DEVELOPMENT AND EVALUATION OF AUXETIC MESHES FOR PELVIC ORGAN PROLAPSE REPAIR

by

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Pelvic organ prolapse (prolapse) is a gynecologic condition that negatively impacts half of all women over the age of 50. It is characterized by the unnatural descent of the pelvic organs into the vaginal canal. Synthetic mesh, commonly used in the treatment of prolapse, is associated with complications, which prompted the FDA to release public health notifications in 2008 and 2011. Research suggests that pore collapse, material stiffness mismatches with vaginal tissue, increased mesh burden, and meshes experiencing permanent deformation with loading all potentially contribute to mesh complications. Thus, the overall goal of this dissertation was to develop an initial prototype mesh that overcomes the problems with current meshes. To accomplish this goal, the behavior of computational, mesh models, with varying auxetic pore geometries, in response to simulated uniaxial loading was assessed via finite element analysis (FEA). Results demonstrated that certain auxetic geometries can prevent pore collapse; however, pore expansion is dependent on the orientation of the auxetic geometry (i.e. the pore) with respect to the loading direction. Next, meshes with auxetic geometries for pores were manufactured from a soft, elastomer polydimethylsiloxane (PDMS), which had a material stiffness that is similar to that of the vagina. The behavior of these meshes was characterized via mechanical testing. Similar to the FEA results, the pores of the mesh expanded in response to tensile loading, and this behavior was dependent on
the orientation of the pore. Additionally, pore expansion was associated with a decrease in mesh burden, and meshes did not permanently deform in response to cyclic loading. Lastly, the host response to elastomeric meshes was assessed via implantation into the abdomens of rodents for 35 days. The default host response to a foreign material was observed with an absence of bridging fibrosis, a phenomenon associated with encapsulation, mesh contraction, and pain. Additionally, the fibrotic response to the elastomeric meshes was less than that of the prototype prolapse mesh, Gynemesh PS. Overall, a novel synthetic mesh was developed which has the potential to improve the current design of prolapse meshes and decrease the risk of mesh related complications.
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PREFACE

In the United States of America, obtaining a quality education, from grade school to post-secondary, is not fundamentally obtainable for all. Unfortunately, this is especially true for those who are economically disadvantaged and living within the inner city, the majority being Black. It is, therefore, not surprising that few Blacks earn a PhD. According to the Doctorate Recipients from U.S. Universities Report, 54,070 research doctoral degrees were awarded in 2014, and of these, 62.9% were awarded to U.S. citizens and permanent residents with only 6.4% being awarded to African Americans compared to 73% White (National Center for Science and Engineering Statistics, Directorate for Social, Behavioral, and Economic Sciences). Among the STEM PhD holders, Blacks comprised 5.9% of those within the life sciences (72.2% White), 3.5% in the physical sciences (75.5% White), and 4.1% in engineering (69.5% White). These numbers are disheartening and put into perspective for me how truly blessed I am to have the opportunity to pursue and obtain a PhD in Bioengineering, a task that once was not possible and still to this day is difficult to achieve for those of African descent living in the United States. As an African American athlete and female bioengineer, I am living proof that Blacks have much more to offer than simply our athletic abilities and talents. We have incredible potential to excel in academics and STEM (science, technology, engineering, and mathematics). Simply provide us an opportunity and watch us work! I therefore dedicate my dissertation to my African American Community and to all of the under-represented minorities who have ever considered or are pursuing an
education and career within STEM. My matriculation through graduate school was not easy and there were definitely many bumps along the way. However, with hard work, a tenacious attitude to finish, motivation, faith, and a strong support system, I made it through and so can you!

As I reflect on my educational journey from grade school to now, I would not be in the position that I am today without a solid foundation and strong support system. I would be remiss if I did not acknowledge those persons that have helped me along the way. First and foremost, I give honor and praise to God for without Him I am nothing. He gave me the strength, courage, and ability to excel in academia and STEM. Throughout undergraduate and graduate school, I never had to worry about paying for school, nor did my parents, and that was all because of God’s blessings and favor over my education. Additionally, God blessed me with two strong parents that were able to provide for their children and made sure that we had everything that we needed. Children do not get to pick their parents and my life could have been completely different had I been born to different parents, but God had me in His hands and picked the perfect parents for me.

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am truly grateful for my parents and do not take them or the life that I have because of them for granted. Outside of my parents, I would also like to thank my family for their prayers and support along my PhD journey, and I thank my siblings for motivating me to excel and finish. I express my sincere gratitude to my Godmother, Yolanda Geiger for ensuring that I had a personal relationship with God.

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1.0 INTRODUCTION

Pelvic floor disorders (PFDs) is a broad term that collectively refers to conditions in which the function of the tissues and muscles within the pelvis are impaired. These conditions are often associated with a variety of symptoms including pain, problems with voiding, and organ protrusion. PFDs including pelvic organ prolapse (prolapse or POP), fecal incontinence, and urinary incontinence, often negatively affect a woman’s quality of life, self-image, and voiding function [1,2]. The risk factors associated with PFDs include pregnancy, aging, parity (defined as the number of vaginal deliveries), mode of delivery, and obesity [3-6]. In 2010, 28.1 million women in the United States were afflicted with at least one PFD [7] and with the advancing age of the US population, this number is expected to increase. By 2050, it is estimated that 43.8 million women will have at least one PFD [7]. The economic burden of PFDs is substantial. In 2005-2006, the direct ambulatory cost of PFDs was $412 million [8], and it is anticipated that this number will increase concomitantly with the expected increase in the number of women with PFDs.

Pelvic organ prolapse is one of the most common PFDs affecting nearly half of all women over the age of 50 with a prevalence of 30% for women between the ages of 20 and 59 [9]. The International Urogynecological Association (IUGA)/International Continence Society (ICS) defines pelvic organ prolapse as the descent of the anterior vaginal wall, posterior vaginal wall, the uterus (cervix), or the apex of the vagina (vaginal vault or cuff scar after hysterectomy) [10]. Elderly and parous women have a higher incidence of prolapse relative to young and nulliparous
women [3,4,6]. By the age of 80, 12.6% of women will have at least one corrective surgery for pelvic organ prolapse and 30% of those will undergo a reoperation [11,12]. The surgical cost of prolapse is substantial, totaling over $1 billion in 1997 [13]. Women with prolapse often experience feelings of and/or see a bulge/protrusion from their vagina, pelvic pressure, dyspareunia (pain with sexual intercourse), and complications with urinary and bowel functions [10,14,15]. Additionally, women with pelvic organ prolapse, particularly those with advanced prolapse, also have feelings of embarrassment, social isolation, and decreased quality of life and self-image [2].

Treatment for prolapse is divided into two categories: conservative and surgical. The conservative approach is often used for women who are not healthy enough or wish not to have surgery and for those with symptoms that are not problematic. Surgical treatment is, therefore, used for all other women with prolapse seeking treatment. Synthetic meshes are commonly used in the surgical repair of prolapse. These devices are porous and are manufactured by knitting polymeric fibers together. As with any medical device implanted into the human body, there are some positive (e.g. relief of symptoms) and negative (e.g. mesh complications and recurrent prolapse) patient outcomes associated with the use of synthetic mesh in the surgical repair of prolapse. An in-depth discussion of synthetic mesh and prolapse will be discussed later on in this chapter.

The purpose of this chapter is to set the foundation for the background knowledge that is required to understand the projects and goals of this dissertation. This chapter will begin by introducing various aspects of prolapse including clinical presentation, causes, and treatment methods. Next, the devices used in the repair of prolapse will be discussed with a particular emphasis placed on synthetic meshes. The perspective of this chapter will then switch from a
clinical perspective to a mechanical one in which the mechanical evaluation of synthetic meshes will be discussed.

