8-2015

Design and Development of a Selectively Absorbable Multiphasic Hernia Mesh an In Vivo Performance as it Relates to Biocompatibility

Georgios T. Hilas
Clemson University

Follow this and additional works at: https://tigerprints.clemson.edu/all_dissertations

Recommended Citation
https://tigerprints.clemson.edu/all_dissertations/1783

This Dissertation is brought to you for free and open access by the Dissertations at TigerPrints. It has been accepted for inclusion in All Dissertations by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.
DESIGN AND DEVELOPMENT OF A SELECTIVELY ABSORBABLE MULTIPHASIC HERNIA MESH AND IN VIVO PERFORMANCE AS IT RELATES TO BIOCOMPATIBILITY

A Dissertation
Presented to
the Graduate School
of Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Bioengineering

by
Georgios T. Hilas
August 2015

Accepted by:
Dr. Karen J. L. Burg, Committee Chair
Dr. M. Scott Taylor
Dr. Frank Alexis
Dr. Kenneth Webb
ABSTRACT

Hernia repair is one of the most frequently performed surgical operations, with the vast majority of these surgeries employing a “tension-free” repair technique with synthetic surgical meshes. Traditionally, meshes for hernia repair have been designed with high strength in order to produce a robust repair; unfortunately, current designs are unable to respond to the dynamic biological needs of the wound healing process. As a result, patients undergoing mesh hernioplasty often suffer from mesh contraction, reduced wound site compliance, fibrosis, and/or chronic pain. The aim of this dissertation was to design and assess a novel, selectively absorbable mesh system for soft tissue repair which exhibits initially interdependent load-bearing components that transition to independent in situ functional properties. More specifically, the mesh design was constructed to provide (1) a short-term stability phase to protect the developing tissue, (2) a mechanical load transitioning phase for support as the selected absorbable component begins to lose mechanical strength, and (3) a long-term compliant phase to allow mechanical sharing of loads between the deposited tissue and implanted construction.

The designed mesh system was evaluated in a chronic ventral hernia model in rabbits and compared to the clinically relevant predicate, UltraPro™ mesh (a partially absorbable mesh currently marketed by Ethicon). Mechanical evaluation of the resulting mesh/tissue complex at 4, 8, and 12 weeks indicated that, while the designed mesh system resulted in a stiffer repair site initially (as compared to UltraPro™), the mesh...
transitioned into a significantly more compliant repair by 12 weeks. Furthermore, the mechanical contribution of the deposited collagen increased at each time point for UltraPro™, but decreased for the designed constructions. The UltraPro™ result suggests a possible cause for the increased long-term abdominal wall stiffness seen in mesh hernioplasty today (i.e. a cycle of constantly stiffening scar plate). Histopathological assessment indicated that the designed constructions triggered a statistically more intense foreign body response for the novel mesh constructions which allowed rapid integration into abdominal wall. This also led to a lower ratio of Type I/III collagen, although the results are limited due to the longest time point of 12 weeks, at which point the abdominal wall has not reached complete remodeling and maturity and all absorbable portions of the mesh are not completely absorbed. Overall, the results of this study show the capacity of the developed constructions to modulate the tissue response of the healing abdominal wall based on temporal dynamic mesh mechanics. In addition, the novel meshes studied as part of this dissertation have the potential of reducing common complications associated with mesh hernioplasty, including mesh contraction, loss of tissue compliance, and reduction in severity of visceral adhesions. Collectively, these results provide justification for further development and assessment of multi-phasic meshes as those described within this body of work.
DEDICATION

I dedicate this work to my loving wife, Beki, who has been my rock throughout this process. This accomplishment could not have been possible without her support, love, and encouragement. To my parents, Theofanis and Konstantina Hilas, who sacrificed everything to move from the mountains of Greece to America so that their children could have the opportunity for a better life. Finally, to the late Dr. Shalaby W. Shalaby, who guided and pushed me to further my education and realize my full potential. He always believed that although some projects may fail, people never do.
ACKNOWLEDGMENTS

I would like to thank the Shalaby family for their continued support throughout this process. I would also like to thank Dr. M. Scott Taylor for his guidance and positive attitude throughout the execution and preparation of this dissertation. A special thanks to the members of my committee, Dr. Frank Alexis, Dr. Kenneth Webb and especially to my committee chair Dr. Karen Burg, for their time and mentorship at multiple instances throughout my scholastic career thus far.

Thank you to Dr. Joel Corbett who performed all of the animal surgeries and to the staff of the Godley-Snell Research Center at Clemson University who provided assistance and direction throughout this process. A special thanks to Dr. Shawn Peniston whose dissertation work laid the foundation for this research. I would be remiss if I did not offer a generous thanks to my Poly-Med coworkers and close friends who have supported and guided me along this journey.
PREFACE

The first knitted, monofilament, polypropylene surgical mesh was introduced into clinical practice in the 1960s. Today, common surgical meshes used worldwide are still knitted monofilament polypropylene that essentially uses the same basic mesh design invented almost half a century ago. Even partially absorbable meshes that currently exist in the market place continue to “piggyback” upon this first initial mesh design, with the added function of reducing the overall final mass of the patients terminal prosthesis. While the final mass of the implanted device is reduced, the standard non-absorbable portions remaining still realize the same long-term clinical problems of increased rigidity, fibrosis, and chronic pain. Therefore, it is our belief that the current clinical issues associated with mesh hernioplasty may be linked back to the use of poor mesh design. This point is observed in the current hernia repair paradigm, which drives the use of a static mesh construction to repair a dynamic healing situation.

In Chapter 2 the design of a selectively absorbable mesh construction is documented using a mesh construction whereupon the absorbable yarn component effectively maintains the non-absorbable polypropylene yarn in tension; preventing pore deformation and creating a mechanically stable mesh construction. Upon strength loss of the absorbable component in the \textit{in vivo} environment, the non-absorbable yarn is released. This results in a temporal change in mesh extensibility, matching the extensional properties of the native abdominal wall tissue. As the mesh transition time
point is dictated by the absorption characteristics of the degradable yarn, a series of mesh constructions were successfully created each exhibiting a unique biomechanical profile.

To assess the potential clinical effect of initial mesh structural stability followed by long-term mesh extensibility on extra cellular matrix wound quality, as exhibited by the created constructs, the development of a clinically-relevant animal model in rabbits that simulated the wound pathology associated with mature hernia development in humans was developed. This animal model, as described in Chapter 3, resulted in the formation of a mature hernia with the presence of a hernia sac, distinct hernia ring, and various unpredictable visceral adhesions. This outcome allowed analysis of critical hernia repair metrics within Chapters 3 and 4, culminating in the identification of key mesh design parameters required to improve the current clinical outcome of mesh hernioplasty.

The completed work described in this dissertation was funded by a Phase I National Institutes of Health (NIH) Grant, NIH Grant No. 1R43GM112194-01. The intellectual property specific to mesh construction and creation of temporal mechanical properties disclosed within this dissertation is captured in two existing patent applications, US Patent Application 13/445,525 (2012) and US Patent Application 13/858,704 (2013). In addition, portions of Chapter 3 describing the mature ventral hernia animal model were presented at the Society For Biomaterials 2015 Annual Meeting & Exposition and published in the meeting transactions.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>v</td>
</tr>
<tr>
<td>PREFACE</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I. LITERATURE REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>Wound Repair</td>
<td>1</td>
</tr>
<tr>
<td>Fascial Wound Healing Model: The Hernia</td>
<td>7</td>
</tr>
<tr>
<td>Current Hernia Treatment Options: Mesh Hernioplasty</td>
<td>12</td>
</tr>
<tr>
<td>Animal Models Utilized to Investigate Hernia Development</td>
<td>24</td>
</tr>
<tr>
<td>and Treatment</td>
<td></td>
</tr>
<tr>
<td>Methods of Characterizing the Wound Healing Process</td>
<td>30</td>
</tr>
<tr>
<td>Conclusions and New Opportunities</td>
<td>36</td>
</tr>
<tr>
<td>References</td>
<td>39</td>
</tr>
<tr>
<td>II. MULTIPHASIC HERNIA MESH DEVELOPMENT AND EVALUATION</td>
<td>50</td>
</tr>
<tr>
<td>Introduction</td>
<td>50</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>52</td>
</tr>
<tr>
<td>Results</td>
<td>57</td>
</tr>
<tr>
<td>Discussion</td>
<td>65</td>
</tr>
<tr>
<td>Conclusion</td>
<td>71</td>
</tr>
<tr>
<td>References</td>
<td>72</td>
</tr>
</tbody>
</table>
### Table of Contents (Continued)

III. MECHANICAL EVALUATION OF MULTIPHASIC SELECTIVELY ABSORBABLE MESH IN A MATURE VENTRAL HERNIA MODEL .............................................................. 74
   - Introduction ......................................................................................... 74
   - Materials and Methods ....................................................................... 77
   - Results ............................................................................................... 88
   - Discussion ......................................................................................... 98
   - Conclusion ....................................................................................... 105
   - References ....................................................................................... 107

IV. HISTOLOGICAL EVALUATION OF MULTIPHASIC SELECTIVELY ABSORBABLE MESH IN A MATURE VENTRAL HERNIA MODEL ........................................... 110
   - Introduction ....................................................................................... 110
   - Materials and Methods ..................................................................... 115
   - Results ............................................................................................. 118
   - Discussion ......................................................................................... 126
   - Conclusion ....................................................................................... 133
   - References ....................................................................................... 134

V. CONCLUSIONS .................................................................................. 138

V. RECOMMENDATIONS FOR FUTURE WORK ........................................ 141

APPENDIX .................................................................................................. 143
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Animal Models of Mature Ventral Hernia Creation</td>
</tr>
<tr>
<td>1.2</td>
<td>Summary of Key Surgical Mesh Design Variables</td>
</tr>
<tr>
<td>2.1</td>
<td>Material Characterization Methods</td>
</tr>
<tr>
<td>2.2</td>
<td>Polymer Resin Characterization</td>
</tr>
<tr>
<td>2.3</td>
<td>Fiber Characterization</td>
</tr>
<tr>
<td>2.4</td>
<td>Initial Mesh Mechanical Properties</td>
</tr>
<tr>
<td>2.5</td>
<td><em>In vitro</em> Mass Retention Study Results</td>
</tr>
<tr>
<td>3.1</td>
<td>Selectively Absorbable Mesh Constructions</td>
</tr>
<tr>
<td>3.2</td>
<td>Adhesion Scoring System</td>
</tr>
<tr>
<td>3.3</td>
<td>Ventral Hernia Development Results</td>
</tr>
<tr>
<td>3.4</td>
<td>Mesh Contraction for SAM-3, SAM-6, and UltraPro™ Mesh</td>
</tr>
<tr>
<td>3.5</td>
<td>Adhesion Scores for SAM-3, SAM-6, and UltraPro™ Mesh</td>
</tr>
<tr>
<td>3.6</td>
<td>Comparison of <em>In Vivo</em> Conditioned Mechanical Testing Results of Each Construction to <em>In Vivo</em> Result of Mesh/Tissue Complex</td>
</tr>
<tr>
<td>4.1</td>
<td>Histopathological Evaluation of IHC and MT Stained Slides</td>
</tr>
<tr>
<td>4.2</td>
<td>Collective Histopathological Analysis of Mesh Constructions</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.1</td>
<td>Anatomy of the inguinal region</td>
</tr>
<tr>
<td>1.2</td>
<td>Mesh positioning during mesh hernioplasty</td>
</tr>
<tr>
<td>1.3</td>
<td>When the large latency complex (LLC) is anchored in a stiff ECM, myofibroblast contraction can lead to the release of TGF-β1</td>
</tr>
<tr>
<td>1.4</td>
<td>Ventral hernia that develops with the described surgical procedure</td>
</tr>
<tr>
<td>1.5</td>
<td>Abdominal protrusion of ventral hernia 21 days post-op</td>
</tr>
<tr>
<td>1.6</td>
<td>The normal wound healing trajectory</td>
</tr>
<tr>
<td>1.7</td>
<td>Ball burst testing performed with a 10 mm diameter ball pushed through the defect site reinforced with mesh</td>
</tr>
<tr>
<td>1.8</td>
<td>As the wound builds strength over time, less support is required by the surgical mesh but some support may be necessary indefinitely</td>
</tr>
<tr>
<td>2.1</td>
<td>Vypro® II Mesh (left) and UltraPro™ Mesh (right)</td>
</tr>
<tr>
<td>2.2</td>
<td>Interdependent co-knit mesh constructions used for developed meshes</td>
</tr>
<tr>
<td>2.3</td>
<td>Maximum burst load of developed constructions (SAM-3, SAM-6, and SAM-18) along with predicate material (UltraPro™) at various durations of in vitro degradation</td>
</tr>
<tr>
<td>2.4</td>
<td>Elongation at 16N/cm for developed constructions (SAM-3, SAM-6, and SAM-18) along with predicate material (UltraPro™) at various durations of in vitro degradation</td>
</tr>
<tr>
<td>3.1</td>
<td>Final hernia defect creation size and location</td>
</tr>
<tr>
<td>3.2</td>
<td>Suture is passed through the abdominal wall and looped around a mesh pore before being drawn back through abdominal wall</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3</td>
<td>Mechanical testing fixture dimensions (A) and image of loaded mesh/tissue complex sample immediately prior to testing (B)</td>
<td>88</td>
</tr>
<tr>
<td>3.4</td>
<td>Image A – The abdominal wall protrusion of the mature ventral hernia 21 days after defect creation procedure</td>
<td>91</td>
</tr>
<tr>
<td>3.5</td>
<td>Image A – Hernia repaired with SAM-6 12-weeks following hernia repair showing good collagen development over the mesh surface with no visceral adhesions</td>
<td>95</td>
</tr>
<tr>
<td>3.6</td>
<td>Load exerted on mesh/tissue complex to realize an extension of 4 mm following the application of a 0.1 N pre-load for SAM-3, SAM-6, and UltraPro™ at 4, 8, and 12 week implantation time points</td>
<td>97</td>
</tr>
<tr>
<td>3.7</td>
<td>Overlay of SAM-3, UltraPro™, and SAM-6 in vitro mesh testing loads at 4 mm of extension over mesh/tissue complex testing data from Figure 3.6</td>
<td>98</td>
</tr>
<tr>
<td>4.1</td>
<td>The relative order of events and cellular involvement after device implantation (adapted from Morais et al)</td>
<td>113</td>
</tr>
<tr>
<td>4.2</td>
<td>Illustration indicating approximate locations for type I/III collagen analysis for interstitial pore space and subcutaneous side of mesh</td>
<td>118</td>
</tr>
<tr>
<td>4.3</td>
<td>TOP – SAM-3 mesh constructions at 8-weeks implantation time point (MT stain) showing high collagen development in and around mesh construction</td>
<td>120</td>
</tr>
<tr>
<td>4.4</td>
<td>Slides at 10x magnification that were stained with α-SMA for SAM-3 (A, 12 –week), SAM-6 (B, 12-week), UltraPro™ (C, 8-week), and UltraPro™ (D, 12-week)</td>
<td>121</td>
</tr>
<tr>
<td>4.5</td>
<td>Myofibroblast density within interstitial pore space for SAM-3, SAM-6, and UltraPro™ Mesh at 4, 8, and 12-week implantation times</td>
<td>122</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6</td>
<td>Collagen Type I/III ratio for each mesh construction following implantation at 4, 8, and 12-weeks over mesh construction on subcutaneous side</td>
<td>123</td>
</tr>
<tr>
<td>4.7</td>
<td>Collagen Type I/III ratio for each mesh construction following implantation at 4, 8, and 12-weeks within mesh construction</td>
<td>124</td>
</tr>
<tr>
<td>4.8</td>
<td>Images of picro-sirius stained slide for SAM-3, SAM-6, and UltraPro™ at 4 and 12-week implantation times taken at 10x magnification under polarized light</td>
<td>125</td>
</tr>
<tr>
<td>4.9</td>
<td>Left – Diagram of SAM mesh pore indicating inter-filament pore distance and model of granuloma/collagen deposition around mesh fibers</td>
<td>129</td>
</tr>
</tbody>
</table>
CHAPTER I
LITERATURE REVIEW

Today, hernia repair is one of the most common surgical procedures performed, yet difficulties with hernia recurrence, chronic pain, and reduced patient mobility still persist. As such, a multifaceted approach is required to ultimately resolve and improve the clinical outcome of hernioplasty. This type of approach requires an understanding of the body’s expected wound healing response to the developing hernia as well as a critical review of current surgical repair techniques and hernia research paradigms in order to determine current clinical pitfalls.

I. Wound Repair

The wound repair process is one of the most complex and important functions that occurs in the human body. The process requires the activation and recruitment of a large array of cells and cell signaling molecules that collectively act to repair the site of injury. Although the most ideal function of the wound repair process would be to restore original functionality to the injured organ system, the process often results in the formation of fibrotic tissue that exhibits a marked loss in functionality as compared to the native tissue being repaired. The wound repair process can be broken down into four distinct phases: hemostasis, inflammation, proliferation, and remodeling.\(^1\)
1.1. Hemostasis

At the onset of tissue injury, platelets rapidly adhere to the injured endothelial cells and exposed connective tissues in order to stop the loss of blood by forming a platelet plug, beginning the intrinsic coagulation cascade pathway. This platelet plug transitions into a cross-linked fibrin network which prevents the loss of blood and other bodily fluids and also functions as a provisional matrix or scaffold for infiltrating cells. In addition to their involvement in restoring hemostasis, activated platelets also release a slew of growth factors and cytokines that are critical for the next stages of wound repair. This release of growth factors and other chemicals occurs within seconds of the platelets adhering to a surface (native and foreign) due to a process called platelet degranulation. Of these chemicals released, platelet-derived growth factors (PDGFs) are of great importance. PDGFs belong to a family of disulfide-bonded dimeric isoforms consisting of homo- and heterodimer polypeptide chains. This family of growth factors includes PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC, and PDGF-DD. These growth factors are key players in the wound healing process as they have been shown to cause the chemotaxis of neutrophils, macrophages, fibroblasts, and smooth muscle cells to the wound site. Furthermore, PDGFs stimulate the production of extracellular matrix components, including fibronectin, collagen, proteoglycans, and hyaluronic acid.

1.2. Inflammation

The inflammation process often begins in conjunction with and overlaps with hemostasis. This process involves the recruitment of a variety of leukocyte cell types,
growth factors, and cytokines that function to prevent infection and remove any cellular debris from dead or dying tissues, preparing the wound site for the next stage of wound healing, proliferation. This stage is marked by an increase in blood flow and fluid to the inflamed site, which contributes to the classic markers of inflammation: redness, swelling, pain, and heat. First to the site of trauma is the neutrophil, which arrives within hours of the insult. This fast reaction time is possible because neutrophils are constantly flowing through the blood vasculature, monitoring for any signs of distress that are expressed by the endothelial cells lining the blood vessel walls. Neutrophils and other leukocytes travel from the blood stream and to the site of trauma through a process known as diapedesis. First, a family of adhesion molecules (L-selectin, P-selectin, and E-selectin) which are expressed on the surface of leukocytes and endothelial cells triggers initial leukocyte rolling on the blood vessel wall. More specifically, L-selectin is expressed on the surface of leukocytes, while E-selectin and P-selectin are expressed on the surface of damaged or inflamed endothelial cells. It is the interaction of these selectins with their respective ligands that enables primary leukocyte capture, the initial adherence of leukocytes directly to the endothelium. Of these ligands, one of great importance seems to be the glycoprotein ligand PSGL-1, which is a P-selectin ligand expressed on the surface of most leukocytes. This ligand helps facilitate the primary adhesion or capture of leukocytes and has also been shown to cause secondary tethering or capture of leukocytes by binding with L-selectin. Once captured and rolling is initiated, leukocyte activation and arrest occurs, allowing strong adhesion with the endothelium, followed by paracellular and/or transcellular migration through the
endothelium. Various integrins are also involved in this process, namely those belonging to the $\beta_1$-integrin and $\beta_2$-integrin subfamilies. Following closely behind neutrophils, monocytes appear at the wound site 2 to 3 days after initial tissue damage. Once differentiated into macrophages, these cells begin removing any damaged cells and/or tissue, in addition to any bacteria or foreign matter that may have been introduced during the injury. Not only are they critical to this innate immune response, but macrophages are also responsible for releasing a slew of cytokines that affect and regulate multiple aspects of the wound healing process.

