taken at 5x objective magnification on Day 7 of 2 mm mesh. Live cells are colored green. The silk mesh is colored red.

Discussion

The goal of this study was to test a new in vitro method of evaluating mesh characteristics – specifically, the use of a newly designed benchtop loom to vary mesh characteristics for mesh optimization studies. Preliminary experiments evaluated adhesives for use as support materials in the mesh studies and tested in vitro assays and procedures. Based on results from these experiments, an in vitro study was conducted to investigate the response of stem cells to silk mesh of two pore sizes. The proliferation, distribution, and viability of murine D1 mesenchymal stem cells on silk mesh samples produced by the auto-loom were
examined. The samples were monitored for a period of 21 days, with data collected at three time points. Cellular assays and LIVE/DEAD® images indicate that the D1 cells attached to and remained on each silk mesh for the duration of the study. Assays of metabolic activity, cell number, and protein concentration suggest that cells proliferated on all silk meshes.

Cellular activity of the MSCs as measured by the alamarBlue® assay peaked at Day 14 of the study \( (p < 0.05) \) and decreased \( (p < 0.05) \) by Day 21 for meshes with both 1 mm and 2 mm pores. However, metabolic activity of control cells was higher \( (p < 0.05) \) at each subsequent time point. The behavior of mesh seeded cells is similar to that observed by Shi and coworkers in their investigation of MSC differentiation in response to a differentiation-directed culture media \[36\]. They found that albumin production of hepatocyte differentiation directed MSCs peaked at Day 15, and then decreased at the same rate as primary mouse hepatocytes. This suggests that the 3-D morphology of silk fibers could have caused the seeded MSCs to differentiate and follow the life cycle of the cell type into which they differentiated. The difference in 2-D control cell activity compared to 3-D silk seeded cell activity is supported by the research of Wilkinson and coworkers and confirmed specifically for MSCs by Engel and coworkers, who found that surface topology can affect cell morphology and proliferation, and in the case of stem cells, differentiation \[20, 37\].

There was a noticeable difference in alamarBlue assay results between the method refinement and silk experiments. The method refinement results (Figure 3.15) show an average difference in metabolic activity between small and large pore meshes of 97%, while the silk experiment results (Figure 3.20) show only a 5% difference in metabolic activity between 1 mm and 2 mm patches. One possible explanation for this phenomenon is differences in cell attachment and adhesion between the nylon and silk meshes caused attached MSCs to react
differently, i.e. the physicochemical and/or mechanical differences caused vastly different cell responses. The quality of the initial cell/material interaction is crucial in determining subsequent cell action, and adhesion strength plays a role in cell development and differentiation [38, 39]. Hence cell adhesion to an implantable material becomes an important aspect of the total biocompatibility of the implanted device [38, 39]. While the specific chemical structure of the nylon used in this study is unknown, nylon is a synthetic, hydrophilic material, with low coefficient of friction and initiates minimal tissue reaction in vivo [40, 41]. Silk fibroin is natural, crystalline, hydrophobic material containing some hydrophilic secondary structures that elicits a moderate tissue reaction in vivo and promotes cell adhesion [5, 42, 43]. It is possible that the silk meshes promoted attachment and differentiation of the MSCs, while the nylon meshes promoted proliferation of MSCs. This conclusion is supported by the relatively constant number of viable cells (Figure 3.18) on each silk mesh compared to the steady rise in protein production at each time point (Figure 3.19).

A sharp increase (p < 0.05) in protein production from Day 7 to Day 14 for the cellular silk mesh samples, as measured by the BCA Total Protein assay, supports increased cellular activity in the early days of the experiment as cells differentiated. The same sharp initial increase in albumin production can be observed in the work of Shi and coworkers, followed by a decline in protein production [36]. However, a decline in protein production was not observed from Day 14 to 21 in the silk mesh experiment. In fact, the control and 2 mm mesh test groups showed a rise (p< 0.05) in protein concentration during this timeframe, and the 1 mm test groups had no statistically significant change in protein concentration from Day 14 to 21. Without data showing the differentiation pattern of MSCs on the silk mesh it is impossible to know what caused this difference in results, but it is possibly caused by differences in the type
of cells the MSCs differentiated into and/or the relative amount of differentiation that took place.

The silk used in this study was gathered from the cocoons of the *Bombyx mori* silkworm. Silk from this type of worm has been used clinically as a suture material for centuries, and currently is being investigated as a scaffold for tissue engineering applications, as a support structure for hernia repair, and as a degradable drug delivery compound [26, 28, 29, 44, 45]. The mechanical properties of *Bombyx mori* silk threads were briefly investigated of the course of this study. The results of a series of tensile strength tests on thread samples (Figure 3.17) show an increase in degummed silk thread over virgin silk threads containing sericin binding agent. This result is contrary to the tensile strength testing performed by Jiang and coworkers, who found that a sodium carbonate based degumming process reduced fiber strength by roughly 46% [46]. However, there were two major differences between the experiments. Jiang and coworkers tested the strength of individual silk fibers and used only sodium carbonate to degum samples, where this study tested the strength of 8-ply silk threads degummed using a combination of sodium carbonate and olive-oil derived soap. These differences in method could be the cause of the different degummed fiber strengths. This conclusion is reinforced by the similar displacement failure lengths seen between the initial failure point of the raw silk in Figure 3.17 and the control samples of Jiang and colleagues [46]. Regardless of the reasons for differences in the two results, more rigorous mechanical testing of future patch materials should be undertaken to better characterize the experimental meshes and to study changes in cellular behavior on meshes of different mechanical properties. Of particular interest may be scanning electron micrographs of raw, degummed, and cell seeded meshes, which many fibroin studies have relied on for evaluating surface topography and cellular distribution [26, 46, 47].
The LIVE/DEAD images shown in Figures 3.21, 3.22, 3.23, and 3.24 clearly show a substantial amount of MSC attachment to the silk mesh, including penetration of cells into the interior of silk threads. The multi-fiber structure of the meshes turns each thread into a network of crevices and strands, each about the width of a single cell, which greatly increases the available surface area for cell attachment. This makes the structure of the silk mesh more akin to braided or multifilament repair meshes than monofilament designs. Unfortunately, what these images can not show is the presence of or type of differentiation undertaken by the attached MSCs. Other assay results also suggest differentiation, but can neither confirm nor deny its existence. This lack of information on the differentiation of the MSCs is a weakness of these studies, but can be added to future work. Initially, a gas chromatography (GC) test was planned to evaluate the existence of adipose cellular differentiation, but the test had to be eliminated due to over-dilution of sample wells combined with insufficient cellular proliferation. The accuracy of the GC test was such that the margin of error would have been greater than the sample readings. Future testing should minimize the amount of liquid used in freeze/thaw cycles to burst cell membranes in order to concentrate cellular contents.