Mechanics has been widely used to study prosthetic devices in a variety of medical fields (e.g. cardiovascular and orthopaedics) for many decades. However, the evaluation of PFD devices from a mechanical perspective is relatively new and studies are limited. In order to understand and appreciate the mechanics of these meshes, one must be knowledgeable of the mechanical/functional role of synthetic meshes in PFD repairs. Thus, these roles will be discussed with a concentration on synthetic meshes used to repair POP and stress urinary incontinence (SUI) (also referred to as urogynecologic meshes). The next three sections of this chapter will discuss the types of tests that are used to evaluate the mechanics of synthetic meshes. The relevance/limitations and methodology of mechanical tests in regards to synthetic meshes will be emphasized. The chapter will then transition to an application-focused perspective in which the results of mechanical testing of synthetic meshes and the biological implications of these results, as observed experimentally and clinically, will be described. Next, the impact of various textile and structural properties of synthetic meshes on the host response to urogynecologic meshes will be explained. This will provide the framework for the motivation of a new design for synthetic meshes used in prolapse repairs. Finally, this chapter will conclude be introducing the specific aims of this dissertation.
1.1 PELVIC ORGAN PROLAPSE

1.1.1 Clinical Overview

In a well-supported pelvis, the vagina provides anterior support to the urethra and bladder, apical support to the uterus, and posterior support to the rectum. A complex network of connective tissues and muscles provide support to the vagina and also provide indirect support to the pelvic organs [16-18]. For conceptual purposes, the connective tissue support to the vagina is divided into three levels (Figure 1) [16,17]. Level I provides apical support to the vagina via the cardinal and uterosacral ligaments. Level II provides support to the mid-vagina by attaching it to the pelvic sidewalls via the anterior and posterior endopelvic fascia (the so-called paravaginal attachments). Lastly, the distal vagina is supported by the anterior and posterior endopelvic fascia, the pubic symphysis anteriorly, and the perineal body posteriorly, comprising Level III support. Studies have shown that the levator ani muscles (iliococcygeus, pubococcygeus, pubovisceral) also play a key role in providing support to the distal vagina (Figure 2) [16,17,19].
Figure 1: Support to the vagina is provided by connective tissue attachments to the sacrum and pelvic sidewalls, and by a series of striated muscles referred to as the levator ani.

Figure 2: Superior view of the levator ani which consists of striated muscles (pubococcygeus muscle – PCM, iliococcygeus muscle – ILM, pubovisceral muscle – PVM) penetrated by the rectum (R), vagina (V), and urethra (U). The levator ani function to close the bony pelvis.
It is generally understood that a loss of support to the pelvic organs, via damage to or abnormalities in the vagina, muscles, and connective tissues, results in the descent of these organs from their normal anatomical position into the vaginal canal (Figure 3). Clinically, this descent is quantified using the pelvic organ prolapse quantification (POP Q) system, which divides prolapse into 5 stages, 0 to IV. Stage 0 represents no prolapse and stage IV is the most severe case in which there is a complete eversion of the vagina [20]. There are multiple types of pelvic organ prolapse and the name of each type stems from the organ or structure that has descended. For example, uterine/cervical prolapse describes prolapse in which the uterus or uterine cervix has descended and vaginal vault prolapse describes prolapse in which the vaginal vault or apex has descended. Descent of the anterior and posterior walls of the vagina also occurs and this is referred to as anterior and posterior vaginal wall prolapse, respectively. Anterior vaginal wall prolapse is often caused by the descent of the bladder into the anterior vaginal wall (referred to as a cystocele). Posterior vaginal wall prolapse is often a result of the protrusion of the rectum into the posterior vaginal wall (rectocele).
1.1.2 Pathogenesis

The exact etiology of pelvic organ prolapse is unknown. However, there are several risk factors that have been identified as contributors to the development of prolapse. Parity, defined as the number of vaginal deliveries, is considered to be one of the leading risk factors [3,4,6,21,22]. Parous women are 4 times more likely than nulliparous women to develop prolapse after a single vaginal delivery, and this risk nearly doubles after a second delivery [4]. Interestingly,
symptomatic prolapse often develops decades after a women’s last birth, and the changes that are occurring during this time period that result in prolapse development are not clear, although injury to the pelvic supportive connective tissues and muscles is one plausible mechanism. Studies have shown that damage to the levator ani muscles, loss of pelvic organ support, and denervation can occur during vaginal delivery [23-25]. Abnormalities in the levator ani muscles as well as pelvic nerve injuries are associated with pelvic organ prolapse and other PFDs [24,26,27]. Advancing age and obesity have also been established as risk factors for prolapse development [3,12,22]. Other potential risks include: prior hysterectomy, race/ethnicity, obstetric factors (e.g. mode of delivery, infant weight), and connective tissue disorders [14,15].

1.1.3 Treatment Methods

The treatment of pelvic organ prolapse can be broadly categorized into conservative and surgical approaches. The simplest form of treatment is observation. This conservative treatment method is for patients who have early signs/symptoms of prolapse, but are not symptomatic or are not bothered by their symptoms. As the name suggests, conservative management involves a wait and watch approach to assess for progression and to determine if intervention is warranted. Pelvic floor muscle physical therapy (e.g. Kegel’s) is a popular conservative treatment that aims to strengthen the pelvic floor muscles. Insertion of a pessary is an alternative conservative treatment option for patients which involves the insertion of a vaginal device that supports the vagina. Pessaries are the preferred method of treatment for patients with symptomatic pelvic organ prolapse that are either unfit for surgery, awaiting surgery, decline surgery, or for those patients needing temporary relief of pregnancy-related prolapse or incontinence [14]. See Section 1.2.1 for more details regarding pessaries.
Surgical treatment for pelvic organ prolapse consists of obliterative and reconstructive procedures. Obliterative surgery corrects prolapse by returning the pelvic viscera to their position in the pelvis then closing off the vaginal canal either partially or completely. Women treated with this type of surgery are typically elderly, medically compromised, and no longer sexually active. Reconstructive surgery is designed to correct pelvic organ prolapse by relieving any pelvic symptoms while concomitantly maintaining (or improving) vaginal sexual function [14]. Traditionally, reconstructive surgery consisted of using the patient’s native connective tissues and muscles to provide support to weakened and/or compromised pelvic structures. Unfortunately, in large prospective trials the failure rate of these surgeries is high – approximately 40% at 2 years [12,28,29], likely associated with poor integrity of the existing tissue and muscles [30-35]. To overcome these dismal outcomes, surgeons have turned to biomaterials most commonly biologic grafts and synthetic meshes. The next section of this chapter will focus on these materials with an emphasis on synthetic meshes.

1.2 PELVIC ORGAN PROLAPSE REPAIR DEVICES

1.2.1 Pessary

The use of prosthetics in the female pelvis dates back to the fourth century, to Polybus, at which time uterine prolapse was treated by placing half a pomegranate in the vaginal area or by packing the vagina with a sponge [36]. These “prosthetics” were the earliest forms of devices referred to today as pessaries. One of the first “more modern” pessaries was described in the late sixteenth century by Ambrose Pare’ who designed oval-shaped pessaries made out of brass and waxed cork
for uterine prolapse [36]. Essentially, the function of these pessaries was to act as a plug for the vaginal canal, preventing the prolapsed organ(s) from protruding outside of the body. Over the years, the design, materials, and function of pessaries has evolved such that these devices are widely used as a non-surgical treatment option for both pelvic organ prolapse (POP) and stress urinary incontinence (SUI).

Pessaries are used to provide structural support. They are not only used to relieve POP and SUI related symptoms, but they are also used to treat vaginal wind, neonatal prolapse, prolapse in pregnancy, and voiding dysfunction [37-43]. Modern-day pessaries are made primarily of silicone or an alternative inert plastic and fall into one of two main categories: space-filling and support pessaries (Figure 4) [40,44]. Similar to the early pessaries, space-filling pessaries fill the vaginal canal by 1) creating suction between the vaginal walls and the pessary and/or by 2) having a diameter that is larger than the genital hiatus. The cube (suction mechanism), donut (large diameter mechanism), and Gellhorn (suction and large diameter mechanism) are all examples of space-filling pessaries. Instead of simply filling a space, support pessaries are placed in the space between the posterior fornix and the pubic symphysis; thus, providing support to the vagina. The ring pessary is one of the most commonly used support pessaries.
Although successful, pessaries are associated with complications. Common complications include irritation of the vaginal mucosa, bleeding, vaginal excoriation, ulceration, and impaction. Vaginal discharge and vaginal odor are also problems associated with pessary usage [37,40]. From a biomechanical perspective, pessaries that are too large create friction between the device and the vaginal walls, which essentially wears down the walls of the vagina resulting in abrasion, erosion and in extreme cases, fistula formation. Thus, the fit of the pessary is a critical component of success. However, it is important for the reader to keep in mind that erosions in pessaries that fit well are also observed, particularly when the pessary is subjected to a lot of pressure from an obese abdomen or simply from the resulting pressure of a loss of support. Despite these complications, pessaries are a viable option for patients that decline or are unfit for surgery and for those seeking temporary relief of POP and SUI related symptoms.
1.2.2 Biologic Grafts