Resolution of the inflammatory phase is critical for normal healing to continue. Undue continuation of the inflammatory process will inevitably result in the destruction of the surrounding healthy tissue as well as newly deposited tissue, ultimately resulting in excessive scar tissue formation or fibrosis. Interestingly, it has been shown that embryos, which lack the development of an immune system or immune response, often exhibit scar-free wound repair with functional tissue deposition.

1.3. Proliferation

The proliferation stage of wound healing is marked by a large increase in fibroblast cell migration, which often begins 2 – 10 days after injury, the exact time based on the degree/amount of contaminating microbes and damaged tissue. The main function of the fibroblast is to begin synthesizing and depositing collagen and other extracellular matrix (ECM) components into the wound site. A large array of tissue growth factors and cytokines is responsible for initiating and regulating the migration and
proliferation of fibroblast and other mesenchymal cells into the wound site. These growth factor and cytokines include but are not limited to PDGFs (as previously discussed), basic fibroblast growth factors (bFGF), and transforming growth factors alpha and beta (TGF-α and TGF-β). In conjunction with this influx of cells, angiogenesis or vascularization begins, supplying blood and oxygen to the newly forming tissue. This tissue is often referred to as granulation tissue, mainly because of its granular appearance resulting from the high concentration of budding capillaries and thin collagen fibril bundles which are composed predominately of Type III collagen. A review of the literature indicates that approximately 62 – 80% of the collagen found in early granulation tissue is Type III or otherwise immature and less organized collagen. As such, this tissue lacks the strength and stability needed to support normal physiological loading. In one study laparotomy wound strength was examined using a rat model over a period of 84 days and indicated low wound stability within the proliferation stage of wound healing. Maximum tensile strength of the wound at 22 days post-surgery was found to be only 20% (~14 N) of that of the resolved wound at 84 days post-surgery (~62 N). In another study where rat full thickness skin incisions were examined, wound tensile strength was found to be only 3.98% of the tensile strength observed for unwounded or otherwise uncompromised tissue 7-days post-insult. In addition to depositing collagen, some of the fibroblast differentiate into myofibroblasts, which function to contract the wound site and bring the edges of the wound together. The differentiated myofibroblast ability to contract comes from expression of α-smooth muscle actin (α-SMA) and strong mature focal adhesions with the deposited ECM.
Although some contracture is necessary in order to restore mechanical stability to the wound site, unregulated or chronic myofibroblast activation has been shown to lead to fibrosis and loss of tissue functionality.\textsuperscript{22–24}

1.4. Remodeling

The last stage of wound healing is the remodeling phase which can last months to even years after the initial insult. This stage typically begins 2 to 3 weeks following injury and is marked by a reduction in overall cell density as cells previously required to rapidly secrete collagen and fill in the wound site are no longer needed.\textsuperscript{1,25,26} In addition, the amount and type of collagen at the wound site begins to transition from the previously 62 – 80\% level of Type III collagen found in granulation tissue, to approximately 20\% Type III collagen as remodeling of the wound site progresses.\textsuperscript{16,17,25} The major proteinase responsible for this collagen and ECM degradation is a family of enzymes called matrix metalloproteinases (MMPs) which are released from resident tissue fibroblasts and macrophages.\textsuperscript{13,27} Once released, MMP activity is regulated by various endogenous inhibitors, termed tissue inhibitors of metalloproteinases (TIMPs).\textsuperscript{27–29} Therefore, MMP and TIMP activity is tightly controlled to regulate overall ECM remodeling. As the wound site is remodeled and transitioned to predominately Type I collagen, tensile strength of the wound increases dramatically as it approaches a maximum of 70 to 80\% post-injury strength.\textsuperscript{1,2,16} It has been shown that tissues of the human body detect and respond to mechanical stimuli, similar to the process observed in
the remodeling of bone; hence, for proper remodeling to occur, sufficient or physiologically normal stressing of the tissue is required.\textsuperscript{30,31}

\section*{II. Fascial Wound Healing Model: The Hernia}

A hernia is generally defined as a defect in the abdominal wall which allows viscera such as adipose tissue and intestinal loops, which are normally confined to the abdomen, to push out into the preperitoneal space. Interestingly, the term hernia is derived from the Greek word hernois which means “sprouting forth”. Hernias typically present in the form of a hernia sac which contains the herniated tissues; in some situations this presentation is lacking.\textsuperscript{32} Depending on the size and location of the hernia, the bowels can become obstructed, leading to an incarcerated or strangulated hernia.\textsuperscript{32} These types of hernias are considered life threatening and require immediate medical intervention. However, most hernias are reducible or otherwise able to be pushed or manipulated back into the abdomen and are at low risk for incarceration.\textsuperscript{33}

\subsection*{2.1. Epidemiology}

Although traditionally, excessive straining and performance of activities that increase abdominal cavity pressure are thought to initiate hernia development, recent studies indicate that the true cause may be due to abnormal connective tissue metabolism.\textsuperscript{34–39} In one study, skin fibroblasts from patients with ventral hernias were cultured and compared against those without any previous history of hernia.
development.\textsuperscript{36} Through the use of reverse transcriptase-polymerase chain reaction, Si and colleagues were able to show a significant decreased ratio of Type I to Type III procollagen mRNA in the patient group with ventral hernia as compared to the control group. In another study, fibroblasts were harvested from the transversalis fasica of patients diagnosed with direct inguinal hernias and compared to a control in regard to MMP-2 and MMP-9 expression.\textsuperscript{40} MMPs are the major proteinase responsible for collagen degradation and are heavily involved in ECM remodeling. Through immunosorbent assays, a significant over-expression of MMP-2 was discovered in the hernia group as compared to the control, indicating a possible genetic defect involving MMP expression as a cause for an inherent weakness in the fascia, predisposing some patients to hernia development.\textsuperscript{37,40} In addition to exhibiting a change in collagen type, modifications in the amount of elastin found in the fascia of patients with hernias may also by an attributing factor. Fachinelli and colleagues analyzed the elastin content of patients with abdominal wall hernias and found that a greater amount of elastin is present in the fascia of these patients as compared to the control group. Additionally, an association between hernia development and abdominal aortic aneurysm further substantiates that hernia development is most likely due to some type of connective tissue disorder.\textsuperscript{41,42}

\subsection*{2.2. Hernia Types}

Hernias are classified based on their origination, lending to the large number of hernia types. The most common hernias seen in the clinical setting, based on relative
frequency, is as follows (listed in decreasing frequency): inguinal, umbilical, epigastric, incisional, para-umbilical, and femoral.\textsuperscript{43}

\textit{Inguinal Hernia}

Inguinal hernias are by far the most common type of hernias seen, with as high as a 27\% lifetime risk reported in men and 3\% lifetime risk in women.\textsuperscript{44} As the name implies, these hernias are confined to the inguinal region in humans, which is located in the lower portion of the anterior abdominal wall, otherwise known as the groin. There are two types of inguinal hernias: direct and indirect. In a direct inguinal hernia, the hernia protrudes through a weak area in the transversalis fascia known as Hesselbachs triangle, which resides above the inguinal ligament but lateral to the rectus abdominis muscle and medial to the epigastric vessel, as shown in Figure 1.1 below.\textsuperscript{45} These types of hernias are typically thought to be caused by some type of straining (i.e. heavy lifting or chronic coughing) and are a result of direct tearing of the fascia. Alternatively, an indirect inguinal hernia is one where the hernia moves through the inguinal ring down the inguinal canal and, in some extreme cases, descends into the scrotum. These hernias are thought to arise from improper closure of the processus vaginalis following decent of the testis into the scrotum in infants.\textsuperscript{32}
A ventral hernia is generally defined as any external abdominal wall hernia that is found along the mid and upper abdominal wall regions and includes umbilical, epigastric, and incisional hernias. As the name suggest, an umbilical hernia results when viscera is pushed out of the abdominal cavity through the umbilicus and is most frequently seen in children and following pregnancy in women. In young children, these hernias occur due to a congenital malformation of the navel whereupon there is a delayed contraction of the

Figure 1.1. Anatomy of the inguinal region.\textsuperscript{45}

\textit{Ventral Hernia}

A ventral hernia is generally defined as any external abdominal wall hernia that is found along the mid and upper abdominal wall regions and includes umbilical, epigastric, and incisional hernias. As the name suggest, an umbilical hernia results when viscera is pushed out of the abdominal cavity through the umbilicus and is most frequently seen in children and following pregnancy in women. In young children, these hernias occur due to a congenital malformation of the navel whereupon there is a delayed contraction of the
fibro muscular umbilical ring, allowing viscera to push out of the abdominal cavity.\textsuperscript{46,47} Rate of incarceration is very low, with most spontaneously resolving themselves by age 4 or 5.\textsuperscript{47} An epigastric hernia is a hernia that occurs between the xyphoid process and the umbilicus and accounts for up to 3.6\% of all hernias found in adults.\textsuperscript{48} In addition, there is a significant male predominance for these types of hernias (2-3 times more common in men verses women) and a higher incidence is found in patients from 20 to 50 years of age.\textsuperscript{49} The exact pathological mechanism for epigastric herniation is unknown but the general consensus indicates that excessive tension in the epigastric region (most likely due to extensive physical training, coughing, and/or obesity) is the most common factor.\textsuperscript{48,49} Incisional hernias are hernias that develop at a previous surgical site or laparotomy and account for a large percentage of post-surgical complications where abdominal cavity access is required. Studies indicate that as high as 11 – 23\% of patients develop incisional hernia following abdominal surgery, with this doubling if wound infection of the closure site occurs.\textsuperscript{50-52} Interestingly, the incisional hernia can take as long as 2 – 5 years following the initial laparotomy before clinical presentation.\textsuperscript{53-55}

\textit{Femoral Hernia}

A femoral hernia is a hernia that develops through the femoral ring (see Figure 1.1) and travels into the femoral canal inferior to the inguinal ligament. These types of hernias are more common in women and are difficult to diagnose, often being incorrectly identified as inguinal hernias.\textsuperscript{56,57} Although occurring at a much less frequent rate when
compared to inguinal hernias, femoral hernias are associated with a high rate of complication, with approximately 45% of all femoral hernias leading to strangulation 21 months after diagnosis, as compared to 4.5% for inguinal hernias during this same time frame.\textsuperscript{56,58}

\section*{III. Current Hernia Treatment Options: Mesh Hernioplasty}

Approximately 20 million hernia repair procedures are performed annually around the world, making it one of the most common surgical procedures.\textsuperscript{59} The vast majority of these repairs employ a “tension-free” repair technique, which involves the use of synthetic surgical meshes to reinforce the herniated region. Existing mesh technology has been designed to be high strength in order to produce a perceived robust repair. However, these meshes are unable to respond to the dynamic biological needs of the wound healing process. Mesh contraction, increased rigidity over time, entrapment of sensory nerves, and chronic inflammatory responses to the prosthesis, among other mesh related factors, contribute to long-term complications such as chronic pain, increased abdominal wall stiffness and fibrosis.

\subsection*{3.1. Clinically- Relevant Mesh Materials}

Historically, material selection for use in surgical meshes is based on bio-inertness and ability to resist biodegradation. As such, surgical meshes used today are generally made from three basic materials: polypropylene (PP), polyethylene-terephthalate
(PET) and, to a much lesser extent, polytetrafluorethylene (PTFE). Of these, PP is the most commonly used material. PP is a nonpolar and highly hydrophobic polymer with a relatively high degree of crystallinity. Mechanically, this characteristic gives PP its strength and stiffness characteristics, which are then translated to the mesh, especially those constructed with a monofilament fiber form. One of the main disadvantages of its use in mesh construction is its pronounced foreign body reaction, which may contribute to the mechanical stiffness of this material as well as to its hydrophobic nature.\textsuperscript{60,61} PET was introduced as a mesh material for hernia repair more than 30 years ago and continues to be used today. Due to its inherent stiffness, it is used exclusively in multifilament fiber forms for mesh construction. Although considered a non-absorbable material, numerous studies from its use in vascular graft applications indicate molecular weight and material strength reduction over time \textit{in vivo}.\textsuperscript{62–64} Traditionally used only in Europe, marketed by Ethicon Inc. as Merselene®, meshes constructed from PET are slowly gaining traction in the United States. PTFE is not a widely used material for hernia meshes. Its application is often limited to situations where visceral adhesions are of major concern. The characteristic feature of PTFE is its inert nature due to the extreme stability of the carbon-fluorine bond. Processing issues, primarily the inability to melt extrude, leads to laminar structures with very small pore sizes and subsequent poor tissue integration.\textsuperscript{61}

### 3.2. Surgical Procedures

Unlike suture repair, where closures of wounds heal due to primary intention, the edges of a hernia are generally not completely approximated during mesh hernioplasty,
leaving the wound site to heal by secondary intention. In effect, the mesh acts as an *in situ* tissue scaffold for the developing tissue. Mesh hernioplasty can be broken down into three main classifications, as depicted in Figure 1.2: 1) open anterior flat mesh repair, 2) extra-peritoneal mesh repair, and 3) posterior (intra-peritoneal) flat mesh repair.

**Figure 1.2.** Mesh positioning during mesh hernioplasty: 1 – posterior (intra-peritoneal) flat mesh repair, 2 – extra-peritoneal mesh repair, and 3 – open anterior flat mesh repair.\(^{50}\)
Open Anterior Flat Mesh Repair

Also known as an onlay mesh repair, in this procedure the mesh prosthesis is placed over top the abdominal wall defect and secured via suture, staples, or tacks into the abdominal wall musculature. Briefly, the hernia sac is first dissected and opened in order to divide any visceral adhesions and other defects that may cause potential issues following the repair.\textsuperscript{50} Once all adhesions are removed, the sac contents are reduced and a mesh is placed with a 2 - 4 cm overlap of the abdominal wound edges.\textsuperscript{50,65} The mesh is typically anchored using interrupted full-thickness suture (absorbable or non-absorbable) or absorbable tacker clips.\textsuperscript{50,65–67}

Extra-peritoneal Mesh Repair

In an extra-peritoneal or sublay mesh repair, the mesh prosthesis is placed between the abdominal wall musculature (retromuscular) and the peritoneum, in effect preventing direct contact of the viscera with the prosthesis material. This procedure can be completed using both an open surgical procedure or via endoscopic repair.\textsuperscript{50,68} If the peritoneum is compromised, for example if dissection of the hernia sac is required, it must first be repaired prior to mesh placement. Since the mesh is “sandwiched” between the peritoneum and muscle, the bowels provide direct pressure up against the prosthesis, therefore strong fixation is not required but some means of fixation (staples, tacks, or fibrin glue) is generally advised to prevent mesh dislocation during the early postoperative period (~ 1 week).\textsuperscript{66,69} The most common surgical procedure of this type is
known as the totally extraperitoneal (TEP) procedure, which is performed with an endoscopic technique and does not involve entering of the abdominal cavity. In this procedure, a preperitoneal space is created around the herniated region using blunt dissection and air insufflation (alternatively balloon dissection is used in some cases for extra preperitoneal space creation). The herniated tissue is then identified and reduced into the abdominal wall. As previously mentioned, any tears to the peritoneum during preparation of the preperitoneal space or reduction of the hernia must be repaired (typically with absorbable suture) prior to placement of the mesh. Once the mesh is positioned with a 3 – 4 cm overlap of the hernia, desufflation of the preperitoneal space is performed with careful monitoring to ensure the mesh stays in a flat configuration. Alternatively, staples, tacks, or fibrin glue may be used at the cardinal points of the prosthesis to prevent migration.

**Posterior (Intraperitoneal) Flat Mesh Repair**

In an intraperitoneal onlay mesh (IPOM) repair, the prosthesis is positioned underneath the hernia opening, allowing direct contact of the viscera and the prosthesis material. As with extra-peritoneal mesh repair, this procedure can also be performed with an open surgical approach or using laparoscopic technique. The most common surgical procedure of this type is known as the transabdominal preperitoneal (TAPP) procedure, which is performed with a laparoscopic technique. Briefly, the herniated tissue is first reduced back into the abdominal cavity and any significant adhesion (per
the surgeons’ opinion) is examined/repaired prior to mesh placement. The mesh prosthesis is then placed over the hernia opening with at least a 3 – 4 cm overlap of the mesh and the margins of the hernia, with even greater overlap recommended if the opening has a diameter greater than 4 cm. In some cases, surgeons prefer to use a “double crown” fixation technique, where two rows of tacks are placed as this is thought to reduce the risk of recurrence. In addition, the TAPP procedure is the most common non-open surgical hernia repair procedure performed as it has a relatively small learning curve for the surgeon and shorter operation time as compared to the TEP mesh repair.66

3.3. Clinical Complications

To improve the clinical outcome for hernia repair, surgeons began using surgical mesh devices in the 1960s; this approach is now the gold standard of hernia repair due to a perceived reduction in recurrence rates.73 A recent evaluation of clinical data indicates that long-term recurrence rates (≥ 10 years) after inguinal hernia repair with mesh show no advantage and that hernia recurrence may be as common today as it was in the 1980s, prior to the widespread acceptance of mesh hernioplasty (i.e. hernia recurrence may simply only be delayed using current surgical mesh prostheses). Furthermore, a number of long term clinical complications have been introduced. Mesh contraction, increased rigidity over time, entrapment of sensory nerves, and chronic inflammatory responses to the prosthesis, among other mesh-related factors, contribute to long-term complications such as chronic pain, increased abdominal wall stiffness and fibrosis.61,74–78
Chronic Pain

Chronic pain following mesh hernioplasty is a well-known and documented long-term complication that is often referred to as mesh inguinodynia (in the case of inguinal mesh repair), which is believed to be attributed to a number of mesh-related factors.\textsuperscript{74,75} According to recent publications, the prevalence of chronic pain after mesh hernioplasty ranges anywhere from 4 to 38\%, with some reports providing numbers as high as 62\%.\textsuperscript{74,76,77,79–81} The complete etiology is still unknown, but researchers suggest that the following factors are largely responsible: 1) irritation or damage of sensory nerves during surgery, 2) chronic inflammatory response to the mesh prosthesis, and 3) entrapment or compression of sensory nerves from fibrosis in and around the implanted mesh.\textsuperscript{75,77,78,82–85}

Mesh Contraction and Fibrosis

One of the most significant and interconnected issues is that of post-surgical mesh contraction or shrinkage. It is logical to assume that this mesh-related problem is responsible for several long-term complications, as mesh size reduction causing buckling and folding of the prosthesis, and can lead to increased abdominal wall stiffness and discomfort to the patient. Clinical examination of mesh contraction using radiographic measurement indicates a reduction in implanted polypropylene mesh sizes of approximately 8 and 20\% at postoperative month three and ten, respectively.\textsuperscript{85,86} This reduction undoubtedly increases the tension created at the mesh-tissue interface, as the mesh “pulls” inward as it contracts. Since hernia recurrence following mesh hernioplasty
most often occurs at the margins of the mesh (accounting for 99% of all mesh repaired recurrence), contraction of the mesh is a likely culprit responsible for these recurrences.\textsuperscript{87–91} Moreover, excessive scar tissue formation increases the stiffness of the abdominal wall following hernia repair. Compliance testing of explanted polypropylene meshes in one study indicated that 30 times more force was required to physically manipulate explanted meshes than the pristine prostheses.\textsuperscript{92} This increased rigidity not only causes physical restriction of the abdominal wall but, in extreme cases, can lead to erosion of the mesh into surrounding tissues and organs such as the urinary bladder.\textsuperscript{93} Interestingly, erosion from the use of current polypropylene mesh is not limited to hernia repair but is also seen for other surgical uses of these prosthetics. A recent Food and Drug Administration (FDA) safety communication warns about the serious complications associated with trans-vaginal placement of surgical polypropylene mesh for pelvic organ prolapse, indicating that frequent complications of mesh erosion through the vagina have been reported.\textsuperscript{94}

3.4. Mesh Biomechanics

Although the foreign body response to current mesh materials may be responsible for many of the long-term complications currently associated with mesh hernioplasty, the effect of mesh biomechanics may represent the missing link that has been slowing advancements in this field for the past 30 years. Tissues of the human body detect and respond to mechanical stimuli in a manner similar to the process observed in the remodeling of bone (i.e. form follows function).\textsuperscript{30,31} It has been shown that increased or
decreased loading of collagen lattices results in extracellular matrix remodeling via changes not only in structure, but also composition.\textsuperscript{95} Therefore, it is imperative to obtain a good understanding of how mesh biomechanics can be modulated to dynamically assist the wound healing process in order to improve clinical results.