The in vitro methods described in the thesis call for the use of multipotent stem cells as the primary biological evaluator of mesh function. The reason for choosing stem cells over more common tissue cell types is that stem cells have the potential to offer a wider gradation of responses than most differentiated cell types. Furthermore, MSCs produce a host of macromolecules that influence the structure of tissue regeneration at injury sites [48]. By evaluating the in vitro response of MSCs to biomaterial morphology and chemical composition, it may be possible to use stem cells to influence the biological reaction to implanted hernia meshes. Similar research has already produced interesting results. Juffroy and coworkers
demonstrated the differentiation capabilities of MSCs in vitro as well as the spontaneous differentiation of MSCs to form bone-like shingles in an osseous tissue-free in vivo environment [49]. Authors Caplan and Bruder agree that MSCs have a lot to offer the field of tissue engineering, and have deconstructed the recipe to create functional tissue to the following ingredients: extracellular scaffolds to anchor, deliver, and orient cells, bioactive factors to promote growth, and cells capable of responding to these factors by producing the tissue of interest [19].

While osteogenesis is admittedly not the type of differentiation desired for the repair of hernias, this research offers an intriguing look at possibilities for the field of soft tissue repair and provides a model for the study of more clinically relevant stem cells such as adipose-derived varieties.

Conclusion

With respect to the immediate goal of determining the feasibility of an evaluation system comprised of the auto-loom to produce mesh and an in vitro test protocol, it can be concluded that such a system is both possible and likely to produce scientifically relevant results. Mesh was successfully produced using the auto-loom, cells were then successfully cultured on the mesh, and the viability and distribution of those cells were successfully evaluated using a series of in vitro assays.

The in vitro evaluation procedure and auto-loom mesh production represents a first step toward merging the mechanical support of implanted mesh with the cellular stability of directed tissue cultivation. By characterizing the biocompatibility of mesh attributes and
integrating the most beneficial of these traits with cutting edge practices in tissue engineering, it is hoped that one day an ideal, bioresponsive hernia mesh prosthesis can be attained.
References


CONCLUSIONS

A bulleted list of the most important conclusions derived from this thesis work, arranged by topic, is presented below. In addition, a list of recommendations for future work is presented in the next section, also in list format. As the work presented in this thesis is largely preliminary in nature, much potential for future improvements to both the auto-loom and in vitro methods exists.

Regarding the operation of the auto-loom for the production of woven mesh,

- It has been demonstrated that the auto-loom is capable of producing mesh of different pore sizes, specifically one and two millimeter.
- The auto-loom’s four shafts may be used in combination to create up to 16 unique shed patterns.
- Programming for the auto-loom is written in MATLAB script and the auto-loom is controlled through execution of said code through a simple, command line interface.

Regarding the preparation of silk for use in in vitro experimentation,

- The procedure used to degum silk thread by immersion in solution of near-boiling olive oil derived soap and sodium carbonate leaves little to no microscopic trace of sericin on the remaining fibroin brins.

Regarding the adhesion and well plate insertion of mesh samples,

- Circular mesh weaves bordered by EVA based hot-melt provided adequate physical support for weave samples in solution.
- Hot Melt adhesive also served as a stem cell friendly surface that appeared not to negatively impact cell proliferation or viability.
Regarding the attachment and proliferation of MSCs on silk meshes,

- Substantial cell attachment and proliferation occurred over the course of the study as evidenced by LIVE/DEAD assay images.

- A peak in metabolic activity at Week Two combined with sustained protein production growth over the course of the study suggests that differentiation may begin in Week Three of culture, but proliferation continues to occur at least three weeks post-seeding.
RECOMMENDATIONS

• Completely automate auto-loom thread handoff and weave advancement.

• Scale down loom dimensions to further reduce the amount of material needed to string the auto-loom.

• Establish a system for removal of mesh from loom without disrupting pore dimensions.

• Develop a more uniform and repeatable method for adhesive application to mesh samples.

• Continue to test the limits of auto-loom production speed and variability using different thread materials and weave styles.

• Add assays to *in vitro* evaluation protocol that investigate stem cell differentiation on meshes.

• Investigate the effect mesh morphology has on bacterial colonization.

• Test response of adipose derived stem cells to mesh.

• Conduct thorough mechanical analysis of mesh materials.