There are three types of biologic grafts: autologous (grafts derived from the patient), allografts (derived from another human source excluding the patient), and xenografts (derived from an animal source). The advantage of autologous grafts is that they are harvested from the patient; thus, the chance of causing a foreign body response is minimal. These grafts also incorporate well with native tissues [45]. However, using autologous grafts increases the operation time, is associated with harvest site morbidity, and the strength of the repair, and hence long-term durability, is dependent upon the quality of the host tissue [45,46]. Allografts, such as cadaveric fascia lata, on the other hand do not impact operation time nor is there a concern for donor site morbidity, as they are often obtained from cadaveric tissue banks. There is, however, an inherent risk of disease transmission, from donor to host. Processing methods (e.g. chemical or radiation) are used to reduce immunogenicity and to reduce the transmission of diseases. Depending on the processing method utilized, the quality of the allograft, in particular the strength and durability, can be significantly impacted [47]. In addition, allografts are often obtained from elderly patients with multiple comorbidities or prolonged illnesses resulting in inconsistent quality. Thus, the number of quality allografts available to surgeons and patients is limited. Xenografts are more readily available and are currently more frequently used relative to the other biologic grafts. They are often decellularized during preparation to reduce the risk of disease transmission. However, one concern of these grafts is the presence of antigens contained in residual cell membranes of xenografts, particularly the oligosaccharide α-Gal (Galα 1,3-Galβ1-4GlcNAc-R) epitope, which can result in hyperacute rejection following implantation [48]. Nonetheless, xenografts are thought to have higher failure rates over time and this is likely due to a host vs graft response potentially resulting from residual antigenic material [49,50].
1.2.3 Synthetic Mesh

Although used in urogynecologic surgeries since the 1950s, synthetic grafts (referred to as synthetic meshes) became popular in the 1990s as concerns regarding the quality, durability, and strength of biologic grafts increased. The primary purpose of synthetic mesh is to provide structural support to the vagina and hence, the pelvic organs in an effort to reasonably restore the anatomy and function of these structures while concomitantly providing a repair that is more or equally durable to a patient’s own tissue [51]. Synthetic meshes are implanted using one of two primary approaches: transabdominally via sacrocolpopexy or transvaginally in the anterior, apical and/or posterior compartments (Figure 5) [52]. An abdominal sacral colpopexy, often performed laparoscopically or robotically is the “gold-standard” procedure, which provides apical support to the vagina by attaching the vagina through a mesh bridge to the sacrum. During this procedure, two independent mesh straps or arms of a Y mesh are placed between the bladder and the vagina anteriorly and the rectum and the vagina posteriorly. The arms or straps are then anchored to the sacrum effectively restoring the vagina to its normal anatomical position via a mesh bridge. In a transvaginal procedure, the mesh is most often used to attach the vagina to the sacrospinous ligament through a mesh bridge or “arm” or the pelvic sidewall via an attachment or a mesh arm to the arcus tendineus fasciae pelvis. A critical part of this procedure is the presence of full thickness vaginal incisions needed for placement of the mesh. Overall, the abdominal procedure is technically more difficult than the transvaginal, has a longer recovery period, and a higher risk of potential morbidity [53-55]. However, the transvaginal procedure is associated with higher rates of complications and higher reoperation rates, which has led to the release of public health notifications by the Food and Drug Administration (FDA) in 2008 & 2011 (to be discussed in more detail later in this section) and an up-classification of these devices from Class II to Class III.
To date, the majority of synthetic meshes are knitted, lightweight (<45g/m²), wide pore (>1 mm, porosity > 55%) Type I (pore sizes exceed 75 μm) polypropylene. The concept of using synthetic mesh for pelvic organ prolapse and incontinence repairs originates from the repair of abdominal hernias. In the abdominal hernia literature, superior outcomes were demonstrated in patients undergoing a mesh repair versus a native tissue repair [56,57]. Starting in the 1970s,
gynecologists were using meshes indicated for hernia repair to repair prolapse abdominally. By the 1990s, hernia meshes were being used for the transvaginal repair of prolapse and for the surgical treatment of SUI. Up until this point, there were no pre-configured meshes for SUI and pelvic organ prolapse repairs. Thus, surgeons were simply cutting hernia meshes to fit the desired shape needed for a prolapse or SUI repair. As a result, companies began to make meshes specifically designed for these types of repairs. Surgical Fabrics (ProteGen Sling, Boston Scientific Corporation, Malborough, MA, USA) was the first pre-configured mesh brought to market specifically for surgical treatment of SUI in 1996 followed by the Tension-Free Vaginal Tape (TVT™, Ethicon, Somerville, NJ, USA) System in 1998, which introduced the terms sling and tape for mesh use in SUI repair. Four years later, Gynemesh PS (Ethicon, Somerville, NJ, USA) was cleared as the first preconfigured mesh for prolapse repair in the form of the Prolift™ transvaginal mesh kit (Ethicon, Somerville, NJ, USA).

In 1976, a Medical Device Amendments Act was passed which classified medical devices into three different risk-based categories. Class I devices pose the lowest potential risk while Class II and III devices pose greater risks with Class III devices having the greatest risks. While the premarket review process for Class I and II devices is a 510(k) process, Class III devices require premarket approval (PMA). In order to pass the 510(k) process, a device must be shown to be similar and at least as safe and effective as a previously, legally, marketed device that did not require a PMA. This can be done by simply showing that 1) the device has the same intended use and technological characteristics as a previously approved device or 2) has the same intended use and different technological characteristics but does not raise new questions on safety and effectiveness and is at least as safe and effective as a previously approved device [58]. The PMA process on the other hand is stricter, requiring scientific and clinical data to demonstrate the safety
and effectiveness of a device [59]. Given that existing hernia meshes (Class II devices) were legally, already in use prior to the 1976 Medical Device Amendments Act, companies were able to remarket their hernia products for treatment of prolapse and SUI using the 510(k) process; thereby avoiding costly premarket testing of an entirely new device. Unfortunately, this meant that these devices were brought to the market without considering the unique characteristics of the vagina and the complex loading conditions of the female pelvis. Additionally, this meant that surgical meshes indicated for prolapse or SUI were cleared without the need for original clinical studies to demonstrate device safety and effectiveness. Mesh use in prolapse escalated following the introduction of the technically easier transvaginal approach and by 2010 meshes were being used in 1/3 of all cases to repair prolapse and over 80% of SUI surgeries [60]. However, as mesh use increased, so did the incidence of mesh related complications prompting the FDA to issue two public health notifications in 2008 and 2011 warning of complications associated with the transvaginal application of mesh. Complications cited from the MAUDE database included infection, mesh shrinkage, mesh erosion into an adjacent structure, mesh exposure into the vaginal lumen, and pain, with the latter two by far being the most commonly reported (Figure 6). To date, the mechanism(s) responsible for mesh complications is unknown and research in this area, as well as litigation, is increasing.
Not surprisingly, following the release of the public health notifications in 2008 and 2011, litigation surrounding the use of mesh in urogynecologic surgery escalated and resulted in multi-million dollar multi-district litigation causing most mesh companies to discontinue their products. With the increased reporting of complications, the FDA recognized the need for studies regarding these products and has up-classified surgical urogenital meshes from Class II to Class III devices; thus requiring companies to submit a premarket approval (PMA) application to perform small clinical trials demonstrating safety and effectiveness of the device as compared to native tissue repairs.
As it pertains to urogynecologic meshes, biomechanics is a broad term that not only refers to the \textit{ex vivo} and \textit{in vivo} mechanical behaviors of these devices, but also how those behaviors are impacted by their textile properties. Currently, there are numerous synthetic mesh products available on the market with no specific criteria based on scientific data for clinicians and surgeons to select one mesh over another. Mechanical testing of synthetic meshes has proven to be invaluable for distinguishing one mesh from another through the analysis of structural properties and mechanical behavior of mesh in response to various boundary (i.e. the imposed boundaries that restrict and/or limit movement in a given direction(s)) and loading (i.e. how deformation or force is applied) conditions. All of these tests have assumptions and limitations that must be understood in order to properly conduct and interpret the results. Additionally, it is important to note that not all mechanical tests aim to simulate \textit{in vivo} scenarios. Limitations in sample size and available testing methods often hinder the physiological relevance of some testing methods. As a result, it is often challenging to correlate the results of mechanical tests to patient outcomes. Therefore, understanding the limitations of each test and appropriately interpreting the results based on boundary conditions is critical for describing the mechanical behavior of synthetic meshes, that often times could not be obtained otherwise.