\textit{Early Wound Stability}

Mechanical disruption of the wound site during initial collagen deposition, prior to the establishment of sufficient strength, can lead to hernia recurrence, especially in incisional/ventral hernia cases.\textsuperscript{37} Pollock and colleagues examined the long-term rate of incisional hernia development after initial failure or wound disruption of laparotomies following abdominal surgeries.\textsuperscript{54} It was shown that 17 of 18 patients (94\%) with laparotomies that were mechanically disrupted (wound-edge gap greater than 12 mm at 1-month follow-up) within the first month resulted in hernia formation within 3 years. In another retrospective study, the computed tomography (CT) scans of 64 patients that had undergone midline laparotomies were examined to determine the ability of predicting an incisional hernia occurrence.\textsuperscript{55} Interestingly, the researchers were able to predict if an incisional hernia was forthcoming 92\% of the time by measuring the distance between the left and right rectus abdominis muscles approximately 1 month after surgery. These results indicate that wound stability during the early phases of collagen deposition may be required to prevent damage to the delicate and developing granulation tissue.
The mechanical properties of present day hernia meshes are vastly different than the mechanical properties of the native abdominal wall. Mechanical testing of human abdominal wall samples reveals an elasticity of 18 – 32% at a physiological force of 16N/cm.\textsuperscript{96,97} In contrast, mechanical testing data of current hernia meshes reveals elasticity values that can range anywhere from 4 – 32% at 16 N/cm, with the vast majority (seven out of nine products tested in the cited study) below 16%.\textsuperscript{98} This long-term lack of elasticity will reduce patient mobility and discomfort at the implantation site as well as cause patient sensation of the mesh prosthesis. Additionally, any tissue encompassed by the mesh is, in effect, stress shielded. This stress-shielding effect could be responsible, in part, for mesh contraction; a lack of mechanical tension within tissues is known to signal fibroblasts to contract in order to re-establish a perceived loss of tissue integrity.\textsuperscript{22} Brown and colleagues examined this phenomenon within three-dimensional collagen lattices that were seeded with fibroblasts.\textsuperscript{99} Briefly, a small amount of tension was maintained on the collagen lattices after seeding with human dermal fibroblasts. The tension across the lattices was then reduced by approximately 53% and the force generated by the seeded cells was monitored. Unloading the scaffold in this manner resulted in an immediate increase in force generation by the resident human dermal fibroblasts, with tensions across the collagen lattice reaching those prior to the unloading step at 2 hours post-unloading. In essence, the seeded fibroblasts are acting in a way to maintain what is perceived as normal tensional homeostasis. Therefore, a mesh
construction that eventually matches the mechanical properties of the native abdominal wall may improve clinical outcome by sharing the load with the native tissue.

Long-term mesh extensibility may also reduce the number of activated myofibroblasts, which are known for their ability to cause wound site contracture. The biomechanics of the repaired wound site plays a key role in initiating and controlling myofibroblast differentiation and function. For example, when fibroblasts are grown on a soft substrate, differentiation of myofibroblasts is suppressed. Yeung and colleagues investigated this phenomenon by culturing fibroblast on polyacrylamide gels with varying elastic moduli ranging from 180 to 16,000 Pa. Through fluorescence staining of the actin fibers, it was shown that stress fiber development and orientation increased with substrate rigidity. Furthermore, the myofibroblast phenotype was only produced on substrates with an elastic modulus of 3000 Pa or higher. In another study, it was shown that ECM compliance also controls myofibroblast focal adhesion size as well as α-SMA (hallmark of myofibroblast differentiation) localization. When myofibroblasts were cultured on low modulus/flexible substrates (9.6 kPa), focal adhesion size was approximately 1.5 to 5.0 μm long. Alternatively, when cultured on high modulus/stiff substrates (16 kPa), focal adhesion size was approximately 8.5 to 9.9 μm long. This is important because, as focal adhesion size increases, the amount of stress capable of being applied by the myofibroblast also increases. In addition to increasing focal adhesion size, contraction of myofibroblasts against a stiff substrate can also cause the release of latent TGF-β1, a growth factor known to cause myofibroblast differentiation. The proposed mechanism, as shown in Figure 1.3, is the release of TGF-β1 from the large
latency complex (LLC) used for cell attachment to the ECM. As suggested by these studies, a mesh design that remains stiff and non-compliant for the life of the patient may initiate an ECM-stiffening cycle through chronic myofibroblast activation.

Figure 1.3. A – When the large latency complex (LLC) is anchored in a stiff ECM, myofibroblast contraction can lead to the release of TGF-β1. B – A compliant ECM may absorb the deformations generated by myofibroblast contraction and prevent the release of latent TGF-β1.
IV. Animal Models Used to Investigate Hernia Development and Treatment

One of the main tools bioengineers employ in studying medical devices, such as surgical meshes, is the animal model. To gain the most useful information from this testing, the applicability of the animal model is of utmost importance as it must, as closely as possible, mimic the human situation. This in vivo assessment allows researchers to discover potential adverse reactions to a device prior to testing in a clinical setting. Current animal models used to study hernia repair are not perfect and often do not replicate the chronic wound pathology associated with hernias in humans. These models can generally be broken down into two main categories or approaches. The first involves resection or en-bloc removal of a section of the abdominal wall in the animal followed by immediate repair of the created defect via mesh hernioplasty. The second approach can be further broken down into two steps: 1) creating the abdominal wall defect, usually by making a fascial incision through the abdominal wall and 2) allowing the defect to mature into a ventral hernia (2 – 4 weeks) followed by evaluating and repairing via mesh hernioplasty. Below, the advantages and disadvantages of each are further discussed.
Acute Defect Creation Model

In the acute defect creation procedure, a section of the abdominal wall musculature is removed from the animal and repaired using mesh hernioplasty, all in the same surgery. The procedure results in a “clean” surgical site for mesh placement, where the fascial edges of the created hernia are otherwise healthy and devoid of the ongoing chronic inflammatory response generally found in clinical hernias in humans. A large number of these type of procedures has been described, typically using a rat model that is generally considered as being “validated” in this field for the study of hernia mesh performance.104–111 Briefly, in this surgical procedure, a midline incision is first made through the skin, followed by blunt dissection to separate the skin from the abdominal wall musculature, creating a workable subcutaneous surgical space. A full-thickness defect is then created distal to the xiphoid process, where a small section of the rectus muscle (including peritoneum) is removed. Immediately following defect creation, a mesh is used to repair the created hernia by either using an on-lay approach or by suturing the mesh directly to the fascial edges of the created wound. Similar procedures have been reported using rabbit, pig, and sheep models.112–116 The main benefit with these larger animal species is that the abdominal wall forces in large animals is thought to more closely replicate the biomechanical abdominal wall forces found in humans, thus creating a more clinically relevant situation.103 In addition to en bloc removal of an actual section of the abdominal wall, a simple laparotomy through the abdominal wall muscle and peritoneum, whereby all of the native tissue is preserved, is also reported in the literature.117,118
The overall advantage of the acute defect creation model is complete control and good repeatability over the created defect, as this is completely surgically created within a single procedure (i.e. the defect site will have almost the exact same presentation across each animal within a study). In addition, study costs are drastically reduced as only one operation is required per animal. The main disadvantage is that the created defect site will not be representative of a true mature hernia, which typically presents with a hernia sac, various visceral adhesions, and/or compromised fascial edges. This disadvantage increases the risk of missing important biocompatibility concerns until pivotal trials are conducted in humans (i.e. a positive outcome in the acute defect creation model does not necessarily translate to a positive outcome in the clinical setting).

**Mature Defect Creation Model**

As the name implies, the ideal result with the mature defect creation procedure is creation of a mature hernia that has a similar presentation as would be found in the clinical setting. This approach requires two separate operations: one to create the abdominal wall defect and one to repair the hernia after it is allowed to mature for 3 – 5 weeks. A number of approaches have been examined with various sized animal species. The first, described by DuBay and colleagues using Sprague-Dawley rats, involves creation of a laparotomy located distal to the xyphoid process which is partially repaired using only two throws or knots of a 5-0 plain catgut suture.¹¹⁹,¹²⁰ Since the catgut suture is rapidly absorbed (≤ 1 week), physiological load is introduced back onto the healing
wound prior to sufficient strength development. As mentioned in Section 1.3, a study of
wound tensile strength development in an incisional rat model indicated that at 7-days post-op the wound strength is only approximately 3.98% that of the native unwounded tissue.\textsuperscript{21} The low strength led to the creation of a chronic ventral hernia as the created wound ultimately failed, allowing viscera to push out into the preperitoneal space as shown in Figure 1.4A and 1.4B.

In a similar mature hernia creation procedure developed using rabbits, a median laparotomy is created approximately 10 – 12 cm from the xyphoid process that is 4 cm in length.\textsuperscript{121} Unlike the procedure described previously in rats, the laparotomy is left completely unrepaired and the skin is simply sutured closed overtop. In this fashion, the hernia is present throughout the entirety of the maturation period which, for this particular study, was 30 days, after which, the developed hernia sac was isolated and resected, then repaired with a polypropylene mesh.
The two procedures described above involve laparotomies that travel through the peritoneum and into the abdominal cavity. Additional procedures have been investigated that involve a more subtle approach, where the peritoneum is completely preserved in the initial creation surgery.\textsuperscript{122,123} Jenkins and colleagues developed such a procedure in a porcine model (Yucatan Mini-pigs) for mature ventral hernia creation.\textsuperscript{122} Briefly, a 5-cm longitudinal incision is made into the skin, subcutaneous fat, fascia, and abdominal wall muscle layers, but not through the peritoneum. The abdominal wall musculature is then left unrepaird while the skin is closed overtop with interrupted 3-0 poly-$p$-dioxanone (PDS) suture. In addition, a small amount of cyanoacrylate-based dermal glue was used over the sutured site to create a barrier against fecal contamination. The created defects were then left untreated for 21 days, resulting in the creation of mature ventral hernias.
In this way, the viscera were never allowed to come in direct contact with the subcutaneous tissue, forming a true hernia sac. In a similar procedure developed in rabbits, a 3 cm² section of the abdominal wall musculature is removed while preserving the peritoneum.\textsuperscript{123} Within 3 weeks postoperative, a mature ventral hernia develops with a distinct hernia sac, as shown in Figure 1.5.

\textbf{Figure 1.5.} Abdominal protrusion of ventral hernia 21 days post-op (A).

Dissected hernia sac as prepared for resection (B).

The main advantage of using a mature defect creation model is that it most closely simulates the wound pathology associated with mature hernia development in humans, often resulting in a defined hernia ring, hernia sac, and visceral adhesions. Table 1.1 below provides a summary of the reported mature defect creation models to date. As compared to the acute defect creation model, the number of studies employing mature ventral hernia models is scarce, although these models seem to provide more clinically relevant scenarios for studying hernia repair. One of the main reasons for the lack of use
may be the increased cost of this type of animal study, as it requires two separate surgical operations for creation and repair. The ventral hernia surgical approach also increases the length of time the animals must be maintained as there is a 21 to 30 day hernia maturation period before the actual mesh being studied can be implanted. In addition, because the hernia is allowed to mature on its own, the clinical presentation from animal to animal is different, although this may also be seen as a positive as it increases the chance of finding situations where the studied product may be contraindicated.

Table 1.1. Animal Models of Mature Ventral Hernia Creation.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Animal Species</th>
<th>Peritoneum Preserved (Yes/No)</th>
<th>Maturation Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dubay et al.\textsuperscript{119}</td>
<td>2005</td>
<td>Sprague-Dawley Rats</td>
<td>No</td>
<td>28 days</td>
</tr>
<tr>
<td>Silva et al.\textsuperscript{123}</td>
<td>2009</td>
<td>New Zealand White Rabbits</td>
<td>Yes</td>
<td>21 days</td>
</tr>
<tr>
<td>Minossi et al.\textsuperscript{121}</td>
<td>2010</td>
<td>New Zealand White Rabbits</td>
<td>No</td>
<td>30 days</td>
</tr>
<tr>
<td>Jenkins et al.\textsuperscript{122}</td>
<td>2010</td>
<td>Yucatan Minipigs</td>
<td>Yes</td>
<td>21 days</td>
</tr>
</tbody>
</table>

V. Methods of Characterizing the Wound Healing Process

The purpose of the wound healing process is to restore functional integrity to the compromised area. In order to determine how well a specific therapy or device is assisting in this endeavor, one must be able to evaluate key markers for success. Fortunately, a number of tools are available to grade and assess the progress of the wound healing process. These include but are not limited to mechanical testing to determine the
elasticity and ultimate strength of the wounded area, histological analysis to determine overall cell densities and specific cell types at the wound site, as well as determination of ECM quality and protein composition. The wound healing trajectory is a series of precisely orchestrated events as shown in Figure 1.6. Through testing, one can determine where along this trajectory a specific device is aiding or retarding the wound healing process in order to improve clinical outcomes.

![Figure 1.6. The normal wound healing trajectory.](image)
5.1. Mechanical Testing

Mechanical testing is used to determine the overall integrity of the healing wound as it is the overall product of the wound healing process (i.e. the main purpose of wound healing is to re-establish integrity and strength). A chronic inflammatory response to a material may lead to excessive collagen deposition and fibrosis, causing loss of mobility (higher modulus of elasticity), while chronic infection may lead to excessive atrophy of the surrounding tissue due to a high density of immune cells, leading to poor strength development and ECM deposition. In addition to assessing the overall wound integrity, this type of testing can also be used to determine how well a device is integrated with the native tissue.

The vast majority of mechanical testing on healing wounds, especially in cases of soft tissue repair with a surgical mesh, is often simple tensile testing on small strips of the tissue/mesh complex as this allows researchers to maximize the use of explanted tissue and, depending on strip size, often permits multiple runs from each animal. In a study examining a porcine-derived acellular dermal matrix for incisional hernia repair, the explanted tissue was cut into 1 x 6 cm strips which were then tested using an Instron 5865 tensile tester fitted with pneumatic grips. In another study using a rabbit incisional model that was repaired with a standard polypropylene mesh, the researchers cut the explanted region into three 1-cm wide strips which allowed one strip for mechanical testing, one for histology, and one for protein analysis. Paul and colleagues successfully studied incisional wound strength development in a rat model.
which used 0.3 cm x 3.0 cm long strips for tensile testing, allowing up to six strips to be created per animal.20 Fresh tissue has a tendency to slip in tensile grips, which are generally designed for the testing of textile fabrics. The researchers were able to overcome this slippage by lining the grips with Emery paper (grade P400) and securing the tissue strips to the fixture using Loctite 406 adhesive. Although testing of tensile strips in this fashion has a number of advantages, one main disadvantage is that the loading of the tissue is not physiological. Load on soft tissues of the abdominal wall is generally not confined to one axis. As such, ball-burst testing for abdominal wound healing models may be more appropriate, as it more closely mimics how the tissue would be loaded in vivo.

In a study of the biomechanical assessment of an absorbable silk-derived surgical mesh in an abdominal wall defect rat model, ball-burst testing of the mesh-tissue complex was successfully conducted.105 Full-thickness explants were harvested with an approximate size of 35 x 35 mm. Ball burst testing was conducted with an Instron 8871 mechanical testing equipment with a stainless steel burst fixture and a 10-mm diameter stainless steel ball. The sample was loaded on the burst fixture with the mesh-tissue complex centered under the loading axis (see Figure 1.7 below) and burst with the stainless steel ball traveling at a rate of 60 mm/min. The final surface area of the tested explant was 272 mm². Ultimate burst strength and stiffness (reported in N/mm) were recorded. Unfortunately, this type of testing results in destruction of the entire explant and requires additional animals if histological assessment is planned.
Figure 1.7. Ball burst testing; a 10 mm diameter ball pushed through the defect site reinforced with mesh.\cite{105}

5.2. Histological Analysis

Histology is performed to examine the cellular and extracellular matrix composition, specifically to examine the type and amount of cellular infiltration into the wound site, degree of vascularity, and the quality of the ECM connective tissue that is deposited.

Cellular Examination

A number of stains are available but typically hematoxylin and eosin (H&E) is employed as a general or routine stain for cellular identification and to determine general granuloma thickness in animal studies of hernia repair.\cite{105,109,110,116} Masson’s trichrome staining can be used to examine collagen formation and orientation as well as for cellular identification, often proving to be a better general stain than H&E. In a study of the histopathologic host response of a polypropylene surgical mesh in a rat abdominal wall
defect model, Masson’s trichrome staining was successfully used to evaluate a number of aspects related to surgical mesh biocompatibility, including vascularization, quantification of inflammatory cells, collagen quality, and general tissue identification. However, when specificity is required, immunohistochemistry (IHC) staining can be employed, for example, to target staining of a specific cell line. A classic marker, indicative of a foreign body reaction and continuation of the inflammatory phase of the wound healing process, the macrophage can be targeted by IHC staining for CD68. CD68 is a transmembrane glycoprotein that is highly expressed by monocytes and tissue macrophages. Junge and colleagues used IHC staining to examine infiltrating macrophages when examining surgical mesh biocompatibility, showing high concentrations of macrophages on the surface of the mesh fibers. In the same study, immunofluorescent staining of activated myofibroblasts was conducted by using antibodies against α-SMA.

**ECM Evaluation**

As discussed in Section 1.4, wound strength dramatically increases during the remodeling phase of the wound healing process, as the ratio of Type I (mature) to Type III (immature) collagen increases. Therefore this ratio can be considered a critical to quality characteristic for ECM evaluation in the healing wound. A number of methods have been developed to quantify the ratio of Type I to Type III collagen. One commonly used approach is to stain tissue slides with picrosirius red to view by cross polarization microscopy (CPM). When viewed under polarized light, Type I collagen looke orange
to red while Type III collagen appears green. Through the use of various types of image analysis software, a number of investigators have been able to generate a ratio based on these differing shades of color. In addition to CPM with Sirius red staining, IHC can also be used to separately stain for Type I and III collagen. Total collagen to protein ratios can also be assessed using picrosirius and fast-green staining. In this procedure, tissue “blocks” are stained with Sirius red (collagen stain) and fast-green (protein stain) separately. The stain is then dissolved off the tissue using 0.1 N sodium-hydroxide in methanol and examined using spectro-photometrical analysis at the specific wavelength of each dye. The absorbance of each can then be compared to obtain a ratio of collagen to total protein at the wound site.

VI. Conclusions and New Opportunities

Based on a review of the literature it is apparent that a surgical mesh construction that is structurally stiff for the lifetime of the patient is not ideal and may be responsible for many of the long-term complications seen with surgical mesh in soft tissue repair situations. Traditionally, from an engineering perspective, hernia mesh was constructed to be of high strength to produce a perceived robust repair; however, this approach lacks the biological consideration of the wound healing process, which is dynamic and constantly evolving. Therefore, soft-tissues repair devices are required to act as in situ tissue engineering devices that modulate their properties with the changing needs of the wound healing process, as shown in Figure 1.8 below.
**Figure 1.8.** As the wound builds strength over time, less support is required by the surgical mesh, but some support may be necessary indefinitely.

The ideal mesh would possess early mesh stability to prevent early wound disruption and long-term mesh extensibility to facilitate the development of functional tissue in order to realize an improved clinical outcome. Investigation into a fully absorbable surgical mesh has identified a mesh construction capable of meeting these design variables. However, further research is required to determine how long the initial repair site needs to be stabilized prior to introduction-sharing of physiological loads. In addition, as hernia development is thought to be an outcome of abnormal connective tissue formation, the requirement of some stabilization from a permanent implant is likely needed for long-term success. Table 1.2 below provides a summary of key surgical mesh design variables.
Table 1.2. Summary of Key Surgical Mesh Design Variables.