Many of the mechanical tests used to assess the behavior and properties of vaginal tissue are also used in the assessment of synthetic meshes. However, it is important to note that there are some key differences between the two that ultimately impact the applicability of the testing method, the experimental protocol, and the interpretation of the results. The structure/composition of synthetic meshes is drastically different from that of vaginal tissue. Synthetic meshes are made of polymeric fibers that are often knitted together to create a porous, knitted fabric. To date the
majority of synthetic meshes are composed of polypropylene fibers; however, materials such as polyethylene terephthalate and polytetrafluoroethylene have also been utilized. The stiffness of these materials is extremely high relative to vaginal tissue. For example, the material stiffness of a polypropylene fiber is 1.5-2.0 GPa, polyethylene terephthalate 2.0-2.7 GPa, and polytetrafluoroethylene 0.4 GPa. In order to create a mesh with a stiffness that is significantly less than that of the individual polymeric fibers, the fibers are knitted together. Knitting results in a porous structure, which allows the fibers to move relative to each other when loaded; thereby, decreasing stiffness. From a mechanics perspective, the porous nature of synthetic meshes classifies them as discontinuous materials. Vaginal tissue on the other hand is composed of cells, proteins, fibers (e.g. collagen and elastin), connective tissue, muscle, and water. As a result, vaginal tissue is often assumed to be a continuous material (also referred to as a continuum, i.e. a material with density at every location in the sample, no voids) and this level of continuity is assumed at the level of fibers. In contrast, the discontinuity of synthetic meshes restricts the use of analyses that are applicable only to continuous materials. Discontinuity also limits the information that can be obtained from mechanical tests to those of structural properties (e.g. load, elongation, stiffness, and energy absorbed) or normalized structural properties (e.g. membrane tension), which will be described in the next few sections. Additionally, the knit pattern of synthetic meshes limits the shape of meshes used for testing. For example, vaginal tissue is often cut into a dog-bone shape for uniaxial tensile testing in order to achieve a uniform strain field in the center of the sample. Creating a dog-bone shaped mesh disrupts the knit pattern thereby altering its mechanical behavior. Moreover, the intertwined network of fibers causes very non-uniform and local deformations to the overall mesh structure. In the next few sections, the various mechanical tests...
that are used to evaluate urogynecologic meshes as well as the \textit{ex vivo} and \textit{in vivo} mechanical behaviors of urogynecologic mesh will be discussed.

1.3.1 Mechanical Tests Utilized to Evaluate Urogynecologic Mesh

1.3.1.1 Uniaxial Tensile Test

A uniaxial tensile test (tensile test) is one of the most common analyses used to evaluate synthetic meshes. The methodology utilized in a tensile test stems from standard testing methods for textiles (ASTM D5035-11, Standard Test Method for Breaking Force and Elongation of Textile Fabrics, Strip Method and ISO 13934-1, Textiles – Tensile Properties of Fabrics – Part 1: Determination of Maximum Force and Elongation at Maximum Force Using the Strip Method) and is slightly modified to accommodate testing of urogynecologic meshes. By this method, a uniaxial load is applied which happens to simulate loading conditions that are similar to those following an abdominal sacral colpopexy. It involves first securing the opposing ends of the mesh, usually with clamps. While one end of the clamped mesh is rigidly attached to the base of a testing machine, the other end is attached to a force sensor (load-cell) that is attached to the crosshead of the testing machine (Figure 7). Slack is then removed from the mesh through the application of a minimal preload. Finally, the mesh is elongated as the crosshead moves upward at a constant rate. Often the mesh is elongated to failure and this is referred to as a load to failure test. This testing method is a relatively simple but powerful tool that is used to determine the structural properties of synthetic mesh, to predict the behavior of synthetic mesh in response to various loading conditions, and to compare synthetic mesh products.
During a load to failure test, the load and corresponding elongation are recorded and used to generate a load-elongation curve. The shape of this curve is typically nonlinear consisting of three regions: an initial toe region, a linear region (that may be bilinear), and a failure region (Figure 7). This curve is then used to determine the structural properties of synthetic meshes: the ultimate load (N), ultimate elongation (mm), relative elongation (mm/mm), stiffness (N/mm), and energy absorbed (N mm). The ultimate load represents the maximum amount of force that the mesh can withstand prior to failure. Ultimate elongation corresponds to the amount that the mesh has elongated at failure. Relative elongation is the normalized version of elongation in which the amount that the mesh elongated is divided by the initial length of the mesh. Stiffness is a measure of the resistance of the mesh to elongation. For load-elongation curves that can be approximated as bilinear, two values for stiffness are typically calculated, low stiffness (the minimum stiffness over a defined interval of elongation) and high stiffness (defined as the highest stiffness over a
specified range of elongation). The low stiffness region corresponds to the initial range of elongation in which the mesh is easily pulled apart whereas the high stiffness region represents an increased resistance to deformation that occurs after most of the mesh is reoriented along the direction of loading.

Structural properties of synthetic meshes are often obtained from uniaxial tests in which the mesh is loaded to failure. Arguably, reporting structural properties at failure is not the most clinically relevant data given that the loads at failure are substantially higher than the loads that meshes will experience under normal physiological conditions. Thus, the ultimate load at failure and high stiffness, although used by vendors to market a “stronger mesh”, have little relevance to the behavior of a mesh that is observed clinically. The other aforementioned properties, however, provide a better understanding of the clinically relevant behavior of mesh since physiologic stresses most likely occur within the low range of elongation.

One of the biggest limitations of performing uniaxial tensile tests on synthetic meshes is size. The size of the mesh sample will impact the results obtained and this is largely due to the boundary conditions dictating how force is transferred through the mesh. Thus, caution should be exercised when comparing the results obtained from different labs and when interpreting the in vivo behavior of mesh. As a way to potentially overcome the size limitation, labs will often normalize the results (e.g. report membrane tension); however, despite normalization, the size of the mesh is still a concern. Additionally, uniaxial tensile testing loads only one axis of the mesh at a time. However, in vivo synthetic meshes are expected to experience loads from multiple directions. In order to evaluate the behavior of the mesh from multiple axes (e.g. along the longitudinal and transverse axes) using this technique, samples along each axis are cut and tested separately. Consequently, the interaction between the two axes is not evaluated. Some researchers
use planar biaxial mechanical testing to overcome this limitation [61-63]; however, this method is not entirely appropriate and has limitations that will be described in the next section of this chapter.

1.3.1.2 Planar Biaxial Mechanical Test

Planar biaxial mechanical testing is a technique that involves applying forces or displacements along two perpendicular axes simultaneously. This technique is used to assess the biomechanical behavior of thin soft tissues that are naturally planar or can be made into planar specimens [64,65]. Experimentally, biaxial mechanical testing can be conducted on synthetic meshes; however, there are several important assumptions of planar biaxial mechanical testing that makes this type of testing inappropriate for describing the overall mechanical behavior of synthetic meshes.

One major assumption of planar biaxial mechanical testing is that stress and strain are in the x-y plane and zero in the direction perpendicular to this plane. Thus, testing should be restricted to thin specimens that are continuous or the assumption of a continuum can be assumed on some level of scale. This is not the case for synthetic mesh, which is a porous material; therefore, biaxial testing of synthetic mesh should be treated as a structural test. Additionally, when conducting planar biaxial mechanical tests, the method used to grip the specimen should be chosen such that uniform loading along the edges is achieved and that the local stresses imposed by the gripping method becomes negligible within the central region of the specimen [66]. In some studies that have used biaxial tests to assess the mechanics of synthetic mesh, the edges of the mesh were heat sealed to allow placement of sutures within this portion of the mesh [63]. However, heat-sealing alters the mechanical behavior of the mesh [67]. Additionally, heat-sealing restricts movement of the lateral edges (free movement of edges is a requirement of biaxial mechanical tests). Thus, while in biaxial mechanical testing, heat-sealing may aid with gripping synthetic mesh samples, the
results obtained do not accurately depict the mechanical behavior of the mesh, which essentially diminishes the validity of the results.

The size of the specimen being tested is also important to consider. It is assumed that the stress and strain field within the central region of the specimen is homogeneous; therefore, the central region should be small and located away from the edges to avoid any effects of gripping that may affect the stress and strain field within this region. Additionally, homogeneity of the stress and strain field is mainly dependent on the material within this region. Neilsen et al showed that for materials that are isotropic (biomechanical behavior is independent of testing direction) and homogeneous, the stress and strain field within the central region is relatively uniform [64]. However, for heterogeneous and/or anisotropic materials, in the case of synthetic mesh, the stress and strain distribution within the central region is highly non-uniform. In fact, for the reasons mentioned earlier, discussions of stress and strain above and beyond what might be located in an individual fiber are inappropriate. However, it is possible to get load and displacement data with large enough mesh samples; the larger the sample, the less the effects of the boundary edges. Given the limitations discussed above with planar biaxial mechanical testing of synthetic mesh, experimental studies in conjunction with computational analyses are necessary to extract significant meaning from these tests. At best, it should be reported as a bi-directional structural test used to identify the degree of axial coupling for a particular knit pattern.