<table>
<thead>
<tr>
<th>Design Variable</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial/Early Mesh Stability</td>
<td>Prevent early wound disruption which has been shown to lead to hernia recurrence. Distribute stress to multiple attachment points when a load is applied.</td>
</tr>
</tbody>
</table>
| Long-term Mesh Extensibility     | Prevent…  
• …mechanical mismatch between the surgical mesh and highly elastic abdominal wall.  
• …long-term loss of mechanical loading (stress-shielding) of the tissue encompassed by the mesh.  
• …loss of abdominal wall mobility. |
| Mesh Transition Point            | Selection of the most appropriate time to begin transitioning the mechanical load onto the tissue is imperative for clinical success. Adequate collagen proliferation to develop surgical site stability is required prior to introduction of load. |
References


CHAPTER II
MULTIPHASIC HERNIA MESH DEVELOPMENT AND EVALUATION OF TEMPORAL MECHANICAL PROPERTIES

I. Introduction

An estimated 20 million hernia repair procedures are performed annually around the world making it one of the most common surgical procedures.¹ These are most commonly confined to the inguinal region in men but can present in various locations surrounding the abdomen. Today, most hernias are repaired through the use of a “tension-free” approach where the herniated area is reinforced through the use of a synthetic surgical mesh. In a recent report by the Food and Drug Administration (FDA), non-mesh or suture repair represents less than 10% of all hernia repair techniques.² As such, any advancements in the field of surgical mesh design and development has the potential to affect a large population of patients through the reduction of current long-term complications.

Usher introduced the first knitted monofilament polypropylene mesh into clinical practice in 1963.³ Today, common surgical mesh used worldwide are still knitted monofilament polypropylene that essentially use the same basic mesh design invented by Usher over half a century ago. Partially absorbable surgical meshes that currently exist in the marketplace still “piggyback” upon Usher’s initial design. Two of the most popular, Vypro® II Mesh (polypropylene/polyglycolide-co-lactide 90:10) and UltraPro™ Mesh
(polypropylene/polyglycolide-co-caprolactone 75:25), are shown in Figure 2.1. The purpose of these current partially absorbable mesh constructions is to reduce the overall final mass of a patient’s terminal prosthesis. As such, the degradable yarn component, or partially absorbable portions, of the mesh is simply plied in with a standard non-absorbable polypropylene yarn. While this effectively reduces the final mass of the device, the standard non-absorbable portions still realize the same long-term problems of rigidity and increased rigidity over time (as discussed in the previous chapter). In other words, the final outcome of current partially absorbable meshes is a non-absorbable mesh which has no significant change in extensional characteristics of the mesh upon degradation of the absorbable component. It is thought by the present researcher that a significant change in the extensional characteristics of the surgical mesh is required to develop functional tissue and improve the clinical outcome of mesh hernioplasty.

Figure 2.1. Vypro® II Mesh (left) and UltraPro™ Mesh (right).

The specific aim of the present study is to develop and evaluate the temporal mechanical properties of various absorbable and non-absorbable bi-component mesh
constructions when placed in an *in vivo*-like environment. To this end, the following items were completed and are discussed: 1) synthesis and characterization of various absorbable candidate materials and subsequent conversion into mono or multi-filament fibers, 2) creation of bi-component warp-knit selectively absorbable surgical mesh variants using the developed absorbable fibers along with standard non-absorbable fibers, and 3) evaluation of the *in vitro* conditioned physical and mechanical properties of each developed bi-component surgical mesh system with comparison to a current predicate device (UltraPro™ Mesh).

II. Materials and Methods

*Polymer Synthesis and Yarn Formation*

A total of three degradable fiber-forming polymer systems were created for this phase of work, as described in detail below:

- Tri-axial, segmented co-polyester polymer (MG-17) – This material was created to realize a 2 to 4 week strength retention profile. MG-17 consists of a 8/92 (wt.%) poly(trimethylene carbonate)/95:5 glycolide:L-lactide and is synthesized using procedures previously described by Shalaby.\(^4\) Briefly, the method entails (1) preparing a poly-axial pre-polymer by placing under a nitrogen atmosphere a mixture of trimethylene carbonate (TMC) monomer, trimethylolpropane as the tri-functional initiator, and stannous octanoate as the catalyst in a pre-dried stainless-
steel resin kettle equipped for mechanical stirring; (2) allowing the pre-polymer to form by heating at 160°C until practically complete conversion is achieved (as determined by GPC); (3) adding a mixture of glycolide and l-lactide to the viscous pre-polymer at 140°C; (4) conducting the end-grafting at 160°C in the melt followed by solid state polymerization (no stirring) until essentially complete conversion of the monomers is achieved.

- Linear, hydrophilic co-polyester polymer (USD-6) – A linear polyester was produced from 1,4-dioxane-2-one using a polyethylene glycol (PEG) initiator to obtain a fiber with a functional use period of approximately 6 – 8 weeks.

- Linear, segmented co-polyester polymer (USLG-1) – A fiber-forming, swellable co-polyester, USLG-1, was prepared to obtain a fiber with a functional use period of approximately 16 – 20 weeks. The material composition for USLG-1 consists of 94/6 l-lactide/glycolide that is initiated with PEG 20000, where the PEG component makes up approximately 8 wt.% of the final polymer. Synthesis conditions are similar to those described for MG-17, in that synthesis is initiated in the melt followed by solid state polymerization at 160°C until essentially complete conversion of the monomers is realized.

Conversion of MG-17 and USLG-1 into a multifilament yarn was conducted using a custom ¾ inch single screw melt extruder (Alex James and Associates Inc.) equipped with a metering pump, 10-hole spinneret, and air quench/diffuser set-up. Fiber orientation was accomplished in-line using a two-stage drawing process with three separate high speed godets heated to 70 - 110°C to achieve a final draw ratio between 2 –
3X with a final fiber denier of 140 – 160 g/9000meters. A non-absorbable multifilament yarn was created with similar methods and specifications using a purchased polypropylene resin. USD-6, due to the slow crystallization rate of poly-dioxanone polymer, was extruded into a monofilament using the same extruder described with the following exceptions: a single-hole spinneret was used with a water quench bath oriented directly below the spinneret. This monofilament was then oriented using a slow-speed two-stage drawing method to achieve a final diameter of 0.15 – 0.20 mm.

*Polymer and Yarn Characterization*

The materials used as part of this study were characterized at various stages of development using the analytical methods outlined in Table 2.1.

**Table 2.1. Material Characterization Methods.**

<table>
<thead>
<tr>
<th>Characterization Method</th>
<th>Equipment</th>
<th>Reference</th>
<th>Output/Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential Scanning Calorimetry (DSC)</td>
<td>Perkin Elmer Pyris 6 DSC</td>
<td>ISO 11357-1, ISO 11357-3</td>
<td>Thermal transitions</td>
</tr>
<tr>
<td>Solution Viscosity</td>
<td>Tube Viscometer</td>
<td>ASTM D2857-95(2007)</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>Fiber Tensile</td>
<td>MTS Synergie w/ 500 Newton Load Cell</td>
<td>ASTM D2256-09</td>
<td>Maximum break strength</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Elongation at break</td>
</tr>
</tbody>
</table>

To determine the melting temperature ($T_m$) and degree of crystallinity (Heat of Fusion, $\Delta H_f$) of the raw polymer and subsequent fiber, a DSC was conducted using the following method: a 5 – 10 mg sample was heated from 20 to 240°C at a rate of
Analysis of the resulting thermal graph was conducted using Perkin Elmer Pyris 6 Software Version 7.0 using the sigmodal function, where applicable, in accordance to ISO 11357-3 “Plastics Differential Scanning Calorimetry: Determination of temperature and enthalpy of melting and crystallization”. Molecular weight of the polymer was determined by one of two ways: gel permeation chromatography (GPC) and/or solution viscosity. The available GPC uses dichloromethane (DCM) as the mobile phase solvent; therefore only high lactide materials could be analyzed with this system. For all others, including high lactide materials, solution viscosity was performed in HFIP (0.1 g/dL sample concentration, Cannon-Fenske Routine Size 50 Viscometer) following applicable ASTM and Poly-Med specific standards as listed in Table 2.1. Fiber tensile testing was conducted using MTS Synergie mechanical testing equipment and software equipped with a 500 Newton load cell and fiber testing grips as described by ASTM D2256-09 “Standard Test Method for Tensile Properties of Yarns by the Single-Strand Method”. Mechanical properties of breaking strength and elongation at break were recorded.

Creation of Warp-knit Mesh Constructs

Bi-component warp-knit meshes were produced on an 18-guage LIBA Racop 4-O knitting machine using a total of four (4) separately warped beams of yarn. Beams 1 and 2 were warped with the created polypropylene yarn using a 1-ply configuration with 89 individual ends per beam. Beams 3 and 4, depending on the mesh construction type, were either warped with MG-17, USD-6, or USLG-1 using a 1-ply configuration and 89
ends per beam. Herein, mesh containing MG-17, USD-6, or USLG-1 will be referred to respectively as SAM-3, SAM-6, and SAM-18 mesh. The following describes the 4-bar knitting pattern which was conducted at 30 courses per inch to create each mesh prototype: guide bars 1 and 2 (containing polypropylene yarn) followed a sand-fly net knit pattern (pattern notation: [Bar 1] 1-0/1-2/2-3/2-1 and [Bar 2] 2-3/2-1/1-0/1-2) and guide bars 3 and 4 (containing the absorbable yarn component) followed a marquisette knit pattern (pattern notation: [Bar 3] 1-0/0-1/3// and [Bar 4] 0-0/3-3//). After knitting, SAM-3 and SAM-18 mesh were annealed while constrained in the wale and course directions at 130°C for 1 hour. SAM-6, due to the lower melting point of USD-6, was annealed at 80°C for 2 hours.

**Mesh Characterization**

The mechanical properties of each developed mesh was evaluated using an MTS Synergie 200 equipped with a 500 Newton load cell and a ball burst testing fixture made to the physical characteristics as described in ASTM D3787-01 “Test Method for Bursting Strength of Textiles Constant-Rate-of-Travers (CRT) Ball Burst Test”. As such, the final burst fixture geometry utilized a 25.4 mm polished steel ball and 44.45 mm diameter inside opening. Each mesh sample was cut into 57 x 57 mm square pieces for burst testing. Prior to testing, samples were pre-conditioned in saline solution at room temperature for 1-hour. A 0.1 N pre-load was used for each test to remove any slack in the mesh at which point the testing was initiated with the ball traveling 2.54 cm/min. until central mesh rupture occurred. For each test two characteristics were recorded: (1) the
maximum load prior to break and (2) the extension at a load of 71 N (used to determine % elongation at 16N/cm). This value was determine as the burst testing fixture has an opening of 4.44 cm: 4.44 cm x 16N/cm is equal to a straight load of 71N. In addition to this mechanical testing, mesh aerial density (g/m²) was determine for each construction.

In vitro conditioned burst testing was conducted in phosphate buffered saline (PBS, 100 mM, pH 7.4, 0.01% NaN₃) at 37°C for periods up to 26 weeks to assess changes in mesh mechanical properties over time. To determine the degree and rate of mass loss for these selectively absorbable mesh systems, mass loss testing was conducted as described by ASTM F1635-11 “Standard Test Method for In Vitro Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants” in standard (PBS, pH 7.4, 37°C) and accelerated (PBS, pH 7.4, 50°C) environments. Based on the Arrhenius Equation with an aging factor (Q₁₀) of 2.0, an accelerated aging factor (AAF) of 2.46 is estimated at 50°C.

III. Results

In order to develop fibers with various degradable profiles a total of three polyester based polymers were created and characterized as shown in Table 2.2. A minimum of 500 grams of each material was synthesized to allow sufficient fiber conversion for use in each mesh construction. The first material, MG-17, is a fast degrading high glycolide exhibiting a characteristic polyglycolide melting event between 210 – 230°C with an inherent viscosity over 1.0 dL/g. Next, a PEG initiated
polydioxanone (PDO) was synthesized with a resulting melting temperature between 105 – 110°C and an inherent viscosity of 1.43 dL/g. A melting event for the PEG portion of this material was not readily apparent on the thermograph but a sharp melting peak between 100 – 110 indicates good 1,4-dioxane-2-one monomer conversion. The final absorbable polymer created was a PEG initiated poly(l-lactide) with a melting point between 175 – 180°C, indicative of the highly crystalline poly(l-lactide) component. GPC of this material indicates a polydispersity index (PDI) of 2.76 with a molecular weight of 770,325 kDa. Thermal analysis of the polypropylene resin is provided as comparison and used for determination of proper extrusion conditions for fiber formation in subsequent steps.

**Table 2.2. Polymer Resin Characterization.**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Polymer Class</th>
<th>Thermal Properties</th>
<th>Molecular Weight</th>
<th>Inherent Viscosity (dL/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG-17</td>
<td>High Glycolide</td>
<td>T&lt;sub&gt;m&lt;/sub&gt; (°C)</td>
<td>ΔH (J/g)</td>
<td>X&lt;sub&gt;c&lt;/sub&gt;* (%)</td>
</tr>
<tr>
<td>214.3 ± 2.5</td>
<td>72.2 ± 6.07</td>
<td>43.2</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>USD-6</td>
<td>Polydioxanone</td>
<td>107.4 ± 1.6</td>
<td>61.4 ± 0.85</td>
<td>59.7</td>
</tr>
<tr>
<td>USLG-1</td>
<td>High Lactide</td>
<td>176.6 ± 0.6</td>
<td>70.41 ± 2.96</td>
<td>75.1</td>
</tr>
<tr>
<td>PP</td>
<td>Polypropylene</td>
<td>166.6 ± 1.1</td>
<td>68.1 ± 1.51</td>
<td>---</td>
</tr>
</tbody>
</table>

*ΔH<sub>m</sub>(glycolide) = 191.2 J/g<sup>5</sup>, ΔH<sub>m</sub>(PPD) = 102.9 J/g<sup>6</sup>, and ΔH<sub>m</sub> (lactide) = 93.7 J/g<sup>5</sup>
Polymers MG-17 and USLG-1 were extruded into a 10-filament count fiber with a target denier range of 140 – 160 g/9000m while the USD-6 polymer was extruded into a monofilament with a target diameter of 0.15 – 0.20 mm. Final characterization of each fiber can be seen in Table 2.3. The MG-17 multifilament yarn had a final denier of 151.6 g/9000m with a tenacity greater than 4.0 gf/denier; indicative of good fiber orientation development during the extrusion. By comparison, the USLG-1 multifilament fiber produced was not nearly as strong with a maximum tensile strength of 4.80 Newtons (N) resulting in a final tenacity of 3.31 gf/denier.

**Table 2.3. Fiber Characterization.**

<table>
<thead>
<tr>
<th>Yarn Type</th>
<th>Filament Count</th>
<th>Denier (g/9000m)</th>
<th>Diam. (mm)</th>
<th>Max. Break Force (N)</th>
<th>Elong. at Break (%)</th>
<th>Tenacity (gf/denier)</th>
<th>Peak Stress (psi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG-17</td>
<td>10</td>
<td>151.6</td>
<td>---</td>
<td>6.14 ± 0.21</td>
<td>65.7 ± 4.28</td>
<td>4.13</td>
<td>---</td>
</tr>
<tr>
<td>USD-6</td>
<td>1</td>
<td>219.5</td>
<td>0.18</td>
<td>11.38 ± 2.09</td>
<td>39.9 ± 7.91</td>
<td>5.28</td>
<td>81.7</td>
</tr>
<tr>
<td>USLG-1</td>
<td>10</td>
<td>147.9</td>
<td>---</td>
<td>4.80 ± 0.19</td>
<td>39.18 ± 2.04</td>
<td>3.31</td>
<td>---</td>
</tr>
<tr>
<td>PP</td>
<td>10</td>
<td>140.6</td>
<td>---</td>
<td>7.78 ± 0.12</td>
<td>97.2 ± 4.58</td>
<td>5.59</td>
<td>---</td>
</tr>
</tbody>
</table>

A total of three different selectively absorbable mesh constructions were created with the aforementioned fibers and compared to a predicate partially-absorbable material
(UltraPro®, Ethicon, Inc.). Each mesh was constructed using a combination of a sand-fly knit pattern (non-absorbable yarn) and a marquisette knit pattern (absorbable yarn). The initial mechanical properties of each developed mesh can be seen below in Table 2.4. In addition, a close up image (Figure 2.2) of the mesh depicts how the absorbable fiber provides initial support to the non-absorbable portion of the mesh.

Figure 2.2. Interdependent co-knit mesh construction used for developed meshes.
Table 2.4. Initial Mesh Mechanical Properties (bolded line item indicates predicate material).

<table>
<thead>
<tr>
<th>Mesh ID</th>
<th>Composition</th>
<th>Max Load</th>
<th>Elongation @ 16N/cm Force (%)</th>
<th>Area Weight (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-3</td>
<td>PP:MG-17</td>
<td>554.5 ± 34.2</td>
<td>7.04 ± 0.29</td>
<td>138.7 ± 2.0</td>
</tr>
<tr>
<td>SAM-6</td>
<td>PP:USD-6</td>
<td>547.5 ± 50.1</td>
<td>9.51 ± 0.38</td>
<td>186.3 ± 3.1</td>
</tr>
<tr>
<td>SAM-18</td>
<td>PP:USLG-1</td>
<td>358.7 ± 22.8</td>
<td>8.76 ± 0.16</td>
<td>133.9 ± 2.2</td>
</tr>
<tr>
<td>UltraPro™</td>
<td>PP:Monocryl</td>
<td>405.3 ± 15.6</td>
<td>11.92 ± 0.45</td>
<td>61.7 ± 1.5</td>
</tr>
</tbody>
</table>

In vitro conditioned mesh burst testing was conducted on each developed mesh in order to evaluate their biomechanical performance over various simulated use periods. In this way, the theoretical mesh transition point (i.e. transition from a mechanically stable construction to a mechanically compliant construction) for each mesh could be determined. The results of this effort can be seen in Figures 2.3 and 2.4. Upon degradation of their respective absorbable components the maximum burst strength for SAM-3, SAM-6, and UltraPro™ Mesh dropped significantly with final burst strength retentions (BSR) of 70.3%, 51.1%, and 50.1%, respectively, while SAM-18 exhibited no significant change over a 26-week period of testing. In addition, all constructions remained well above the 70N physiological loading condition as reported by Junge and colleagues for the human abdominal wall. Over this same testing period the elongation at 16N/cm changed significantly for each of the developed constructions from a
combined average of 8.4% to a final average of 25.5% following sufficient strength loss of each mesh constructions respective absorbable component. This transition point occurred between weeks 2 – 4, 6 – 11, and 17 – 24, respectively, for SAM-3, SAM-6, and SAM-18 mesh constructions. While UltraPro™ saw a marked reduction in burst strength, the extensional properties only marginally changed from an initial value of 11.9% at 16N/cm to a final value of 15.5% at 16N/cm.

**Figure 2.3.** Maximum burst load of developed constructions (SAM-3, SAM-6, and SAM-18) along with predicate material (UltraPro®) at various durations of *in vitro* degradation. Each data point represents the average of a minimum of 5 samples with the data label indicating the final time point taken. († = Values are significantly higher (p-value less than 0.05) than SAM-6, ‡ = Values are significantly higher (p-value less than 0.05) than UltraPro™, ‡ = Final value is significantly lower (p-value less than 0.05) than initial)
Figure 2.4. Elongation at 16N/cm for developed constructions (SAM-3, SAM-6, and SAM-18) along with predicate material (UltraPro®) at various durations of *in vitro* degradation. Each data point represents the average of a minimum of 5 samples with the data label indicating the final time point taken. († = Values are significantly higher (p-value of less than 0.05) then UltraPro™, ‡ = Final elongation values are significantly higher then initial elongation values)
In addition to mechanical burst testing, an *in vitro* conditioned mass loss study was conducted to determine how each selectively absorbable mesh construction degrades and losses mass over time; ultimately allowing determination of the terminal area weight for each mesh. Table 2.5 summarizes the results of this testing. With the exception of the SAM-6 construction, each mesh lost approximately 50% of it’s mass after full degradation of the absorbable yarn component. The terminal area weight for each developed mesh was also significantly higher than the predicate material in all developed constructions. The SAM-18 construction required the longest incubation period to realize full absorption of its absorbable yarn component (USLG-1); as expected for this high lactide polymer variant. A full mass loss characterization study was not conducted on the predicate material but the absorbable component of this mesh was removed using an accelerated method in a high pH PBS (pH 12.0) in order to determine the final % mass retention.
Table 2.5. *In vitro* Mass Retention Study Results (Note: Bolded value indicates terminal area weight of each construction)

<table>
<thead>
<tr>
<th>Mesh ID</th>
<th>Time Point (weeks)</th>
<th>% Mass Retention¹</th>
<th>Area Weight (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-3</td>
<td>4</td>
<td>85.0 ± 0.8</td>
<td>Initial: 138.7 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>64.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>58.8 ± 0.1</td>
<td>Final: 79.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>57.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>57.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>SAM-6</td>
<td>8</td>
<td>92.6 ± 0.1</td>
<td>Initial: 186.3 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>89.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>51.1 ± 1.2</td>
<td>Final: 62.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>34.3 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34²</td>
<td>33.5 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>SAM-18</td>
<td>8</td>
<td>98.8 ± 0.1</td>
<td>Initial: 133.9 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>95.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>81.6 ± 0.4</td>
<td>Final: 71.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>48²</td>
<td>70.1 ± 4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>126²</td>
<td>53.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>UltraPro™</td>
<td>Final³</td>
<td>56.1 ± 0.2</td>
<td>Initial: 61.7 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final: 34.6 ± 0.6</td>
</tr>
</tbody>
</table>

1. Each value is the average of 4 samples.
2. Accelerated conditioning at 50°C.
3. Absorbable component was removed from mesh using an accelerated method with PBS (pH 12.0) over a 1-week period.