1.3.1.3 Ball-burst Test

Ball-burst testing (sometimes referred to as biaxial tensiometry) is another mechanical test that is used to characterize the structural properties of synthetic mesh. This test method involves pushing a steel ball-head through mesh until it ruptures. It has also been used to assess the structural integrity of mesh-tissue complexes (discussed later in this chapter). Ball-burst testing originates
from the American Society of Testing and Materials (ASTM) D6797-15, the Standard Test Method for Bursting Strength of Fabrics - Constant-Rate-of-Extension (CRE) Ball Burst Test. In order to apply this testing method to synthetic meshes that are of the size to be implanted in-vivo, a scaled version of this ASTM standard is often utilized.

The protocol for conducting ball-burst testing is similar to that for uniaxial tensile testing; however, the testing setup is slightly different. Instead of testing rectangular samples of synthetic mesh, square or circular samples are used for testing. The samples are secured between two flat clamps that have a circular hole in the middle for the steel ball-head to pass through. The clamped sample is then mounted onto a custom stand that is attached to the base of a testing machine (Figure 8). The steel ball-head is connected in series with a load cell that is attached to a moveable crosshead.
A small preload is applied (e.g. 0.5 N) to ensure that contact is made between the mesh and the ball head with minimum deformation applied to the mesh. The ball-head is then pushed through the synthetic mesh at a constant rate until it breaks through the mesh (failure). The resulting load and displacement are recorded and used to create a load-displacement curve (Figure 8). Similar to uniaxial tensile tests, the ultimate load, maximum displacement, stiffness, and energy absorbed are determined.

There are some important caveats to consider when conducting ball-burst testing. Experimentally, it is important that during the preparation process the mesh is securely clamped along the entire perimeter and that there is enough space between the edges of the mesh and the hole in the middle of the clamps to ensure that the mesh will not slip out of the clamps during testing. Additionally, the steel ball should be placed in the center of the mesh. Finally, the diameter of the ball-head should be significantly larger than the maximum pore size of the mesh. These considerations are critical for uniform loading of the mesh and for the repeatability of the data from sample to sample. A limitation of ball-burst testing is the boundary conditions, which restrict testing to a small area. This “artificially” increases the stiffness of the mesh and it is also not an accurate reflection of the in vivo boundary (and loading) conditions for synthetic urogynecologic meshes. However, the purpose of ball-burst testing is not to simulate in vivo conditions, but rather it is used in the literature to assess mesh structural properties and to make comparisons between mesh devices. These structural properties can provide useful information and are used in the assessment of mesh-tissue complexes following implantation of synthetic mesh into the abdomen and vagina of animal models. While theoretically, it is possible to determine a maximal stress from this test for a continuous material, this is not valid for mesh, which is a discontinuous material and for which the reporting of mechanical properties is inappropriate.
It is important to note that the ultimate load, maximum displacement, stiffness, and energy absorbed are similar or the same in name as the structural properties reported for a uniaxial tensile test. Though these properties describe the same features of the resulting curves, the values obtained are not to be interpreted the same (see Tables 1 and 2 in Section 1.3.2). These two testing methods, despite being structural tests, have different boundary and loading conditions, which yield different values in structural properties. This demonstrates the importance of maintaining the same testing conditions (i.e. the same boundary and loading conditions and mesh dimensions) when comparisons are to be made between meshes.

1.3.2 Structural Properties

At first glance, most synthetic meshes appear to be very similar. They are lightweight, knitted, have large pores (> 1 mm), and most are composed of polypropylene. However, upon close examination via microscopy and mechanical testing, these meshes are not the same. In terms of textile properties, the knit patterns, pore geometries, and pore diameters of synthetic meshes are, in fact, quite different (Figure 9).

Figure 9: The knit pattern and pore geometry of synthetic meshes are distinct. The dimension of each image is 10 mm x 10 mm.
Uniaxial tensile testing as well as ball-burst testing has shown that the structural properties, particularly stiffness, of synthetic meshes vary considerably from product to product, see Tables 1 and 2 [67-72].

Table 1: Uniaxial structural properties of synthetic mesh for prolapse repair. Data represented as mean ± standard deviation. Data obtained from Shepherd et al, Uniaxial biomechanical properties of seven different vaginally implanted meshes for pelvic organ prolapse, Int. Urogynecol. J. Pelvic Floor Dysfunct. 23 (5) (2012) 613–620.

<table>
<thead>
<tr>
<th>Synthetic Mesh</th>
<th>Low Stiffness (N/mm)</th>
<th>High Stiffness (N/mm)</th>
<th>Load at Mesh Failure (N)</th>
<th>Relative Elongation at Failure (%)</th>
<th>Relative Elongation at Inflection Point (%)</th>
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</thead>
<tbody>
<tr>
<td>Boston Scientific Polyform™</td>
<td>0.1±0.0</td>
<td>1.4±0.1</td>
<td>53.8±4.8</td>
<td>86.5±2.4</td>
<td>39.9±1.5</td>
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<td>Coloplast NovaSilk™</td>
<td>0.1±0.1</td>
<td>0.5±0.1</td>
<td>19.6±4.5</td>
<td>89.4±21.4</td>
<td>44.6±7.5</td>
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<tr>
<td>Gynecare Gynemesh PS™</td>
<td>0.3±0.0</td>
<td>1.4±0.1</td>
<td>46.3±2.6</td>
<td>66.7±4.6</td>
<td>25.0±0.9</td>
</tr>
<tr>
<td>Gynecare Ultrapro™ (Artisyn®)</td>
<td>0.01±0.0</td>
<td>0.2±0.0</td>
<td>7.83±0.7</td>
<td>87.9±5.6</td>
<td>46.5±5.2</td>
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<tr>
<td>Mpathy Smartmesh™</td>
<td>0.2±0.0</td>
<td>0.6±0.0</td>
<td>22.7±1.8</td>
<td>68.5±2.5</td>
<td>29.2±1.0</td>
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Table 2: Ball-burst structural properties of synthetic mesh for prolapse repair. Data represented as mean ± standard deviation. Data obtained from A. Feola et al, Characterizing the ex vivo textile and structural properties of synthetic prolapse mesh products. Int. Urogynecol. J. Pelvic Floor Dysfunct. 24 (4) (2013) 559–564.

<table>
<thead>
<tr>
<th>Synthetic Mesh</th>
<th>Stiffness (N/mm)</th>
<th>Failure Load (N)</th>
<th>Elongation (mm)</th>
<th>Energy Absorbed (J)</th>
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<tr>
<td>Boston Scientific Polyform™</td>
<td>28.0 ± 0.4</td>
<td>108.0 ± 5.7</td>
<td>7.8 ± 0.1</td>
<td>261.0 ± 27.0</td>
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<tr>
<td>Coloplast NovaSilk™</td>
<td>16.0 ± 5.5</td>
<td>54.0 ± 19.0</td>
<td>6.3 ± 0.6</td>
<td>113.0 ± 43.0</td>
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<tr>
<td>Gynecare Gynemesh PS™</td>
<td>28.0 ± 2.7</td>
<td>108.0 ± 8.6</td>
<td>7.3 ± 0.3</td>
<td>288.0 ± 37.0</td>
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<tr>
<td>Gynecare Ultrapro™ (Artisyn®)</td>
<td>22.0 ± 2.8</td>
<td>76.0 ± 12.0</td>
<td>7.3 ± 0.2</td>
<td>170.0 ± 11.0</td>
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<tr>
<td>Mpathy Smartmesh™</td>
<td>11.0 ± 0.9</td>
<td>45.0 ± 3.8</td>
<td>6.7 ± 0.5</td>
<td>109.0 ± 11.0</td>
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</tbody>
</table>

Gynemesh PST™ (Ethicon, Somerville, NJ, USA) is the first synthetic mesh specifically marketed for pelvic organ prolapse repair. This mesh is identical to the Prolene soft hernia mesh (Ethicon, Somerville, NJ, USA). Relative to newer generation meshes, Gynemesh PST™ is currently one of the stiffest meshes available on the market. This is not surprising given the current trend of manufacturing meshes with decreasing weight and porosity, which results in a decrease in stiffness. Additionally, from ball-burst testing, textile properties are shown to correlate with structural properties, with stiffness, ultimate load, and energy absorbed being positively correlated to specific weight and porosity [72].
1.3.3 Characterization of Mechanical Behavior

1.3.3.1 Anisotropy

The structural properties of synthetic meshes are not always independent of the testing direction. This type of behavior is referred to as anisotropy in which the mechanical behavior is dependent on the direction in which the material, in this case mesh, is evaluated. UltraPro (marketed as Prolift+M™ and Artisyn by Gynecare, Inc.) is an example of a prolapse synthetic mesh that has directionally dependent structural properties. Using uniaxial tensile testing, the stiffness of UltraPro in the direction that is parallel to the blue orientation lines was found to be orders of magnitude stiffer than the stiffness in the direction that is perpendicular to the blue orientation lines, 0.258 ± 0.085 N/mm and 0.009 ± 0.002 N/mm, respectively [73]. The anisotropic nature of synthetic meshes is also demonstrated with some abdominal hernia meshes [74]. Understanding and reporting the anisotropic behavior of synthetic meshes for urogynecologic repairs is of utmost importance to surgeons. With this information, surgeons will have a better knowledge of the mesh that they are implanting. Additionally, they will also be able to make more scientifically driven decisions regarding which mesh to implant and the way in which the mesh should be implanted that will result in a good surgical outcome for the patient.

1.3.3.2 Boundary Conditions and Mesh Wrinkling

The boundary conditions that synthetic meshes are subjected to impact the response of these materials to loading. Typically, the top and bottom edges of synthetic meshes are completely constrained during uniaxial tensile tests. In this configuration, loading and deformation is uniformly distributed and the mesh is allowed to contract in the direction perpendicular to loading. However, this configuration constrains out-of-plane deformation and is not the most
physiologically relevant boundary condition. Surgeons use sutures to attach synthetic mesh to the vagina and to anchoring points such as the sacrum and sacrospinous ligament. The number of sutures used and placement of these sutures varies for each surgeon and patient. These sutures essentially become point loads, which result in an uneven distribution of loading and deformation. Barone et al (2015) demonstrated that applying point loads to synthetic meshes results in out-of-plane deformation in response to uniaxial loading and the magnitude of this deformation increases with the number of point loads applied (Figure 10) [75].

Figure 10: Boundary conditions impact mesh deformation. Uniaxial loading of mesh to 10 N of force with and without point loads (i.e. sutures) resulted in out-of plane deformation, observed as mesh wrinkling. Increasing the number of point loads (sutures) resulted in increased mesh wrinkling. Orange dots represent suture attachment points.
Out-of-plane deformation, described clinically as mesh wrinkling/buckling/folding, was mimicked with the introduction of suture induced point loads in the uniaxial test previously mentioned. It is important to note that this behavior was observed at loads that are within the physiologic range, 1 N and 10 N. These results are significant, and it is hypothesized that areas of mesh wrinkling result in an increase in mesh density (i.e. mesh bunching), which may result in an enhanced host foreign body response. Further research is needed to substantiate this claim. Nonetheless, the number of sutures, location of sutures, and the amount of tension applied are important factors for surgeons to consider.

1.3.3.3 Permanent Deformation with Repetitive Loading

Most synthetic meshes experience some degree of permanent deformation (i.e. irreversible deformation) with loading, and this occurs at loads that are well within the physiologic range, 0.5 N to 15 N [70,71]. At lower loads, this occurs because the structure of the mesh is undergoing reorganization, i.e. knots are tightening, fibers are realigning, etc. Permanent deformation or plastic deformation of the polypropylene material itself occurs at much higher loads. Combining this knowledge with the current trend of manufacturing meshes with decreasing stiffness has led surgeons to use synthetic meshes that may be more susceptible to permanent deformation. Permanent deformation may lead to elongation of the mesh that is irreversible but it has not been exclusively associated clinically or experimentally with mesh complications.

1.3.3.4 Pore Size Reduction and Loss of Porosity

The pore size and consequently porosity (defined as the amount of open space relative to the total area of the mesh) tends to decrease when synthetic mesh is loaded [76,77]. Otto et al (2014) demonstrated that a loss of porosity occurred in both the arms and central body of transvaginal
synthetic meshes when mesh samples were elongated to relatively small loads (1000 g maximum applied to the arms and 2000 g maximum applied to the body). In a study conducted by Barone et al (2016), similar results were shown for abdominal sacrocolpopexy polypropylene meshes. A reduction in the pore diameter and a loss in porosity (as much as 87%) were observed for these meshes in response to 5 N and 10 N of tensile load. In both of the previously mentioned studies, a loss in porosity was also associated with a reduction (or complete loss) in the effective porosity for some meshes. Effective porosity is defined as the percentage of pores with dimensions that are greater than 1000 µm in all directions [78]. Previously, 1000 µm was identified as the minimum distance between two polypropylene filaments required to allow for tissue ingrowth and to prevent bridging fibrosis, the merging of the host immune response from adjacent fibers for hernia meshes [79-81]. The implications of bridging fibrosis will be discussed more in Section 1.4.3. Relating this knowledge to the biomechanical behavior of urogynecologic synthetic meshes, decreases in pore size, porosity, and effective porosity potentially reduces the biocompatibility of these meshes. This in turn may lead to an increased risk for poor tissue integration, inflammation, fibrosis, and bridging fibrosis, and it is hypothesized that these parameters will worsen as pore sizes decrease. Collectively, the reduction in mesh biocompatibility may lead to complications and ultimately poor patient outcomes. Future studies are needed to substantiate this claim for urogynecologic meshes.

1.3.4 Clinically Observed Mechanical Behaviors

Surgeons and clinicians have reported that in vivo, synthetic meshes bunch (synonymous with pore collapse), contract/shrink, fold, and wrinkle (buckle) (Figure 11) [82-84]. These behaviors are typically present in patients experiencing complications associated with mesh implantation. Mesh
contraction, often reported as “shrinkage or retraction”, is one of the commonly reported, and particularly problematic, mesh complications. Vaginal pain, dyspareunia (pain with sexual intercourse), and tenderness around the contracted portions of the mesh are all symptoms associated with mesh contraction [82,85]. The mechanisms that are responsible for these behaviors are unclear; however, the implantation method/surgical technique and mesh behavior with loading may be contributing factors. Manufacturers recommend that synthetic meshes be implanted as flat as possible. Despite a flat implantation, deformation of the initial mesh geometry still occurs. Additionally, the way that the mesh is anchored and the amount of tension applied to the mesh may be contributing factors. For example, with transvaginal synthetic meshes that have arms, the angle at which mesh arms are anchored into the pelvic sidewall and/or unequal tensioning placed on the mesh arms may predispose the mesh to deformation. Biological factors such as tissue incorporation and the quality of the tissue in contact with the mesh may also play a role. Ex vivo and in vivo studies are needed to investigate these mechanical behaviors and to determine their etiology.
Figure 11: Removal of mesh is common for women experiencing complications. In both images, the mesh was removed for exposure and pain. In the left image, there is very little tissue incorporated in the area of exposed mesh (green arrow). This is also the area where the mesh appeared to have buckled/folded. In the image on the right, mesh bunching and a loss of porosity was observed.

1.4 HOST RESPONSE TO MESH: INFLUENCING FACTORS

In a perfect world, the ideal synthetic mesh is biocompatible, inert, non-carcinogenic, strong, able to be sterilized, non-modified by body tissue, has minimal risk for infection or rejection, convenient, and affordable [51,86]. However, as one can imagine, currently there is no synthetic mesh that meets all of these requirements. Based on the literature, there are a number of factors that can influence the overall response of the host to mesh (e.g. material, structural properties such as stiffness), and much of this information was acquired from the abdominal hernia literature. This is largely due to urogynecologic meshes previously being classified as Class II devices requiring 510(k) approval (the predecessor devices being hernia meshes); therefore, rigorous scientific testing and clinical data were not required for synthetic meshes to be used in urogynecologic surgeries. However, following the Food and Drug Administration Public Health Notifications
released in 2008 and 2011 regarding complications associated with synthetic meshes for prolapse repairs, more in vivo studies are emerging. Thus, in the next few sections, data from the urogynecology and abdominal hernia literature will be used to discuss some of the key textile and structural properties that influence the host response to synthetic mesh. Specifically, the complications (e.g. infection, pain, fistula formation, etc) associated with various textile and structural properties will be emphasized. It is important to note that mesh failure, which is, defined as recurrent prolapse or incontinence is not considered a complication in this dissertation and therefore, will not be discussed. Additionally, it is essential to keep in mind that although the textile and structural properties will be discussed separately, it is likely that a combination of these factors dictates the overall host response to mesh.