### IV. Discussion

A total of three bioabsorbable polymers and yarns thereof were created for the mesh development portion of this study. These can generally be classified as a high glycolide (MG-17), poly-dioxanone (USD-6), and a high lactide (USLG-1) material. As
shown in Table 2.2, each polymer had a distinct melting point which represents the crystalline portion of each respective polymers major chemical component with no exothermic (crystallization) events visible in any of the thermographs. The presence of sufficient crystallinity in the raw material is necessary to aid in extrusion processing as an amorphous material will tend to cause bridging issues in the feed zone of the extruder and indicates poor crystallization potential due to polymer impurities and/or lack of sufficient polymerization. MG-17 was the first material synthesized for this work with a fiber degradation target or functional use period of 2 – 4 weeks. Thermal analysis of this material indicates a % glycolide crystallinity of 43.2% (ΔH of 72.2 J/g) when compared to the heat of fusion of a fully crystalline poly-glycolide (ΔH of 191.2 J/g). The reduced crystallinity of MG-17 here is likely due to its tri-functional structure as well as the presence of TMC and L-lactide monomers which will further act to disrupt the polyglycolide repeat unit preventing a high degree of crystallinity. This “scrambling” technique was employed in order to increase the flexibility of the final fiber as well as insure sufficient degradation within the required time frame as a higher crystalline material will be both less compliant and have a longer degradation profile. The second material, USD-6, was created to realize a degradation profile of 6 – 8 weeks when converted into a monofilament fiber. As such, the bulk of this material is comprised of poly(p-dioxanone) (PPD), which historically has a functional use period of 6 – 10 weeks in vivo when extruded and oriented into a monofilament suture. In a study conducted by Ray and colleagues the break strength retention of PPD suture (both 2-O and 4-O sizes) was approximately 14% at 8-weeks when implanted in the posterior dorsal subcutaneous
of Long Evans rats.\textsuperscript{8} It is important to note that while USD-6 can largely be considered a PPD, a small portion (%) of each polymer segment contains a PEG modality. This will act to increase the hydrophilicity of the bulk material as well as impart a higher degree of chain mobility. The third and final absorbable material, USLG-1, was created to realize a degradation profile of 16 – 20 weeks which was accomplished through the creation of a PEG initiated poly-L-lactide co-polymer. Traditionally, high l-lactide materials have shown long strength retention profiles. In one \textit{in vitro} study, the % retained strength of a molded poly-L-lactide device was found to be as high as 50\% at 30-weeks in an environmental conditioning at 37°C in a saline buffered solution.\textsuperscript{5} By incorporating a very hydrophilic polymer segment such a PEG in the center of each polymer chain, moisture is readily absorbed into the material to significantly increase the degradation rate. Furthermore, a small amount of glycolide is used to cause crystalline disruption and accelerate the degradation process. Thermal analysis of this material (Table 2.2) indicates a high crystallinity resin with a heat of fusion of 70.41 J/g (75.1\% L-lactide crystallinity). In addition, GPC analysis reveals a PDI of 2.76 indicating that the polymer chain size/length homogeneity is less than ideal as PDIs of less than 2.0 are preferred.

Each of the absorbable polymers mentioned above were extruded into a mono or multifilament fiber for use in mesh development. The main driver for selection of mono or multifilament fiber forms was to obtain fibers from each polymer that would result in a “soft” feeling mesh construction. Multifilament fibers are made up of a bundle of micro-filaments; therefore they are able to contour to bends with a smaller turn radius resulting in more compliant constructions. As such inherently stiff materials, due to a high degree
of crystallinity and/or a lack of bond mobility, were extruded into a multifilament fiber construction. These materials include MG-17, USLG-1, and polypropylene. USD-6 was extruded into a monofilament fiber form as PPD based polymers and co-polymers result in more compliant constructions with a lower modulus than glycolide and lactide based absorbable polymers. In addition, the slow crystallization rate of PPD polymers limits their use in a traditional multifilament extrusion line which relies on rapid crystallization rates or set-up of the material between the extruded spin head and the initial take-up rolls which is needed for in-line fiber orientation.

Each of the developed fibers were used to construct a bi-component mesh using a unique interdependent knit construction as previously reported by Peniston and colleagues. By using absorbable components with varied strength retention profiles we were able to create meshes possessing unique mechanical transition periods and fulfill the key surgical mesh design variables as discussed previously (see Chapter 1, Table 1.2). These transition periods are marked by a distinct and significant change in mesh extensional properties while maintaining sufficient strength to prevent mesh rupture. As shown in Figure 4, each developed mesh (SAM-3, SAM-6, and SAM-18) begins at an elongation at a force of 16N/cm of less than 10% which is lower than the current predicate material UltraPro™ (elongation of 11.92% at the same force). It has been reported that mechanical disruption of the wound site during initial collagen deposition can lead to hernia recurrence, particularly in incisional and ventral hernia repair cases. Therefore by having a mesh construction that remains stiff and shields the wound during this critical wound healing period, a reduction in ventral hernioplasty recurrence
may be realized. This stress shielding phase has varied durations based on each mesh construction: SAM-3 maintains an elongation of less than 10% for 2 weeks, SAM-6 maintains an elongation of less than 11% for 6 weeks, and SAM-18 maintains an elongation of less than 10% for 13 weeks. After this stress shielding phase, the developed meshes transition to a construction possessing a high degree of extension that matches the extensional profile of the native human abdominal wall which has been reported to be between 18 – 32% at a load of 16N/cm$^{14,15}$ The transition phase duration varies in lengths due to the bulk degradation mechanics of the selected absorbable materials. SAM-3 mesh which is constructed with a fast absorbing high glycolide material transitions from a stiff construct to a more compliant structure over a relatively short period (2-weeks) while SAM-6 and SAM-18 each have longer transition periods (5-weeks for SAM-6 and 8-weeks for SAM-18) as dictated by their respective absorbable components. By ultimately sharing the load with the native tissue, it is the thought of the present researcher that a more functional repair will be realized with a reduction of current clinical complications associated with mesh hernioplasty (i.e. fibrosis, chronic pain, reduced mobility, and recurrence). It is interesting to note that as each construction transitions into its more compliant form a significant drop in overall burst strength is noted in all meshes except for the SAM-18 mesh construction. As previously mentioned the absorbable component for SAM-18 is a 10-filament yarn of USLG-1 which had the lowest tensile break force of all the yarns created. Therefore this yarn is not contributing as greatly to the ultimate strength of the mesh construction as it fails in tensile much sooner than the PP fibers.
Examination of the initial and final area weight of these selectively absorbable mesh constructions (Table 2.5) reveals that each developed mesh is significantly heavier than the selected predicate material. A review of the literature concerning density of surgical meshes indicates that constructions with an areal density of greater than 70 g/m² are considered heavy weight meshes while an areal density of less than 40 g/m² is generally considered a light weight mesh.16–19 Today, surgeons are moving towards the use of light weight mesh constructions as this generally translates to a more flexible repair and reduced foreign body reaction as less material is terminally implanted into the patient. In addition, various clinical studies indicate that the use of heavy weight mesh constructions is attributed with a higher risk of adverse events such as fistula formation and chronic pain.18–20 While the developed selectively absorbable meshes have an initial areal density of 130 – 190 g/m² (much higher than ideal), after degradation and mass loss of their respective absorbable components the terminal areal density falls in the range of 60 – 80 g/m². The higher initial density construction imparts better handling characteristics for the surgeon while still minimizing the amount of material implanted long-term in the patient. As compared to predicate heavy weight meshes (namely Prolene™, Surgipro™, and Marlex™) which are composed entirely with a monofilament polypropylene fiber form, the developed constructions employ the use of multifilament polypropylene yarn which will result in a more flexible and compliant construction possibly reducing the associated risk as seen with higher areal density meshes.
V. Conclusion

The present study resulted in the development of three selectively absorbable mesh constructions each exhibiting a distinct temporal mechanical profile. The successful application of various absorbable polymeric systems to a unique co-knit construction resulted in meshes displaying (1) early mechanical stability to stress-shield the initial hernia repair site, (2) a gradual transfer of mechanical loads back onto the remodeling tissue, and (3) long-term compliance with extensibility similar to the human abdominal wall. The initial mechanical stability phase was modulated in each construction with mesh transitions occurring between 2 – 4 weeks (SAM-3), 6 – 11 weeks (SAM-6), or 17 – 24 weeks (SAM-18). By creating a suit of meshes in this fashion, the most appropriate time to begin introducing load back onto the tissue may be further explored in a clinical setting.
References


CHAPTER III
MECHANICAL EVALUATION OF MULTIPHASIC SELECTIVELY ABSORBABLE MESH IN A MATURE VENTRAL HERNIA MODEL

I. Introduction

Investigation of new biomaterials and devices often requires testing in animals to show the potential effectiveness of the device in a living model. The applicability of the animal model used is of utmost importance as it must, as closely as possible, mimic the human situation. This allows researchers to discover potential adverse reactions to the device prior to testing in a human clinical setting as well as verify that the design criteria used to develop the device are appropriate for the intended application, which can also be considered “Design Validation” in subsequent FDA submission documents. As hernia repair is one of the most common surgical procedures performed\(^1\), development and use of a hernia model that accurately replicates the wound pathology associated with mature hernias in humans is very important in studying new device design as this has the potential of affecting a large number of patients.

An acute hernia defect creation animal model is typically employed in the study of new hernia mesh products as this procedure is well documented in the literature\(^2\)–\(^14\), reduces, as much as possible, the amount of variability seen from animal to animal, and is less costly as the hernia creation and repair surgery are all performed in one surgery. In
this procedure a small section of the abdominal wall is resected (including muscle and peritoneal layers) in order to create the hernia model in the animal. In this way, the created hernias from animal to animal are nearly identical as they are completely generated in the surgical procedure typically using some sort of template to aid in repeatability. Immediately following hernia defect creation, the surgical mesh of interest is used to repair the hernia by either using an on-lay approach, where the mesh is simply sutured over the defect site, or by suturing the mesh directly to the facial edges of the created wound. At pre-determined time points the animal is euthanized and mesh is explanted for analysis of mesh performance.

In a more difficult but possibly more clinically relevant animal model, a defect is created in the abdominal wall in an initial hernia creation surgery. The created defect is allowed to mature resulting in the formation of a distinct hernia ring and sack with many of the complications and challenges seen in the clinical setting. This type of hernia maturation is typically seen in the human situation where the hernia develops over a period of weeks to months before it is successfully identified and repaired. A number of mature ventral hernia models have been reported in the literature including rat\textsuperscript{15,16}, rabbit\textsuperscript{17,18}, and porcine\textsuperscript{19} animal models with various procedures for creating the mature defect in the abdominal wall. In the procedure first reported by DuBay and colleagues, a 5 cm full-thickness incision is created in the abdominal wall of Sprague-Dawley rats\textsuperscript{15}. The created defect is temporarily repaired using a rapidly absorbing suture (5-0 Plain Catgut). Since the suture fails prior to the development of adequate tensile strength to prevent wound dehiscence, a ventral hernia is created within 1 to 2 weeks. In another
approach, the peritoneum is left completely intact and a small portion of the abdominal wall musculature is either compromised or excised in the location desired for hernia creation. Two such procedures have been successfully performed and described in a porcine and rabbit animal model. In the reported porcine model, a simple 5 cm incision is made through the muscle layers taking special care not to cut into the peritoneal layer. With the muscle layers compromised the, skin is sutured closed over top and the defect is allowed to mature into a ventral hernia over a period of 3 weeks. In this procedure, the abdominal wall musculature is completely preserved and simply cutting through the muscle layers is enough to result in hernia creation. Alternatively, in the reported rabbit model an area of the muscle must be completely resected in order to result in hernia development. In this procedure, a 3 x 1 cm metal frame was used as a guide to remove a 3 cm² section of the abdominal wall musculature, while preserving the peritoneum, located longitudinally along the linea alba on the umbilicus scar. The skin was closed over the created defect and allowed to mature over a period of 21 days. With this procedure the authors reported the formation of a stable ventral hernia in all animals with the presence of a hernia ring, sac, and visceral and/or omental adhesions. It was also noted that attempts at removing smaller areas of the abdominal wall muscle (2 cm² area) did not promote reliable hernia formation while removal of larger areas of the abdominal wall musculature (≥ 4 cm² area) was associated with high rates of complication (skin dehiscence and posterior wound infection). A third and possibly more reliable procedure is reported by Minossi that involves simply making a 4 cm full-thickness incision (through the muscle and peritoneal layers) approximately 10 to 12 cm below the xyphoid
process. Unlike the previous procedure reported by DuBay where the created incision is temporarily repaired using catgut suture, in this case the incision through the abdominal wall was left open as the skin is closed over top. The created defect was then allowed to mature for a period of 30 days resulting in an incisional hernia.

In the present study segment, the bi-component meshes that were developed and discussed as part of Chapter 2 will be evaluated in a clinically relevant animal model that simulates the wound pathology associated with mature hernia development in humans. As such, the following items were completed and are discussed: 1) creation of a clinically relevant mature ventral hernia animal model in rabbits and 2) evaluation of developed meshes against predicate material as it relates to mesh repair site mobility, post-surgical mesh contraction, visceral adhesion formation, and incidence of hernia recurrence.

II. Materials and Methods

Warp-knit Mesh Constructs

A total of three separate mesh constructions, including the predicate material, were examined in this study. Table 3.1 provides a summary of each mesh composition including a brief overview of key performance features. As discussed in Chapter 2, a total of three selectively absorbable constructions were developed to exhibit biomechanical phase transitions between 2 – 4 weeks (SAM-3), 6 – 11 weeks (SAM-6), and 17 – 24 weeks (SAM-18). As the phase transition for SAM-18 is greater than the
longest implantation for this in vivo study it is being excluded from this round of evaluation.

Table 3.1. Selectively Absorbable Mesh Constructions

<table>
<thead>
<tr>
<th>Mesh Type</th>
<th>Fiber Type</th>
<th>Fiber Description</th>
<th>Phase Transition Period*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM 3</td>
<td>Non-absorbable</td>
<td>10-filament yarn of polypropylene</td>
<td>2 – 4 weeks</td>
</tr>
<tr>
<td></td>
<td>Absorbable</td>
<td>10-filament yarn of a polyglycolide</td>
<td></td>
</tr>
<tr>
<td>SAM 6</td>
<td>Non-absorbable</td>
<td>10-filament yarn of polypropylene</td>
<td>6 – 11 weeks</td>
</tr>
<tr>
<td></td>
<td>Absorbable</td>
<td>Monofilament yarn of a poly-dioxanone</td>
<td></td>
</tr>
<tr>
<td>UltraPro™</td>
<td>Non-absorbable</td>
<td>Monofilament of polypropylene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absorbable</td>
<td>Monofilament of a polyglycolide-co-</td>
<td>No significant transition in extensional properties seen during in vitro testing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>caprolactone</td>
<td></td>
</tr>
</tbody>
</table>

*Period over which the mesh transitions from a stable construction (< 10% extension at 16N/cm load) to a more compliant construction (> 18% extension at 16N/cm load)

Development of Hernia Animal Model

All animal work was performed at the Godley-Snell Research Center (Clemson University) under Animal Use Protocol 2013-005 using female New Zealand White (NZW) rabbits that were at least 5 to 6 months of age at the time of the initial surgery. This age range was selected to ensure that the animals would not be significantly changing in size as this may have led to mesh complications and resulted in confounding
results. Prior to surgery each animal received 1.1 mg/kg Acepromazine, 33 mg/kg Ketamine HCL, 0.02 – 0.05 mg/kg Atropine, and 0.05 mg/kg Buprnnorphine as pre-anesthetics. During each surgery 1 – 4% isoflurane in oxygen was used to maintain anesthesia. At the conclusion of each surgery (both hernia creation and mesh repair) a Fentanyl patch (Duragesic 25 ug/hr) was placed either on the sternum or directly on the back of the animal as a multimodal analgesia. When the pre-determined study time point was reached, animals were given heparin (500 units) by subcutaneous injection prior to euthanasia with Fatal Plus® (pentobarbital sodium) administered intravenously at 1.0 ml/10 pounds.

**Hernia Creation Procedure A – Pilot Animal No. 1**

Starting 2 cm below the xiphoid process a midline incision measuring approximately 4 cm was created through the skin and subcutaneous fat. Blunt dissection was performed around the incision to create a workable subcutaneous surgical space. Approximately 3 cm below the xiphoid process and centered along the linea alba a 3 x 1 cm section of the abdominal wall musculature was excised while taking special care to leave the peritoneum intact. Following defect creation skin closure was accomplished using 3-O PDS™ II suture (Ethicon Inc.). A light application of Tissumend™ II (Veterinary Product Laboratories) absorbable tissue adhesive was applied to the skin incision site to aid in wound closure and to provide a physical barrier for potential contamination. The abdominal wall defect was allowed to mature for 36 days, at which
point the animal was euthanized and examined for the development of a mature ventral hernia.

_Hernia Creation Procedure B – Pilot Animal No. 2_

Approximately 13 cm below the xiphoid process and 2 cm away from the linea alba (lower left side of abdomen) a 4 cm incision was created through the skin and subcutaneous fat. Blunt dissection was performed to create a workable subcutaneous surgical space. A 3 x 1 cm section of the abdominal wall musculature was excised while leaving the peritoneum intact. A 2 cm incision was then created through the peritoneum while being careful not to damage any of the inter-abdominal contents. Two knots of Vicryl™ 2-O (polyglactin 910) suture were placed 0.5 cm in on each side of the incision going into the peritoneum in order to prevent viscera from pushing directly into the subcutaneous space. Skin closure was accomplished as described for _Hernia Creation Procedure A_. The created abdominal wall defect was allowed to mature for 35 days, at which point the animal was euthanized and examined for hernia creation.

_Hernia Creation Procedure C – Pilot Animal No. 3_

Approximately 13 cm below the xiphoid process and 2 cm away from the linea alba (lower left side of abdomen) a 3 x 1 cm section of the abdominal wall musculature was excised and a 3 cm incision through the peritoneum was created. Skin closure over the created defect was accomplished as previously described. Animal was euthanized 10 days following creation surgery as humane endpoints were reached prior to the planned conclusion date. This included lack of appetite, weight loss of greater than 10%, and a
lack of urination which indicated a possible entrapment of the bladder in the created hernia.

_Hernia Creation Procedure D – Pilot Animal No. 4_

Approximately 3 cm below the xiphoid process and 2 cm away from the linea alba or abdominal mid-line (upper left side of abdomen) a 1.5 cm full-thickness incision was created through the abdominal wall muscle layers and peritoneum taking special care not to damage the underlying viscera. Skin closure of the created defect was accomplished as previously described. The abdominal wall defect was allowed to mature for a period of 23 days, at which point an exploration surgery was conducted to determine the outcome.

_Final Hernia Creation Procedure_

The final hernia creation procedure employed for Animals 5 – 30 was conducted as described for _Hernia Creation Procedure D_ except that the full-thickness incision into the abdominal cavity was increased from 1.5 cm to 3.0 cm in length. Figure 1 shows the final positioning and size of the created defect model. In addition, the defect was allowed to mature 21 to 35 days prior to mesh hernioplasty to repair the resulting ventral hernia.
Figure 3.1. Final hernia defect creation size and location.