1.4.1 Filament Type and Knit Pattern

Synthetic meshes can be broadly categorized into two types based on the composition of the mesh fibers: monofilament and multifilament. As the name suggests, monofilament meshes have fibers that are composed of a single filament whereas multifilament meshes are composed of more than one filament. In the case of multifilament meshes, the filaments that comprise the fibers are braided, double braided, twisted, or coated and this creates small spaces, termed interstices, which can be <10 μm. Theoretically, these interstices put multifilament meshes at a disadvantage as they would allow for the passage of small bacteria (<1 μm) but prevent the passage of larger immune cells which typically remove the bacteria such as macrophages and leukocytes (approximate diameter of 16-20 μm and 9-15 μm, respectively) [87]. In the literature, multifilament meshes are associated with infection. In vitro and in vivo studies using animal models have shown that multifilament meshes induce biofilm growth, harbor bacteria for longer periods, and bacteria
spreads more on multifilament meshes relative to monofilament meshes [88-91]. Additionally, incidences of infection with multifilament meshes have also been demonstrated clinically when used in urogynecologic repairs [92,93].

During the fabrication process, mesh fibers are either woven or knitted. Woven meshes are comprised of two tightly packed, parallel sets of filament. One set is aligned along the length of the mesh (referred to as the warp direction) while the other runs over and under the warp direction filaments in an alternating pattern (the weft direction). This latter set of filaments is perpendicular to the warp direction. Unlike woven meshes, knitted meshes are constructed from a continuous filament that is interlocked/looped around another filament. Based on these fabrication differences, woven and knitted patterns have distinct advantages and disadvantages [51,94]. Woven meshes are advantageous for shape memory, strength, and the mechanical behavior/structural properties of these meshes typically are the same in the warp and weft directions. However, the tightly packed nature of woven mesh fibers limits the overall porosity and the ability of these meshes to conform to surfaces (i.e. tissues). Knitted meshes on the other hand are more porous than woven meshes and are highly conformable/flexible; yet, they are not as strong as woven meshes and the mechanical behavior/structural properties can be directionally dependent (i.e. they are anisotropic). Despite these limitations, knitted meshes are the preferred type for urogynecologic meshes, as woven meshes are associated with higher rates of complications [51,95].

1.4.2 Material

Along with the filament type and knit pattern, the material(s) from which synthetic mesh is constructed is another important factor in the host response to mesh. Synthetic mesh can be hydrophilic or hydrophobic. Hydrophilic materials attract water (have a low contact angle)
whereas hydrophobic materials repel water (have a high contact angle). The wettability (contact angle) of the material is thought to influence the attachment of bacteria with hydrophilic materials believed to be more attractive for bacterial strains; however, the results in the literature are contradictory regarding this property [96-99].

Historically, a variety of materials were used in the manufacturing of synthetic mesh with some of these materials being absorbable (aka an absorbable synthetic mesh), non-absorbable (aka a non-absorbable mesh), and there are instances in which both absorbable and non-absorbable materials are utilized. Synthetic meshes constructed using both types of materials are referred to as composite meshes. Absorbable meshes are advantageous from a host response perspective as they have less risk of rejection [100]. Additionally, since these meshes are not permanent, complications associated with the long-term use and presence of a permanent mesh (e.g. mesh erosion) is drastically diminished. However, absorbable meshes are mechanically dis-advantaged, as they do not provide the long-term strength and durability that a permanent implant provides which ultimately limits the use of these meshes in urogynecologic repairs. Regardless of the type of mesh, there are positive and negative (in the form of complications) host responses to mesh.

Literature is scarce regarding the use of absorbable mesh in urogynecologic surgery. Dexon (polyglycolic acid) and Vicryl (polyglactin 910) meshes are the two most commonly reported absorbable meshes. Complications associated with the use of these meshes include: the formation of fistulas, pelvic infections, and mesh erosion (though rare) [101-104]. The primary non-absorbable meshes discussed in the literature are composed of expanded polytetrafluoroethylene (ePTFE, Gore-tex), polyethylene terephthalate (Mersilene), and polypropylene (Marlex, Prolene, Atrium, Gynemesh). Of these meshes, the response to Gore-tex is the least favorable clinically with high rates of complications reported. High rates of erosion (~11-16%), sinus tract formations,
and infection have been observed with Gore-tex suburethral slings and prolapse meshes [92,105-107]. Additionally, the removal rate for Gore-tex slings was reported as high as 35% [106]. Mersilene mesh, a polyester woven mesh, is another mesh that has had unfavorable results clinically with a reported erosion rate of 8% [108]. Interestingly, the fibers of both Gore-tex and Mersilene meshes are multifilament which contain small interstices that are prone to harbor bacteria and infection [88-91]. Thus, it is argued that the response to Gore-tex and Mersilene meshes is a response to the construction of the mesh fibers just as much as (if not more than) it is to the material itself.

Of the materials utilized in the construction of synthetic mesh, polypropylene is currently the most commonly used material. Polypropylene meshes are often knitted and monofilament. Early reports (1970’s to early 90’s) on the clinical use of polypropylene mesh for SUI and prolapse repair demonstrate success/cure rates of 70% to 100% [87]. Some of the complications cited in these early studies for SUI repair included voiding dysfunction (22%), bladder obstruction, and chronic cystitis which are related to over-tensioning of the mesh [109-111]. Early retrospective studies and case series report high success rates (at least 92%) for prolapse repair with low complications, likely associated with incomplete follow up or to short term follow up [87]. More recent prospective randomized studies (in the early 2000’s) show similar trends in terms of high success rates observed for polypropylene meshes; however, complication rates are higher than anticipated [45]. Mesh erosion, recurrent prolapse, dyspareunia, and pain are complications associated with polypropylene mesh [82,112-117]. These complications are not rare and played a major role in the release of the FDA Public Health Notifications in 2008 and 2011.
1.4.3 Pore Size and Porosity

Much of the information obtained regarding the impact of pore size and porosity (defined as the amount of void space in a mesh relative to the mesh area) on the biocompatibility of synthetic meshes stems from the abdominal hernia literature. In the late 90’s, Amid devised a classification system that classifies synthetic hernia meshes based on pore size, and this system was adapted for urogynecologic meshes. Accordingly meshes are categorized as follows:

- Type I meshes (totally macroporous) – pore sizes exceed 75 μm
- Type II meshes (totally microporous) – pore sizes are less than 10 μm
- Type III meshes – macroporous with multifilamentous or microporous components
- Type IV meshes – contain submicron level pores [118].

Recent literature suggests that the pore size and porosity of synthetic meshes can significantly impact the reaction of the tissue (i.e. the host) to the mesh. Meshes with large pores and high porosity yield better tissue integration with increased collagen deposition between pores and decreased inflammation and fibrous relative to meshes with small pores and low porosity [79,119-121]. The adhesive potential of a mesh is also associated with pore size. Meshes with small pores induce stronger adhesion formation relative to meshes with large pores [122]. Additionally, pore size is inversely proportional to bridging fibrosis [79,119]. Bridging fibrosis is a process by which the foreign body response to a mesh fiber overlaps/merges with that of a neighboring fiber, which can lead to contraction, encapsulation, and pain (Figure 12). Thus, when pore sizes are small, the risk of bridging fibrosis increases whereas with larger pores this risk is decreased. For polypropylene meshes, 1 mm is identified as the optimal minimal pore diameter needed to allow for tissue ingrowth and for the prevention of bridging fibrous [79].
1.4.4 Stiffness

Stiffness is a critical structural property of synthetic meshes and one that can affect the overall mechanical integrity and remodeling response of the vagina following mesh implantation. A mesh must be stiff enough to restore normal anatomy to the vagina and prevent recurrent prolapse (or incontinence) while concomitantly being able to withstand \textit{in vivo} forces. However, there is a limit to the stiffness of biomedical materials. Synthetic meshes that are too stiff can lead to a
phenomenon referred to as stress shielding. Stress shielding results when there is a discrepancy in stiffness between adjacent materials such that the stiffer material bears the majority of the load while shielding the less stiff material from the load it normally experiences. The resulting lack of loading of the less stiff material can lead to a maladaptive remodeling response associated with degeneration and atrophy. This phenomenon has historically been observed in orthopedic research, particularly in the case of bone healing/remodeling. Studies have shown that bone mineral loss, bone resorption, and a decrease in the mechanical properties of bone were all consequences of the implantation of bone stabilizers (e.g. plates, rods, etc) that were too stiff [123-125]. Of more relevance to the vagina, a soft tissue, the negative effects of stress shielding is also observed with tendons, which is also a soft tissue [126-128].