Mesh Repair Surgical Procedure

Hernia Assessment - After 21 to 35 days of maturation the mesh hernioplasty surgery was scheduled and the created ventral hernia was repaired using the following procedure. The animal was placed under general anesthesia as previously described and shaved/prepped for surgery. Prior to moving into the surgical suit the developed hernia was palpated in order to obtain a general idea of its severity and size as this was used to determine the most appropriate location for re-entry into the subcutaneous space. If the created hernia exhibited a distinct bulge that was at least 3 cm in diameter an incision into
the skin was made approximately 1 cm towards the midline of the animal away from the hernia edge. This was done to reduce the risk of damaging underlying viscera which often were resting just below the skin. Similarly, if the developed hernia was less than 3 cm the previous incision point was used to obtain access into the subcutaneous space. Once the skin was compromised careful blunt dissection was performed around the periphery of the hernia sac in order to create a workable subcutaneous space. The hernia sac was then carefully dissected in order to remove any visceral adhesions, if present, and the hernia contents were re-approximated back into the abdominal cavity. The hernia ring was examined and if deemed necessary, surgically modified to reduce the abdominal wall thickness at its edges as often these were covered in scar tissue developed during the hernia maturation process. The length and width of the hernia ring was then measured and the selected mesh variant (SAM-3, SAM-6, or UltraPro™) was cut to be 1 cm greater in width and length in order to realize a minimum 1 cm overlap with the native tissue after the repair process.

*Mesh Attachment* – For each repair the mesh was attached using a posterior (intraperitoneal) flat mesh repair technique using 4-O PDS™ II Suture (Ethicon, Inc.). In this procedure, the mesh is positioned posterior to the hernia which allows direct contact of the mesh with the viscera. In order to accomplish this in an open mesh repair scenario (see Figure 3.2), each attaching suture was (1) first passed through the abdomen approximately 2 cm away from the hernia ring edge and up through the hernia opening, (2) looped through and around a mesh pore at the edge of the mesh being attached, and (3) passed back into the hernia opening along the path of the first needle (within 0.5 cm).
This was repeated until 6 anchor sites were created, 3 on each side of the mesh located longitudinally. The suture points were then drawn in which pulled and tucked the mesh into the intraperitoneal position. For all repairs, the mesh was placed with the textile wale (machine direction) running down the length of the animal and mesh textile course (cross machine direction) positioned side to side. This was conducted to minimize any potential variation due to inherent differences that are generally seen in warp knit textiles when comparing the wale and course direction mechanical properties. When mesh positioning was satisfactory (i.e. no curling or slack seen in the mesh) each suture point was tied and skin closure was accomplished as previously noted with a running suture of 3-O PDS™ II (Ethicon, Inc.) followed by a light application of an absorbable tissue adhesive Tissuemend™ II (Veterinary Products Laboratories). Animals were then housed for select periods of time as laid out in the Study Design section to evaluate each mesh construction. In addition, Hemoclip™ titanium ligating clips (Teleflex Medical, size small) were placed at each of the four corners of the mesh prior to implantation in order to allow x-ray visualization for positioning and size.
Figure 3.2. Suture is passed through the abdominal wall and looped around a mesh pore before being drawn back through abdominal wall (A). Suture points are pulled tight and tied which pulls the mesh into the intraperitoneal position (B). View of repair site after all anchor points are tied off indicating positioning of attachment points along sides of mesh and hernia (C).
Study Design

Each mesh construction was evaluated at 4, 8, and 12 weeks with a total of 3 animals per mesh and study time point. Periodically, abdominal wall x-rays were taken of select animals in order to evaluate mesh positioning and size changes throughout the study. At predetermined time points the animals were euthanized and the mesh/tissue complex was extracted. During the extraction process any visceral adhesions to the mesh were noted and scored accordingly along with gross observation. The mesh/tissue complex was placed between two gauze pads that were soaked in saline solution and transported immediately back to the lab for mechanical testing. After mechanical testing, tissue was fixed in 10% neutral buffered formalin (NBF) for histological evaluation (Note: Histological evaluation will not be discussed as part of this chapter).

Mesh Size Measurement – As titanium ligating clips were placed at the corners of the mesh prior to implantation, x-ray imaging as well as direct measurement during euthanasia was used to determine the change in mesh size. Both initial and subsequent measurements of the mesh were analyzed at each time point using ImageJ 1.48v image analysis software.

Adhesion Scoring – An adhesion scoring system was adopted from Leach and colleagues and was based on a combined score examining overall adhesion and related morphology. The severity or strength of each adhesion was not assessed as this would require attempting to pull and remove the adhesions which may have damaged the
mesh/tissue complex for mechanical and histological assessment. The adhesion scoring system is outlined in Table 3.2. With this system, a maximum score of 10 is possible.

Table 3.2. Adhesion Scoring System.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion Coverage</td>
<td>0</td>
<td>No adhesions</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1 – 25% involvement</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26 – 50% involvement</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>51 – 75% involvement</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>76 – 100% involvement</td>
</tr>
<tr>
<td>Morphology</td>
<td>0</td>
<td>No adhesions</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Filmy avascular adhesions</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Vascular or opaque adhesions</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Direct attachment of mesh with intestine or other structures</td>
</tr>
</tbody>
</table>

Total Adhesion Score = Adhesion Coverage + Morphology

Mechanical Testing – A special burst testing fixture was developed for testing each explanted mesh/tissue complex. The fixture consisted of an aluminum plate with a 2 cm diameter opening that was modified to accept 18 gauge needles as shown in Figure 3.3. The bursting pin consisted of a stainless steel pin with a 1.4 cm radius rounded and polished end. Testing was conducted on a MTS Synergie mechanical testing unit equipment with a 500 Newton load cell. The sample was loaded onto the pin plate so that the mesh/tissue complex was centered over the fixture opening. The bursting pin was
then lowered until a 0.1 Newton pre-load was realized at which point the test was initiated at a testing speed of 13 cm/min. to a maximum extension of 4 mm. This extension was chosen as not to damage the tissue for histological analysis but still allow evaluation of the mesh/tissue complex stiffness.

![Mechanical testing fixture dimensions (A) and image of loaded mesh/tissue complex sample immediately prior to testing (B).](image)

**Figure 3.3.** Mechanical testing fixture dimensions (A) and image of loaded mesh/tissue complex sample immediately prior to testing (B).

### III. Results

A summary of the hernia creation development phase can be seen in Table 3.3. Examination of the first pilot animal 36 days following hernia creation surgical procedure indicated no visual signs of a mature ventral hernia. Palpation of the defect site indicated no protrusion or weak points in the abdominal wall. These findings were confirmed following euthanasia and surgical exploration of the abdominal wall which
indicated that the created defect healed nicely with no visceral adhesions. Similar results were noted for hernia creation procedure B with no signs of hernia creation and healing of the defect site with a collagenous plug. In contrast, hernia creation procedure C resulted in the creation of a large ventral hernia with various animal complications. These included significant weight loss, lack of appetite, and presence of blood in the urine. As such, the animal was euthanized only 10 days into the study as humane end points were reached. Upon surgical examination, it was discovered that the herniated area was very large (5 cm in diameter) and included portions of the bladder, large intestine, and uterine horn. In hernia creation procedure D, the hernia defect size was reduced and moved away from the lower abdomen to avoid strangulation of critical organs. Exploration surgery performed 23 days post-op revealed the creation of a small ventral hernia in the abdominal wall that measured 1 cm (longitudinally) x 0.5 cm with a distinct hernia ring. In the final surgical creation model the full thickness laparotomy was increased to 3 cm in order to facilitate the creation of a larger ventral hernia for mesh repair. See Figure 3.4 for images of resulting mature ventral hernia.
**Table 3.3. Ventral Hernia Development Results.**

<table>
<thead>
<tr>
<th>Hernia Creation Procedure</th>
<th>Surgical Technique</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Created a 3 x 1 cm defect along the linea alba while keeping peritoneum intact.</td>
<td>No hernia maturation, defect healed over with collagenous plug.</td>
</tr>
<tr>
<td>B</td>
<td>Created a 3 x 1 cm defect along the linea alba, peritoneum was compromised and temporarily repaired with 2 knots of absorbable suture.</td>
<td>No hernia maturation, defect healed over with collagenous plug.</td>
</tr>
<tr>
<td>C</td>
<td>Created a 3 x 1 cm defect in the lower left quadrant of the abdominal wall, peritoneum was compromised with a 3 cm incision.</td>
<td>Resulted in a large ventral hernia with strangulation of critical internal organs, animal euthanized 10 days post op as humane end points reached.</td>
</tr>
<tr>
<td>D</td>
<td>Created a 2 cm full thickness laparotomy in the upper left quadrant that traveled through the muscle and peritoneal layers.</td>
<td>Resulted in a small mature ventral hernia 1 x 0.5 cm in size.</td>
</tr>
<tr>
<td>FINAL</td>
<td>Created a 3 cm full thickness laparotomy in the upper left quadrant that traveled through the muscle and peritoneal layers.</td>
<td>Resulted in a mature ventral hernia measuring 2 x 0.5 cm.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cumulative Results of Final Hernia Creation Procedure</th>
<th>Average Hernia Area (cm²)</th>
<th>Maximum Value (cm²)</th>
<th>Minimum Value (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.22</td>
<td>5.06</td>
<td>0.61</td>
</tr>
</tbody>
</table>
Figure 3.4. Image A – The abdominal wall protrusion of the mature ventral hernia 21 days after defect creation procedure. Image B – Hernia sac in the process of being dissected and approximated back into the abdominal cavity. Image C – Prior to mesh repair after hernia has been approximated and all adhesions addressed. Image D – After intraperitoneal mesh repair using described method prior to skin closure.

The created mature ventral hernias were repaired using an intraperitoneal mesh repair technique with the mesh of interest as described in the Methods section of this chapter. An image of the final repaired hernia site can be seen in Figure 3.4 (Image D). As titanium tacks were placed at the corners of each mesh prior to repair, x-ray imaging was used to determine size changes of each construction in both the textile wale and...
course directions. In some instances, final x-ray images prior to euthanasia were not available. For these, direct measurement following the euthanasia were substituted in order to allow for evaluation of mesh contraction in each respective direction. Examination of this data indicates a large amount of variability in regards to size change for each construction as shown in Table 3.4. Overall each construction appears to reduce in the length (textile wale direction) more so than in the width (textile course direction) although statistically this was unfounded with the chosen sample size. Furthermore, it is important to note that the largest single sample contraction value was obtained for the UltraPro™ mesh material with a % contraction/shrinkage of over 50% after 12-weeks of implantation.

Table 3.4. Mesh Contraction for SAM-3, SAM-6, and UltraPro™ Mesh.

<table>
<thead>
<tr>
<th>Time Point (weeks)</th>
<th>% Change in Dimension</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Width</td>
</tr>
<tr>
<td>4</td>
<td>4.4 ± 5.1</td>
<td>(4.5) ± 6.5</td>
</tr>
<tr>
<td></td>
<td>Area = 0.3 ± 9.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Highest Recorded = 10.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16.3 ± 8.9</td>
<td>(5.9) ± 5.6</td>
</tr>
<tr>
<td></td>
<td>Area = 21.9 ± 8.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Highest Recorded = 30.4</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>13.6 ± 4.7</td>
<td>(18.2) ± 13.5</td>
</tr>
<tr>
<td></td>
<td>Area = (2.5) ± 12.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Highest Recorded = 10.9</td>
<td></td>
</tr>
</tbody>
</table>

*No statistical differences between data sets were discovered.*
Animals were euthanized at pre-determined time points ranging from 4 to 12 weeks after hernia repair as discussed in the methods section of this chapter. Immediately following euthanasia, the abdominal cavity was opened to allow for exploration in order to assess the presence of recurrence and grade any observed visceral adhesions to the mesh surface. Table 3.5 summarizes the findings from each animal and time point. No recurrence was observed in any of the studied mesh time points. In addition, adhesion scoring indicates that UltraPro™ resulted in a higher amount of morphologically concerning adhesions although statistically this was unfounded with the minimal sample set tested. As discussed in the methods section, a value of 6 for morphological assessment indicates a direct adhesion of visceral organs to the mesh surface. This was observed in five separate instances within the UltraPro™ testing group (all 12-week animals tested and two 8-week time points) while only being observed once in the SAM-6 (8-week, Animal 3) and SAM-3 (12-week, Animal 2) mesh groups. In addition the only mesh sample to obtain an adhesion score of “zero” was a SAM-6 mesh after a 12-week implantation period which can be seen in Figure 3.5.
Table 3.5. Adhesion Scores for SAM-3, SAM-6, and UltraPro™ Mesh.

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Time Point (weeks)</th>
<th>Animal No.</th>
<th>Adhesion Score</th>
<th>Time Point Average</th>
<th>Overall Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-3</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>SAM-6</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>UltraPro™</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

*No statistical differences within or between data sets were discovered.
**Figure 3.5.** Image A – Hernia repaired with SAM-6 12-weeks following hernia repair showing good collagen development over the mesh surface with no visceral adhesions. Image B – Hernia repaired with UltraPro™ 12-weeks following hernia repair showing very little collagen development over the mesh surface and multiple direct visceral adhesions to the mesh surface.

The mesh/tissue complex was mechanically tested using the described testing fixture by extending a stainless steel bursting pin 4 mm into the tissue following a 0.1 N preload. In this testing scenario a higher force is indicative of a stiffer mesh/tissue complex. Mechanical testing of the mesh/tissue complex (Figure 3.6) indicates that all tested constructions required a significantly higher amount of force to extend 4 mm than the native tissue at every time point tested. At the 4-week implantation period, the SAM-6 construction required a significantly larger amount of force in order to extend the burst fixture rod 4 mm into the tissue as compared to the predicate material UltraPro™. By the 12 week time point the mesh/tissue complex for both SAM-3 and SAM-6 constructions
have significantly increased in extensibility as indicated by the lower resulting force at
the same extension distance while the UltraPro™ material remains mechanically the
same across the 12 week testing period. In addition, the SAM-6 mesh/tissue complex is
more extensible at 12 weeks than the UltraPro™ mesh. Figure 3.7 provides an overlay of
the in vitro mechanical testing data for each construction under similar testing conditions,
showing the relative addition to mechanical stiffness of the developing collagen.
Comparison of the in vitro mechanical testing data with the values obtained for the
mesh/tissue complex during the animal study reveals the collagen contribution to the final
mechanical stiffness of each construction as shown in Table 3.6.

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Time Point</th>
<th>Load at 4 mm of Extension (N)</th>
<th>Collagen Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week</td>
<td>in vitro</td>
<td>in vivo</td>
</tr>
<tr>
<td>SAM-3</td>
<td>0</td>
<td>7.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.88</td>
<td>5.68 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.22</td>
<td>3.10 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.22</td>
<td>3.12 ± 0.77</td>
</tr>
<tr>
<td>SAM-6</td>
<td>0</td>
<td>6.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.21</td>
<td>8.62 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.11</td>
<td>3.58 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.73</td>
<td>2.55 ± 0.43</td>
</tr>
<tr>
<td>UltraPro™</td>
<td>0</td>
<td>3.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.75</td>
<td>3.46 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.22</td>
<td>3.08 ± 1.13</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.22</td>
<td>3.27 ± 0.09</td>
</tr>
</tbody>
</table>

Table. 3.6. Comparison of In Vitro Conditioned Mechanical Testing Results of Each Construction to In Vivo Result of Mesh/Tissue Complex.
Figure 3.6. Load exerted on mesh/tissue complex to realize an extension of 4 mm following the application of a 0.1 N pre-load for SAM-3, SAM-6, and UltraPro™ at 4, 8, and 12 week implantation time points.

(† = Values are significantly higher (p-value less than 0.05) than UltraPro™, ‡ = Values are significantly higher (p-value less than 0.05) than Native Tissue, ⱡ = Value is significantly lower (p-value less than 0.05) than UltraPro™)
IV. Discussion

Animal Model Development – In order to examine the effect of mesh biomechanics on the wound healing process as it relates to ventral hernia repair, the use of an animal model which effectively replicates the wound pathology associated with mature hernia development in humans was required. Therefore the first segment of this research was focused on creating such a model using New Zealand White rabbits. A
review of the literature identified two potential procedures for attaining this model type in
the chosen animal species. The first described by Silva and colleagues\textsuperscript{18} utilizes a less
aggressive approach where a small area of the abdominal wall muscle layer is removed
while preserving the peritoneum. This approach was attempted in Hernia Creation
Procedure A (HCP-A) where a 3 x 1 cm section of the abdominal wall musculature was
removed while preserving the peritoneum. The defect was allowed to mature over a
course of 35 days culminating in the formation of a collagenous plug at the defect site.
Therefore this procedure did not result in the formation of a mature ventral hernia as
previously described. As a limited number of pilot animals were available for this
segment, slightly more aggressive approaches were taken with each successive hernia
creation procedure until the desired result was reached. In HCP-B the peritoneum was
compromised in addition to removing a 3 x 1 cm area of the abdominal wall musculature.
Prior to closing, two throws of a fast absorbing suture were placed at the edges of the
created wound to provide temporary support and prevent immediate herniation. The
center most portion of the created defect was left open in order to increase the probability
of ventral hernia creation. As with HCP-A, this also resulted in a “healed” situation
where a collagenous plug filled in the weakened area over a 35 day study period. In the
subsequent hernia creation procedure, HCP-C, the defect site was moved to the lower left
abdominal wall quadrant as this area is inherently weaker in the NZW rabbit. In addition
to removing a 3 x 1 cm section of the abdominal wall muscle layer, a 3 cm lateral
incision through the peritoneum was created and left open. This resulted in a large
ventral hernia with strangulation of various critical organs, therefore for HCP-D the
A successful mature ventral hernia was created with HCP-D although the size of the resulting hernia ring was smaller than desired for proper mesh assessment and made the hernia repair difficult due to the limited surgical space. In order to create a larger and possibly more reliable ventral hernia, the incision into the muscle and peritoneal layers was increased to 3 cm. Examination of the final hernia creation procedure over the course of this study (28 total animals) reveals a large deal of variability in the size or area of the resulting hernia as indicated in Table 3.3. This is similar to what is seen in the human situation as hernias come in various shapes and sizes. In addition, this procedure resulted in the formation of a distinct hernia sac and ring. The hernia ring was found as a ring of fibrous tissue that was significantly stiffer than the virgin muscle and peritoneal layers. As such, the hernia creation segment of this study was considered successful but potentially introduced a large amount of variability into the study design. As previously discussed, researchers often opt for a simple acute hernia creation and repair model as this limits the amount of variability seen from animal to animal making comparisons between different mesh designs easier. While this seems like the best decision from a research point of view it potentially hides real world variables that physicians face day to day and may be the reason some mesh designs perform well during animal trials but result in similar complication rates when transferred into the clinical setting.

**Implantation and Mesh Performance** – Each mesh was implanted using an intraperitoneal onlay mesh repair technique that was adapted for open repair. The transabdominal preperitoneal (TAPP) procedure is the laparoscopic version of the one
used in this study and the most common procedure used for mesh hernioplasty.\textsuperscript{21} In both cases, the mesh is placed over the hernia ring in an intraperitoneal position which allows direct contact of the mesh with the viscera. Since direct contact with the viscera occurs, a higher probability of visceral adhesions exists with reports as high as 64 to 76\% after mesh repair.\textsuperscript{22–24} In this study we observed a large number of direct intestinal adhesions with the predicate mesh material UltraPro™ as exhibited in Figure 3.5. Although a significant difference (\textit{p}-value < 0.05) in adhesion score was not found when comparing the developed mesh constructions (SAM-3 and SAM-6) with the predicate material, a less clinically severe adhesive reaction was seen as the vast majority of the adhesions occurring to the developed constructs consisted of soft adherence of the fatty omentum which has a low likelihood of resulting in clinical complications. It is thought that the rough/stiff character of a monofilament surgical mesh, such as UltraPro™, results in abrasion of the soft internal viscera leading to an increased likelihood of direct intestinal adhesion. It is important to note that adhesion assessment was a secondary output of this study and the tenacity of the adhesions were not assessed as this risked affecting the mechanical and subsequent histological analysis.

The issue of hernia mesh contraction is widely reported in the literature with decreases in mesh size of 10 to 50 \% after mesh hernioplasty.\textsuperscript{25–29} In this study we found a large variance in the amount of mesh contraction in both the textile wale and course directions of each construction as shown in Table 3.4. The highest contraction rates were observed in the textile wale direction for SAM-3, SAM-6, and UltraPro™ with 18.6, 28.1, and 35.0\% contraction, respectively. Conversely, in the textile course direction a
minimal change in size was typically observed, with SAM-3 and SAM-6 constructions often increasing in length with an average increase of 18.2 and 9.5%, respectively, at 12-week post-implantation. This is likely due to the developed mesh design as the absorbable component is placed in a lay-in stitch configuration which inherently adds more support in the course direction. As this mesh component is hydrolytically degraded and loses strength, ability to extend in the course direction is now greater than the meshes ability to extend in the wale direction. The overall large amount of variability reported within this phase of the study may be a result of the hernia creation model itself as each mature ventral hernia had a slightly different presentation from animal to animal. In addition, the effect of visceral adhesions on mesh contraction is unknown and may play a key factor, particularly as the highest reported contraction values were found for UltraPro™ which also exhibited the most direct visceral adhesions. The highest single sample value for overall areal mesh contraction (50.5%) was also reported for UltraPro™ mesh which is alarming considering the small sample size of three animals for 12-week implantation time points.

One of the main goals of this research was to assess the potential clinical effect of a mesh that exhibits a biomechanically changing profile where the construction is initially stiff to protect the healing tissue but ultimately compliant to allow for adequate wound remodeling. Examination of the mechanical testing conducted on the mesh/tissue complex, Figure 6, immediately following explanation indicates that the predicate material UltraPro™ exhibits little to no change in mechanical properties over the 12-week testing period. This result mirrors the findings from the in vitro testing portion of
this research (Chapter 2) which determined that UltraPro™, with more than 40% mass loss of its absorbable component, showed minor changes in extensional properties over this testing period. The lack of change in the UltraPro™ construction is largely pattern driven and not simply a function of material loss due to degradation of the absorbable component fiber. Alternatively, the SAM-6 mesh/tissue complex is significantly stiffer at the 4-week implantation time and gradually transitions into a more compliant construction over the 12-week testing period as the respective degradable component loses strength. As the SAM-3 mesh was designed to transition over a 2 – 4 week period, this process is significantly underway at the first explant time of 4-weeks. For this reason we do not see a statistically significant difference between the SAM-3 mesh/tissue complex and the UltraPro™ mesh/tissue complex. In addition, it was determined that the SAM-6 construction is significantly more mechanically compliant at 12-weeks than both SAM-3 and UltraPro™ meshes while remaining significantly higher than the native tissue values. It is important to note that the selectively absorbable mesh systems of this discussion were designed to exhibit a long-term % mechanical extension (at a force of 16 N/cm) of greater than 18% in order to match the extensional characteristic of the native human abdominal wall tissue. To realize a 16 N/cm force using the developed fixture would have required the application of a 32 Newton load onto the test specimen. Unfortunately we were unable to stress the mesh/tissue samples as part of this study to this degree of loading as the rabbit abdominal wall is much weaker than that found in humans and would have likely resulted in significant damage to the specimen. It is thought that were we able to extend to the mesh designed input of 16N/cm, a much more
distinct difference between the SAM-6 and SAM-3 construction would have been observed in comparison to the UltraPro™ mesh at every time point tested, specifically indicating much more compliance after 12-weeks of implantation.

A review of the *in vitro* conditioned mechanical testing data in Figure 3.7 and Table 3.6 for these constructions at an extension of 4 mm reveals the contribution of the developing collagen to the overall stiffness of the repair site. It appears that more collagen is being deposited on the SAM-3 and SAM-6 constructions at 4 and 8 week time points as compared to UltraPro™. We see no measurable collagen contribution in the UltraPro™ mesh especially at the 4 week time point which matches our gross observations which noted very little collagen deposition on this construction. This difference in collagen deposition is likely due to the higher density of the SAM-3 and SAM-6 constructions (138.7 and 186.3 g/m²) as compared to the UltraPro™ material (61.7 g/m²). Furthermore, the differences in multifilament and monofilament yarn forms for each construction as noted in Table 3.1 will also affect this response. Since multifilament fibers inherently have higher surface areas as compared to monofilaments, a more intense foreign body response and subsequent collagen deposition is expected with these types of constructions. In addition, we see that the collagen contribution of UltraPro™ increases over time while the collagen contribution to the developed constructs, especially in the case for the SAM-6 mesh, decreases over each study period. This shift is expected to continue well past the longest time point chosen for this study of 12-weeks with final UltraPro™ repair being significantly stiffer than the native tissue.
while the developed constructions move towards a more compliant and clinically acceptable repair.

V. Conclusion

The present study resulted in the development of a mature ventral hernia model in rabbits that recreated all of the wound pathology associated with chronic hernia development in humans including the presence of a distinct hernia sack, hernia ring, and various unpredictable visceral adhesions. This allowed in vivo evaluation of the created selectively absorbable mesh constructions in a high relevance animal model with comparison against a commonly used predicate partially absorbable material. The design intent of the developed selectively absorbable mesh constructions (SAM-3 and SAM-6) was to create a mesh that initially stress shields the repair site while gradually transitioning to a more compliant construction over time to allow for proper collagen remodeling and increased repair site mobility. Through mechanical testing of the resulting mesh/tissue complex it was shown that while the use of the developed selectively absorbable meshes initially resulted in a much stiffer repair site as compared to predicate material, this transitioned to a more compliant repair over the course of the 12-week monitoring period as hypothesized due to an in situ modulation in extensional properties. Furthermore, it was noted that the collagen contribution to the mechanical stiffness of the repair site increased over time for the predicate material while this value decreased and/or plateaued in the developed constructions. It is thought that in a long-term study the developed selectively absorbable constructions will continue this trend.
resulting in reduced complication rates and less patient discomfort as the final repair will approach the mechanical properties of the native tissue. Therefore, a selectively absorbable surgical mesh that exhibits the changing biomechanical profile as described in this chapter may lead to a reduction in current clinical complications associated with mesh hernioplasty.
References


CHAPTER IV
HISTOLOGICAL EVALUATION OF MULTIPHASE SELECTIVELY ABSORBABLE MESH IN AN ANIMAL MODEL OF MATURE VENTRAL HERNIA

I. Introduction

The overriding goal of mesh hernioplasty is to repair a defect in the abdominal wall such that the quality of life of the patient is brought back to levels prior to having the hernia. Unfortunately, current treatment options are based on simply reinforcing the hernia defect site in hopes that future herniation is prevented. This has resulted in the development of mesh constructions that mechanically are much different (stronger and less compliant) than the soft tissue that is being repaired.\textsuperscript{1,2} As a result, the long-term compatibility, or lack thereof, for these devices has led to a high rate of clinical complications such as chronic pain, reduced wound site mobility, and fibrosis.\textsuperscript{3–5} Assessment of hernia mesh biocompatibility requires a multifaceted approach as ultimately this is the result of a number of dependent and independent tissue response variables occurring to and because of the implanted prosthesis. The size and severity of the hernia being repaired, the cellular response to the implanted polymer(s) material chemistry, as well as the long-term biomechanical profile of the mesh must all be considered during the evaluation process with a focus on how each may ultimately affect long-term biocompatibility.
Once a medical device such as a hernia mesh is implanted in the body a number of complex foreign body responses begin as dictated by the adsorption of blood and tissue specific proteins onto the device surface and the cellular environment in which the device is being implanted, as depicted in Figure 4.1. The adsorption of blood and tissue specific proteins onto the surface of an implanted medical device is dependent on a number of variables that include polymer crystallinity, hydrophilicity (surface energy), macro/micro porosity, material surface chemical composition, surface area, and amount of terminally implanted material. Protein adsorption results in altered protein folding and configurations, which signals the cells of the body that the implanted prosthesis is a foreign material. Therefore, various leukocytes including neutrophils and macrophages migrate to the implant cite as the inflammatory process is underway. Ultimately, the foreign body response for terminally non-absorbable materials ends in the formation of a fibrous capsule as the body attempts to wall off the material. During this process, macrophages remain active and continue to release a slew of enzymes and toxic peroxides in a continued effort to break down and remove the implanted material resulting in a chronic inflammatory situation. As such, even materials that are traditionally considered to be non-absorbable will show some degree of surface and bulk polymer degradation. This phenomenon is well documented in the use of polyethylene-terephthalate (PET, Dacron) as a vascular graft material where drops in molecular weight and strength loss are report over periods of 10+ years. Instances of “non-absorbable” material breakdown is not limited to PET but also reported in explanted polypropylene mesh material. In a study conducted by Costello and colleagues, analysis of 14
explanted polypropylene meshes (due to chronic pain, recurrence, and/or visceral adhesions) indicated significant surface cracking, roughness, and peeling of the component fibers. In addition, thermal analysis of those explanted materials also indicated significant drops in melting temperature and heat of fusion providing further evidence for in vivo oxidative degradation of polypropylene. Therefore, adequate control and understanding of how mesh chemical and mechanical properties affect cellular compatibility is of utmost importance as a long-term chronic inflammatory response is not ideal for maintenance of implanted material strength as well as ultimate success of the mesh repair.
Figure 4.1. The relative order of events and cellular involvement after device implantation (adapted from Morais et al.\textsuperscript{7}). The left side of the figure outlines the series of chronological events occurring around the implant while the right side outlines specific interactions with the implant surface itself.

In regards to mesh biocompatibility, the degree of foreign body response to resulting mesh prosthesis is also greatly dependent on material area density and mesh pore size.\textsuperscript{3,15} Mesh for hernia repair are loosely classified by mesh density as heavy weight (> 90 g/m\textsuperscript{2}), medium weight (50 – 90 g/m\textsuperscript{2}), light weight (35 – 50 g/m\textsuperscript{2}), or ultra-
light weight (< 35 g/m²). As heavy weight meshes have been associated with increased complication rates (chronic pain, fistula formation, and fibrosis) there has been a general movement towards medium to light weight mesh materials. While the weight/volume of the implanted material plays a significant role in the foreign body response, the size of the mesh pores has been found to be a major driving factor in the ultimate foreign body response as smaller pores (< 0.82 mm inter-filament distance) result in the formation of a bridging scar plate. In this situation, the mesh is completely encapsulated in collagen and results in a less than ideal repair situation as mesh mobility is restricted and abdominal wall function is impaired. Conversely, movement and incorporation of collagen through and into the mesh would result in strong attachment of the mesh to the abdominal wall, reducing the likelihood for detachment or mesh migration.

While the overall foreign body response (in regards to cellular involvement) is a key aspect in predicting compatibility of a hernia repair device, it is common to report collagen quality at the mesh implantation cite, which is generally expressed as Type I to III collagen ratios. Type I collagen (mature) is presented as a highly cross-linked network that provides mechanical tensile strength to tissues while Type III collagen (immature) is loosely integrated and generally found in the provisional matrix of healing wounds. Recent studies into the epidemiology of hernia formation indicate that collagen disease or dysfunction that leads to reduced levels of type I collagen maybe a strong risk factor or cause of incisional and inguinal hernia development. It has been shown that the native tissue (within both skin and fascia) of patients presenting with incisional or
inguinal hernia exhibit reduced collagen type I/III ratios when compared to individuals of the normal population controls.\textsuperscript{23–27} This is further supported in findings indicating a correlation between abdominal aortic aneurisms and hernia development.\textsuperscript{28} Therefore it is generally accepted that a robust mesh repair requires the development of high values of collagen type I/III ratios.\textsuperscript{21,24,29,30} In a study that examined the collagen type I/III ratio of 78 explanted mesh specimens (due to recurrence, chronic pain, and/or infection) it was determined that meshes exhibiting recurrence had an average type I/III collagen ratio of 1.3 while those associated with chronic pain had an average type I/III collagen ratio of 3.4.\textsuperscript{29} In contrast, native healthy fascia tissue is reported to exhibit a 1.3 to 1.9 collagen type I/III content.\textsuperscript{26} Therefore the most ideal repair would conclude with a type I/III collagen ratio of 1.5 to 2.5 in order to provide sufficient strength in order to prevent recurrence but not over-shield the area which may lead to a chronic pain and discomfort.

In the present study, the foreign body response to the developed bi-component meshes was evaluated in a mature ventral hernia rabbit model with direct comparison to the predicate material UltraPro\textsuperscript{TM} Mesh. This entailed examination of the cellular response to each mesh construction as well as determination of collagen quality and maturation (type I/III ratios) over a 3 month testing period.

II. Materials and Methods

Animal Model and Repair Procedure

A detailed description of the mature abdominal wall hernia model and mesh repair procedure can be found in Chapter 3. Briefly, the hernia creation procedure required the
creation of a 3 cm full-thickness incision in the top left quadrant of the rabbit abdominal wall which was allowed to mature for a period of 21 to 35 days. After this initial hernia maturation phase, the created hernia was repaired with either SAM-3, SAM-6, or UltraPro™ mesh using a posterior (intraperitoneal) flat mesh repair technique using 4-O PDS™ II (Ethicon, Inc.) at 6 anchor sites. Each mesh was implanted for a duration of 4, 8, and 12 weeks with 3 total animals per test/time group. It is important to note that the tissue/mesh complex for all animals was subjected to mechanical testing as described in Chapter 3 prior to placing in a histological fixative for slide preparation.

**Histological Evaluation**

Each mesh/tissue complex was fixed in 10% neutral buffered formalin and embedded in paraffin for slide preparation. Various stains were employed to study the cellular and extra-cellular matrix (ECM) response to each material construct as described below. All slide preparation and staining was conducted by a CLIA/GLP certified institution (Histology Tech Services, Inc., Gainesville, FL) as described in Appendix X. Stained slides were examined and graded by a licensed histopathologist to determine the overall cellular reaction to each implanted construct.

*Immunohistochemistry (IHC)* - To evaluate the inflammatory response to each candidate mesh material, IHC was used to specifically stain for activated macrophages. The CD68 antibody (Biocare Medical) was chosen for this work as it is a glycosylated transmembrane protein that is mainly located in lysosomes, therefore, it is a commonly used marker for monocytes and macrophages. For a detailed staining protocol please
see Appendix X. Stained slides were examined to determine overall macrophage density and presence of foreign body giant cells on a scale of 1 (minimal macrophage involvement) to 4 (severe macrophage involvement). In addition to staining for tissue macrophages, IHC staining for activated myofibroblasts was also employed in order to determine the degree of myofibroblast involvement at each time point. The selected marker for this cell type was α-smooth muscle actin (α-SMA, Biocare Medical) as α-SMA is considered the most reliable marker for myofibroblastic differentiation. Slides were photographed on a Leica light microscope at 10x magnification using a 10mp camera. ImageJ 1.48v image analysis software was then used to count the number of cells occupying a 200 x 200 µm box created/centered between the pores of the mesh.

Masson’s trichrome (MT) - MT stain was used to determine the degree of collagen synthesis in and around each construct as it selectively stains collagen a blue to green color. Stained slides were examined to assess collagen deposition around each mesh construction on a scale of 1 (minimal collagen deposition around mesh construction) to 4 (severe/thick layer of collagen around mesh construction).

Picrosirius red - To analyze collagen quality (Type I to Type III ratios) slides were stained with picrosirius red. When viewed under polarized light, picrosirius red stains collagen Type I a bright red color and collagen Type III a light green color. All photographs were taken using a Leica polarizing light microscope with a 10x objective. Images were then analyzed using ImageJ 1.48v image analysis software to determine the ratio between collagen Type I and III using the “Color Histogram” plug-in (available on NIH website) which allowed determination of a color histogram of associated red, green,
and blue pixels. Threshold for the red and green color spectrum were adjusted to achieve reliable results. The final method was verified by conducted analysis on native abdominal wall sections and comparing to values reported in the literature. Collagen typing was conducted on the subcutaneous side of each mesh/tissue complex and within the interstitial pore space of each construction as shown in Figure 4.2.

**Figure 4.2.** Illustration indicating approximate locations for type I/III collagen analysis for interstitial pore space and subcutaneous side of mesh.

### III. Results

Examination of IHC (macrophage) and MT stained slides indicates that a significantly more intense inflammatory response was observed with the SAM-3 and SAM-6 constructions as shown in Table 4.1. In addition, it appears that a higher degree of cyst/seroma formation was found for the UltraPro™ material as compared SAM-3 and SAM-6 materials. Examination of collagen synthesis scores did not reveal any significant differences but the data appears to indicate a slightly higher degree of collagen development in and around the developed mesh constructions as compared to UltraPro™.
Two of the mesh time points could not be scored for collagen synthesis due to the presents of adhesion formation which would have confounded this result. Images of MT stained slides for SAM-3 and UltraPro at 8-weeks are provided in Figure 4.3, indicating increased collagen deposition and cellular response in and around SAM-3 construction as compared to UltraPro™.

Table 4.1. Histopathological Evaluation of IHC and MT Stained Slides. (Note: † indicates value is significantly lower than SAM-3 and SAM-6.)

<table>
<thead>
<tr>
<th>Mesh Type</th>
<th>Time Point</th>
<th>Hernia Defect Size</th>
<th>Cyst/Seroma Formation</th>
<th>Collagen Synthesis</th>
<th>Inflammation Score</th>
<th>Mean Inflammation Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-3</td>
<td>4</td>
<td>15.0</td>
<td>0</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>15.1</td>
<td>0</td>
<td>3</td>
<td>2.8</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.8</td>
<td>0</td>
<td>1.3</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>SAM-6</td>
<td>4</td>
<td>10.6</td>
<td>0</td>
<td>Unable to Evaluate</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>16.8</td>
<td>2</td>
<td>2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13.0</td>
<td>1</td>
<td>2</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>UltraPro™</td>
<td>4</td>
<td>12.4</td>
<td>2</td>
<td>Unable to Evaluate</td>
<td>1.8</td>
<td>2.0 †</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11.8</td>
<td>2</td>
<td>1</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>14.2</td>
<td>2</td>
<td>1.7</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

* Means are based on a scale of 1 (minimal) to 4 (severe) except for cyst/seroma formation with indicates presence or lack of presence of cyst/seroma.
* “Unable to Evaluate” indicates that adhesion formation obscured the grading of this parameter.
Figure 4.3. TOP – SAM-3 mesh construction at 8-weeks implantation time point (MT stain) showing high collagen development in and around mesh construction (Scale Bar = 100µm). BOTTOM – UltraPro™ at 8-weeks implantation (MT stain) showing minimal collagen deposition around mesh construction (Scale Bar = 200µm).
Evaluation of IHC stained slides to α-SMA (Figure 4.4 and 4.5) indicates a high degree of myofibroblast activity for each mesh tested. UltraPro™ showed a statistically significant drop in myofibroblast density from 8-weeks (66.3 cells/0.04 mm$^2$) to 12-weeks (21.3 cells/0.04 mm$^2$). For SAM-3 and SAM-6 mesh constructions, myofibroblast activity remained constant through the 12-week testing period.

**Figure 4.4.** Slides at 10x magnification that were stained with α-SMA for SAM-3 (A, 12-week), SAM-6 (B, 12-week), UltraPro™ (C, 8-week), and UltraPro™ (D, 12-week). Scale bar is at 100 µm.
Figure 4.5. Myofibroblast density within interstitial pore space for SAM-3, SAM-6, and UltraPro™ Mesh at 4, 8, and 12 week implantation times. († = UltraPro™ value at 12-weeks is significantly less than UltraPro™ value at 8-weeks).

Examination of Type I/III collagen ratios on the subcutaneous side (Figure 4.6 and 4.8) for SAM-3 and SAM-6 indicates that overall collagen maturation is trending higher between 4 and 8-week implantation periods (not statistically significant) but remains similar between 8 and 12-weeks. Conversely, UltraPro™ mesh indicated a statistically significant increase in type I/III collagen ratios between 4 and 12 week implantation periods. Statistical analysis of this data set overall revealed no significant differences to mesh type at the time points tested for collagen type I/III on the subcutaneous side the hernia mesh repair in this study. In addition to collagen analysis on the subcutaneous side of each repair, collagen typing was also performed within the
pores of each mesh construction. Examination of collagen type I/III ratios at this location (Figure 4.7) indicated a significantly lower collagen type I/III ratio particularly for the developed meshes, SAM-3 and SAM-6. At the 12-week time point, UltraPro™ had a significantly higher type I/III collagen ratio as compared to SAM-3. Testing of the native abdominal wall tissue (non-herniated side) indicated a Type I/III collagen ratio of $1.82 \pm 0.12$ which falls within reported values that range from 1.3 to 1.9 in humans. While the reference values are from human tissues, testing of the healthy rabbit fascial tissues in the manner allowed validation of the chosen test method for accurately determining collagen type ratios.

**Figure 4.6.** Collagen Type I/III ratio for each mesh construction following implantation at 4, 8, and 12 weeks over mesh construction on the subcutaneous side. († = UltraPro™ at 12-weeks is significantly higher than UltraPro™ at 4-weeks)
Figure 4.7. Collagen Type I/III ratio for each mesh construction following implantation at 4, 8, and 12 weeks within mesh construction. († = UltraPro™ is significantly higher than SAM-3 at 12-weeks)
Figure 4.8. Images of picro-sirius stained slides for SAM-3, SAM-6, and UltraPro™ at 4-week and 12-week implantation times taken at 10x magnification under polarized light. Note how overall ratio and quality of red color (Type I collagen) increases between 4 and 12 weeks for all mesh constructions tested.
IV. Discussion

In this study we were able to successfully evaluate the short-term cellular and extra-cellular matrix (ECM) response for the developed selectively absorbable mesh constructions, SAM-3 and SAM-6, in comparison to the clinically relevant partially absorbable mesh UltraPro™ in a mature ventral hernia model in rabbits. As previously discussed, accurate assessment of mesh acceptability requires a multifaceted approach. As such, in this study the cellular specific response was examined through selective staining for tissue macrophages and activated myofibroblasts while the ECM response was evaluated through the use of Masson’s trichrome and picro-sirius red staining. This culminated in a critical evaluation of key surgical mesh design criteria as it relates to the foreign body response seen in hernia mesh and ultimately to the potential reduction in mesh related clinical issues.

Quick integration of a surgical mesh into the native tissue is required to reduce the risk of mesh migration, displacement, excessive stress on suture points, and contraction – all of which are associated with hernia recurrence. In a pig model for hernia repair, Gonzalez and colleagues were able to show a significant correlation between the degree of mesh tissue integration and a meshes’ ability to resist in situ contraction. This integration process requires quick deposition of collagen, which initially is a process that is predominately driven by the tissue specific response to the implanted material chemistry and surface area. This is supported by numerous investigations which indicate that a strong initial acute inflammatory response immediately after mesh implantation is
associated with greater tissue in-growth and integration of the mesh into the abdominal wall.\textsuperscript{35–37} Examination of the inflammatory response (Table 4.1) to the tested mesh constructions indicates a significantly higher inflammatory score for SAM-3 and SAM-6 constructions as compared to UltraPro\textsuperscript{TM}. While the earliest time point in this study was 4-weeks, we suspect a more intense acute inflammatory response (1 – 2 days post-implantation) for the developed constructions as well due to an the increased area weight and presence of multifilament fibers for these constructions as compared to UltraPro\textsuperscript{TM}. In addition, this appears to be associated with a higher overall degree of collagen synthesis (see Table 4.1 and Figure 4.3) although this was not statistically relevant. This result, however, is supported by mechanical test data for the mesh/tissue complex (Chapter 3) which indicated a larger amount of collagen contribution to overall mechanical integrity at implantation durations of 4 and 8 weeks for SAM-3 and SAM-6 constructions as compared to UltraPro\textsuperscript{TM}. It was also noted previously that UltraPro\textsuperscript{TM} exhibited the highest amount of mesh contraction of the tested mesh groups, which may be attributed to poor initial integration into the native tissue due to the fact that it induced a minimal inflammatory response, especially at the earliest time point of 4-weeks by which time integration is most critical.

Seroma/cyst formation is a common complication of laparoscopic hernia repair with rates being reported as high as 78\%.\textsuperscript{38–40} We reported that 6 out of the 9 UltraPro\textsuperscript{TM} mesh implantations (67\%) resulted in seroma formation while only 3 out of 9 were found for SAM-6 mesh implantations (33\%) and no seroma formation for SAM-3 mesh implantations. This reduction in seroma formation for the developed constructions may
be a function of overall mesh area weight and ability to induce a high degree of collagen deposition, as such a defect filling characteristic of these constructs may be evident. While seroma formation often resolves itself in the clinical setting and its presence alone is generally not considered a mesh repair complication, it has been associated with patient discomfort and in some cases confused with possible recurrence.38

During the wound healing process, myofibroblast are activated in order to restore mechanical stability through wound contracture and synthesis of ECM components collagen types I and III, after which they spontaneously disappear via cell mediated apoptosis as the wound healing process comes to completion.41,42 Examination of myofibroblast activity for each mesh construction indicates the constant presence of this cell type throughout the 12 week testing period for SAM-3 and SAM-6 meshes while a significant drop in myofibroblast cell density is seen for UltraPro™ mesh between 8 and 12-weeks. As these cells are associated with the wound healing process these results correlate with previous findings of increased inflammatory response and collagen deposition for SAM-3 and SAM-6 mesh constructions. Examination of mesh contraction values (Chapter 3), however, did not correlate with increased myofibroblast differentiation as one might suspect as UltraPro™ exhibited the highest amount of contracture at 12-weeks but resulted in the least amount of myofibroblastic activity at the same time point. It is possible that the mesh contraction for UltraPro occurs between the 8 and 12 weeks implantation period as we see a peak in myofibroblast activity at 8-weeks for this construction, indicating that this may still be a myofibroblast driven process but one that has resolved by 12-weeks.
Figure 4.9. Left – Diagram of SAM mesh pore indicating inter-filament pore distance and model of granuloma/collagen deposition around mesh fibers. Right – Diagram of UltraPro™ mesh pore indicating inter-filament pore distance and model of granuloma/collagen deposition around mesh fibers. Black = non-absorbable fiber, Red = absorbable fiber, Blue = collagen deposition

The long-term efficacy of a mesh hernioplasty repair is ultimately dependent on the implanted meshes ability to 1) become fully integrated into the patient’s abdominal wall, 2) allow for a compliant repair site such that patient quality of life is not impeded and sensation of foreign body is minimal, and 3) allow for long-term reconstruction of the abdominal wall such that collagen type and quality approaches that of healthy tissues.
The latter was examined within this study through the evaluation of MT stained slides, as previously discussed, and through the use of picro-sirius staining techniques to determine collagen composition/maturity. Type I/III collagen ratios were determined at two specific locations: above each mesh construction located on the subcutaneous side and within the pores of each construction. Data in Figure 4.5 indicates that UltraPro™ exhibits a significant increase in collagen type I/III ratio between 4 (0.52) and 12-week (1.44) implantation time points on the subcutaneous side of each mesh. In comparison, SAM-3 and SAM-6 constructions trended higher at 8 and 12 weeks, as compared to 4-week implant durations, but this increase was not of statistical relevance. This result mirrors findings by Pascual and colleagues which indicated a faster conversion of type III collagen to type I collagen in UltraPro™ meshes as compared to smaller pore mesh designs. In addition, UltraPro™ exhibited significantly higher collagen Type I/III ratios (1.07) at 12-weeks as compared to SAM-3 (0.40) within the pores of the mesh construction as shown in Figure 4.7. The ability of UltraPro™ mesh to induce higher levels of type I mature collagen, as compared to SAM-3 and SAM-6, within this study is attributed by the author to its low area weight, monofilament fiber form construction, and large pore design. In addition, the pores of the developed constructions is occupied by an absorbable yarn component, as depicted in Figure 4.9, resulting in an initial inter-filament pore distance of 0.65 mm. This leads to a tendency for the collagen to be deposited over the mesh construction versus being laid down within the mesh pores as is the case for UltraPro™ which has an inter-filament pore distance of 2 mm. In addition, as the developed mesh absorbable fibers begin to degrade an inflammatory response is triggered
within the pores of the mesh, which ultimately results in the deposition of more type III collagen. In a long-term study with durations of use where by the absorbable components for SAM-3 and SAM-6 have been completely resorbed it is believed that a more mature collagen lattice would be realized.

Collective analysis of the findings within this study (Table 4.2) indicate that the developed SAM-3 and SAM-6 constructions resulted in a moderate degree of inflammation that remained active through the 12-week study period. Although the initial response is thought to be due to the high area weight and multifilament fiber form of these constructions, the continued activity of tissue macrophages and myofibroblasts is likely in response to absorbable component degradation by-products. This is especially true within the pores of the developed constructions where low levels of type I/III collagen ratios were observed. UltraPro™ mesh benefited from the large pore and monofilament construction in regards to eliciting a higher degree of collagen maturation but these factors also resulted in slow integration into the native tissue and minimal collagen deposition around the mesh construction. As a result, a higher incidence of direct visceral adhesions and mesh contraction was seen as previously reported (Chapter 3). As such, it is the author’s opinion that a higher density construction, as those developed as part of this research, may be more appropriate in a defect filling situation where a section of the abdominal wall is resected and/or absent. This situation routinely presents itself in transverse rectus abdominis myocutaneous (TRAM) flap breast augmentation procedure where a section of the abdominal wall is completed resected.43 Conversely, a hernia where a large majority of the tissue is preserved and simply needs
reinforcement may benefit from a lighter, open pore design as that presented by UltraPro™ but with the long-term changing mechanical profile of the developed constructs, SAM-3 and SAM-6. This data indicates that a one-size-fits-all solution is not applicable to hernia repair as the presentation is highly dependent on location and size of the defect.

Table 4.2. Collective Histopathological Analysis of Mesh Constructions.

<table>
<thead>
<tr>
<th>Mesh Type</th>
<th>Mesh Design</th>
<th>Collective Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM-3</td>
<td>Fiber Form</td>
<td>Moderate degree of inflammation triggered by mesh construction which allowed rapid integration into the native tissue. Inflammatory phase continued throughout the 12-week study period due to timing of absorbable component degradation.</td>
</tr>
<tr>
<td></td>
<td>10-filament PP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-filament PG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absorption Characteristics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial A.W. = 138.7 g/m²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final A.W. = 79.2 g/m² (16 wks.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTP = 2 – 4 wks.</td>
<td></td>
</tr>
<tr>
<td>SAM-6</td>
<td>Fiber Form</td>
<td>Collagen deposition was moderate around periphery of mesh due to dense construction and resulted in reduced levels of type I/III collagen ratios. Placement of absorbable component within pores of developed mesh not ideal as this stunted collagen maturation within mesh construction.</td>
</tr>
<tr>
<td></td>
<td>10-filament PP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monofilament PDO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absorption Characteristics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial A.W. = 186.3 g/m²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final A.W. = 62.4 g/m² (16 wks.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTP = 6 – 11 wks.</td>
<td></td>
</tr>
<tr>
<td>UltraPro™</td>
<td>Fiber Form</td>
<td>Minimal inflammatory response triggered by mesh construction which led to slow integration into the native tissue. This was attributed to large pore and low area weight design as well as complete monofilament fiber form.</td>
</tr>
<tr>
<td></td>
<td>10-filament PP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monofilament PDO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absorption Characteristics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial A.W. = 186.3 g/m²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final A.W. = 62.4 g/m² (16 wks.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTP – no significant transition</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimal collagen deposition in and around mesh construction. Higher degree of collagen maturation specifically within the pores of the mesh construction.</td>
</tr>
</tbody>
</table>

Note: For table above: A.W. = Area Weight, PP = Polypropylene, PG = Polyglycolide, PDO = Polydioxanone, PTP = Phase Transition Point


V. Conclusion

Through the use of various histologically staining techniques and procedures the short-term cellular and extra-cellular matrix response to candidate selectively absorbable surgical mesh constructions, SAM-3 and SAM-6, were accessed in comparison to the predicate material UltraPro™ in a mature ventral hernia rabbit model. A higher ability to induce an acute inflammatory response and subsequent collagen deposition was found for SAM-3 and SAM-6 which resulted in better and more rapid integration of the mesh device within the native tissue. Evaluation of collagen maturity indicated a general trend of higher type I/III collagen ratios within the UltraPro™ study groups when compared to SAM-3 and SAM-6 meshes. This is thought to arise from the smaller inter-filament space found for the developed constructs in comparison to the large pore/light weight design of the UltraPro™ mesh. In addition, a higher tendency for cyst/seroma formation was found in the UltraPro™ mesh groups, which is attributed to the lack of collagen deposition in and around the mesh construction.
References


CHAPTER V
CONCLUSIONS

Although the foreign body response to non-absorbable mesh materials is thought to be responsible for many of the long-term complications currently associated with mesh hernioplasty, little research on the effect of mesh biomechanics has been reported and may represent the missing link that has been limiting advancements in this field for the past 30 years. In this body of work, we outlined the development of a suite of surgical meshes based on identification of critical design inputs. Each construction exhibits an early stability phase followed by a long-term compliance phase which matches the extensional properties of the human abdominal wall. A major contributing factor for hernia development is thought to be abnormal connective tissue formation; therefore, some type of long-term support was identified early on as a critical design characteristic. As such, these developed constructions were created to be selectively absorbable (i.e. at least one component was selected to be non-absorbable) but still display significant shifts in mechanical properties in situ while maintaining an adequate level of strength to prevent central mesh rupture.

In order to accurately determine the effect of these constructions on wound repair, as it relates to mesh hernioplasty, a mature ventral hernia model in rabbits was successfully created which exhibited many of the wound pathology characteristics seen in the human hernia situation: presence of a distinct hernia sack, hernia ring, and various
unpredictable visceral adhesions. In addition, UltraPro™ mesh was chosen as a predicate material, which is a commonly used partially absorbable mesh allowing side-by-side comparison with the designed hernia mesh system.

Conclusions of in vivo experimentation indicated that, while the initial repair site was mechanically much stiffer for the developed constructs as compared to the native tissue and UltraPro™ mesh, the developed construct transitioned to a more compliant repair over the course of the 12-week testing period. In contrast, UltraPro™ mesh did not exhibit any significant change in mechanical compliance even though an almost 50% drop in material mass occurred over this same testing period. In addition, comparison of in vivo and in vitro mechanical testing indicated that the contribution of collagen development to the stiffness of the mesh/tissue complex in the predicate testing group increased over time while this mechanical component decreased or plateaued in the developed mesh test groups. Histopathological analysis indicated that the developed constructs triggered a moderate inflammatory response which allowed rapid integration into the native tissue, while UltraPro™ exhibited slow integration which resulted in a higher incidence of direct visceral adhesion and seroma formation. Collagen maturation (type I/III collagen ratios) was found to be higher in the predicate group, although this is attributed to the placement of the absorbable component in the developed constructions, which is thought to result in increased type III collagen deposition in this short-term study. Collectively, the findings of this research indicate the potential of the experimental mesh design to reduce certain complications associated with mesh hernioplasty, by exhibiting a dynamic mechanical wound response with rapid tissue
ingrowth, including reduced wound site mobility and mesh contracture with a reduction in the severity of adhesions.
CHAPTER VI
RECOMMENDATIONS FOR FUTURE WORK

Based on the outcomes of the research discussed in this dissertation, future work should attempt to:

- Examine the developed constructs in an *in vivo* model using a larger animal (pigs or sheep) where the abdominal wall mechanics are more closely comparable to humans
  - As predicate mesh materials as well as the ones developed as part of this research are designed for human end use, the physiological loads these constructions are designed for may never be realized in a small animal model
- Evaluate the developed constructs long-term in order to realize the true effect of load transfer on mesh hernioplasty outcome
  - Ideally the chosen end points would allow complete mass absorption and resolution of the absorbable components (5 to 8 months)
  - Majority of mesh related issues are exhibited years after mesh placement, especially in the case of hernia recurrence
- Create light-weight variants of the constructions described within this research using monofilament fiber forms and open pore design
- Reduced risk of chronic inflammation and development of a thick scar plate with this type of design
- Placement of the absorbable mesh stabilizing component away from the pore so that collagen maturation is not impeded

- Examine the developed constructs with the effect of mesh construction on visceral adhesions included as a primary endpoint
APPENDIX

Slides were stained by Histology Tech Services using the provided methods. Tissue was fixed in 10% neutral buffered formalin immediately following mechanical testing. Tissue sections were provided in standard histology tissue cassettes for processing. Each staining protocol is described below:

**Picro-Sirius Red**

1. Deparaffinize and hydrate to distilled water.
2. Stain in Picro-sirius red for 30 minutes to 1 hour.
3. Rapidly wash in running tap water for a few seconds.
4. Counterstain with Harris Hematoxylin or 0.001% fast green solution for 15 minutes.
5. Dehydrate rapidly in 3 changes of 100% Alcohol.
6. Clear in xylene and coverslip using a xylene based mounting medium.

**Masson’s Trichrome**

1. Deparaffinize and hydrate to distilled water.
2. Mordant in Bouin’s for 1 hour at 56°C.
3. Cool and wash in running tap water until yellow color disappears.
4. Rinse in distilled water.
5. Place in Weigert’s iron hematoxylin for 10 minutes or in Harris hematoxylin for 7-8 minutes.
6. Rinse in distilled water.
7. Biebrich scarlet acid-fuchsin solution for 2 minutes. Rinse in distilled water.

8. Phosphomolybdic-phosphotungstic acid solution for 10-15 min. when using [aniline blue](#). (Use aqueous phosphotungstic acid 5% for 15 min. when using light green counterstain.)

9. Aniline blue for 5 min. (Or light green for 1-7 min).

10. Rinse in distilled water.

11. Dehydrate in 95% alc., 100% alc., and clear in Xylene. Coverslip.

**IHC General Staining Procedure:**

1. Tissue sections are procured from the paraffin at a 4 micron thickness and placed on adhesive slides in order of the specimen list given for each phase of a study.

2. The slides are air heated for 15 minutes in a 65°C Roto Dry oven.

3. The slides are then cooled and deparaffinized through xylene and graduated alcohols to water.

4. Soak slides for 3 minutes in distilled water.

5. Rinse slides for 2 X 3 minutes in TBS Buffer.

6. Perform HIER. Follow the ‘Full HIER’ protocol in SOP-IHC-32 (Substituting Diva Decloaker for Trilogy).

7. Slides are then placed on the [Dako Autostainer Plus](#) (See below) using the appropriate program run (Auto-program CD68 Biocare). See the "Neg Control" Auto-program for Negative Control.

8. When the staining run is complete, remove slides and place in a rack within a staining dish filled with distilled water.

9. Counterstain. Place slides in staining rack and in Reserve Hematoxylin for 90 seconds.

10. Rinse for 1 minute in tap water.
11. Dip slides for 30 times in High Def.
12. Rinse slides for 1 minute in running tap water.
13. Dip slides for 30 seconds in Reserve Bluing Reagent.
14. Rinse slides for 1 minute in running tap water.
15. To dehydrate, dip slides in 95% alcohol 5 times, then immediately submerge slides in a series of three 100% alcohol stations for 45 Seconds each.
16. Clear slides in a series of three Xylene stations for 30 seconds to 1 minute each.
17. Use Cytoseal XYL to coverslip out of Xylene.

**Dako Auto Stainer Plus: CD68**

1. TBS Buffer Rinse
2. Peroxidazed 7 Minutes
3. TBS Buffer Rinse*
4. Background Sniper 7 Minutes
5. TBS Buffer Rinse*
6. CD68 30 Minutes
7. TBS Buffer Rinse*
8. Mach 3 Probe 10 Minutes
9. TBS Buffer Rinse*
10. Mach 3 Mouse HRP-Polymer 10 Minutes
11. TBS Buffer Rinse*
12. DAB Chromagen 5 Minutes
13. TBS Buffer Rinse
14. TBS Buffer Rinse
15. Distilled Water Rinse*

*Until all slides have received rinse whether 1 slide or 48 slides, which would vary in time.

**Smooth Muscle Actin (SMA):**

1. Deparaffinize slides and rehydrate.
2. Rinse slides for 3 minutes in DH2O.
3. Perform HIER. (SOP-IHC-32)
4. After 3 minute gentle water rinse, place slides for 3 minutes in Tris Buffer, gently dip to agitate.
5. Prepare a 1:100 dilution of Alpha Smooth Muscle Actin Primary Antibody (30 minutes before use), as directed above. For the Negative Control use Antibody diluent.
6. Rinse slides for 2 X 3 minutes in Tris Buffer.
7. Place slides in Hydrogen Peroxide and incubate for 10 minutes.
8. Rinse slides for 2 X 3 minutes in Tris Buffer.

**Dako Auto Stainer Plus: Smooth Muscle Actin (SMA)**

1. TBS Buffer Rinse
2. SMA 30 Minutes
3. TBS Buffer Rinse*
4. Anti-Mouse IgG 30 Minutes
5. TBS Buffer Rinse*

6. Vector ABC Kit 30 Minutes

7. TBS Buffer Rinse*

8. DAB Chromagen 10 Minutes

9. TBS Buffer Rinse

10. TBS Buffer Rinse

11. Distilled Water Rinse*

*Until all slides have received rinse whether 1 slide or 48 slides, which would vary in time.