In studies conducted by Feola et al 2013, Jallah et al 2015, and Liang et al 2013, synthetic meshes with different values of structural stiffness were implanted into non-human primates via an abdominal sacrocolpopexy following a hysterectomy [129-131]. The meshes, and respective stiffness, implanted were as follows: 1) Gynemesh PS – 0.29 ± 0.015 N/mm, 2) Smartmesh (no absorbable component) – 0.18 ± 0.026 N/mm, 3) UltraPro (with an absorbable component, least stiff orientation) – 0.009 ± 0.0016 N/mm, and 4) UltraPro (with an absorbable component, higher stiffness orientation) – 0.258 ± 0.085 N/mm. After three months, the mesh-vagina complexes (MVCs) were explanted and analyzed. Overall, the results of these studies suggested that synthetic mesh negatively impacts the structural properties of the underlying and newly incorporated vagina. Specifically, it was estimated that the vaginal tissue contribution to the overall stiffness of the MVC was significantly reduced with Gynemesh PS relative to sham (no mesh implanted). Additionally, the contractile ability of the smooth muscle was negatively impacted following implantation, with the greatest, negative impact observed following implantation with Gynemesh
PS. Gynemesh PS was associated with an 80% decrease in the force generated by the vaginal smooth muscle relative to sham [129]. Congruent with these results, the organization and thickness of the smooth muscle layer were drastically reduced (Figure 13) in the presence of Gynemesh PS, and this was also associated with a decrease in nerve innervation [130,131].

![Figure 13](image)

Figure 13: Synthetic mesh can negatively impact the morphology of vaginal tissue. Implantation of Gynemesh PS resulted in a decrease in smooth muscle organization and volume, and a significant amount of apoptosis can be seen surrounding the fibers of the mesh (*). Smooth muscle is fluorescently labeled red.

Sham – no mesh was implanted. E – epithelium, S – subepithelium, M – muscularis, and A – adventitia.

Lastly, the composition of the vaginal tissue was also impacted by mesh. Significant decreases in the collagen and elastin content and increased collagenase activity as well as increased glycosaminoglycan content were all consequences of implantation with Gynemesh PS [131]. Interestingly, these degenerative effects of mesh were related to the stiffness of the mesh as Gynemesh PS was the stiffest mesh that was implanted in these studies and it had the greatest negative impact on the vagina. Additionally, it was the mesh associated with the greatest amount of deformation. The negative impact of mesh on the smooth muscle is particularly concerning.
given that studies have shown that women with pelvic organ prolapse have a decreased fractional area of smooth muscle, increased smooth muscle apoptosis, and disorganization of the smooth muscle layer [30,132,133]. Thus, implanting current synthetic meshes into an already comprised vagina may increase the chances of inducing a maladaptive remodeling response, which could ultimately lead to complications, such as mesh erosion.

1.4.5 Weight and Surface Area

Weight is defined as the mass per unit area of synthetic mesh and is representative of the “heaviness” of the mesh (ASTM D4850-13, Standard Terminology Relating to Fabrics and Fabric Test Methods). Synthetic meshes are commonly classified as either heavyweight or lightweight. Relative to heavyweight meshes, increased incorporation of tissue within the pores of the mesh as well as a reduction in fibrosis and inflammation is observed with lightweight meshes [79,81,134,135]. Additionally, the potential for adhesions and pain are reduced with lightweight meshes [122,136]. In studies conducted by Klinge et al, the mobility of the abdominal wall was less restricted with the hernia mesh containing a reduced amount of polypropylene [81,135]. Abdominal wall restriction is directly correlated with the degree of fibrosis; hence, the formation of a scar or connective tissue around the mesh fibers decreases mobility [137].

Surface area is another property of synthetic mesh shown to impact the foreign body response [122,138]. It is a measure of the amount of material that is in contact with the tissue. Early research showed that the smaller the area of mesh exposed to the tissue, the lesser the foreign body response to the mesh (a larger surface area induces a strong and active inflammatory tissue response) [95,137]. Surface area is of particular concern with multifilament meshes as the surface...
area increases by at least 50% for multifilament threads; thus, providing a greater surface upon which bacteria can adhere relative to monofilament meshes [139].

The weight and surface area of synthetic mesh all focus on the amount of material in contact with the tissue. Results from the studies presented in this section (see previous two paragraphs of this section for references) suggest that lightweight and low surface area meshes are ideal, and this was the general consensus over the last decade. Though these two properties are important, the role of pore size cannot be excluded. Many of the lightweight meshes that demonstrated favorable outcomes had larger pores, and this is not surprising given that in theory an increase in pore size should decrease the amount of material in contact with the host. However, a heavyweight mesh with large pores could have a decreased host response relative to a lightweight mesh with small pores. With pore size being the common link, these findings suggest that pore size may have a stronger influence on the host response to mesh than the overall amount of material. Evidence from a study conducted by Weyhe et al 2006 is supportive of this claim [140]. Implantation of a heavyweight large pore mesh resulted in a significantly less fibrotic response relative to a lightweight microporous mesh, and the total number of macrophages was also significantly lower for the heavyweight mesh. Thus, pore size is a critical property that plays a dominating role for tissue integration and in the foreign body response, ultimately impacting the overall biocompatibility of synthetic mesh.

1.5 MOTIVATION AND SPECIFIC AIMS

Pelvic organ prolapse is a gynecologic condition that negatively impacts a woman’s quality of life and is most commonly observed in postmenopausal, parous women [2,9]. For cases in which
conservative treatment methods are not effective, synthetic mesh is often employed, comprising one-third of the surgeries to repair prolapse [60]. Currently, most synthetic mesh products are knitted, lightweight (<45 g/m²), wide pore (>1 mm, porosity > 55%) Type I polypropylene devices. Though successful, mesh repairs are associated with significant morbidities/complications prompting the FDA to issue two public health notifications (2008 & 2011) declaring that there are complications associated with synthetic meshes used for prolapse repairs and that these complications are not rare [60]. Complications cited include infection, mesh shrinkage/contraction, mesh erosion or exposure, and pain with the latter two being the most commonly reported. Rates of complications for surgical repair of prolapse utilizing mesh are reported to be as high as 15% [141]. Too often complications require repeat (sometimes multiple) revision surgeries and in some cases the pain that prompted surgery persists in spite of removal of the mesh. To date, millions of dollars have been awarded to patients for mesh related complications causing leading manufacturers of mesh to discontinue products based on escalating costs and not on science. The pathogenesis of the complications associated with synthetic meshes is unknown; however, recent research has begun to shed light on possible mechanisms. Specifically, in our lab, we have identified specific properties and behaviors of current synthetic meshes that may be contributing to mesh complications.

Synthetic meshes used in urogynecologic repairs are simply hernia repair meshes that were cut/shaped for application in the female pelvis with no consideration of the unique properties of the vagina and loading conditions imposed on it that contribute to both normal and abnormal support. From a design and mechanical perspective, this is considerably problematic given that the composition and functional role of the tissues within the female pelvis drastically differ from those of the abdomen. In the abdomen, loading is directed more uniformly (circumferentially) along the
edges of the mesh causing the pores of the mesh to remain open. However, meshes used in prolapse repair are subjected to more uniaxial/unidirectional forces, which predisposes them to pore collapse (Figure 14).

Figure 14: The loading conditions applied to abdominal hernia meshes drastically differ from meshes used in prolapse repair. Abdominal hernia meshes are loaded uniformly along all edges of the mesh, which helps to keep pores open. The forces applied to abdominal sacrocolpopexy and transvaginal meshes are more uniaxial/unidirectional which favors pore collapse.

Thus, the pores of most synthetic meshes collapse when subjected to small loads decreasing the overall effective porosity of the mesh (no pores > than 1 mm). For example, subjecting Gynemesh PS to just 10 N (~2.2 lbs) of force, a force that is well within the physiologic range, resulted in the reduction of the porosity from an initial value of approximately 60% to <15% (Figure 15) [77].
Figure 15: Gynemesh PS undergoes significant pore collapse when subjected to 10 N of uniaxial force with a concomitant (and significant) decrease in the porosity from 60% at 0 N to 15% at 10 N.

In addition to pore collapse, mesh wrinkling (also referred to as mesh bunching or folding) is observed. Mesh wrinkling is a result of the collapse of the mesh along its transverse direction in response to longitudinal loading. This causes areas of high mesh density and non-uniform force distributions that may be linked to localized and enhanced tissue responses possibly resulting in complications [75]. In a preliminary finite element analysis conducted in our lab of a commercially available synthetic mesh product, the areas of high mesh burden (defined as mesh per volume of tissue) were located in the areas of the mesh where the pores had significantly collapsed (Figure 16). Interesting, these areas have been identified clinically as areas where patients being treated for mesh contraction are experiencing vaginal pain (exacerbated with movement) and dyspareunia (pain with sexual intercourse) with the most severe pain located between the fixation arms and the center of the mesh [82]. These findings are significant and demonstrate the negative affect that
decreasing pore size and porosity due to pore collapse with loading have on the overall biocompatibility of synthetic mesh for prolapse repair.

Figure 16: Preliminary finite element analysis results of tensioning on mesh arms. Solid model of Restorelle DirectFix Anterior in response to no tension (a) and a representative model in response to 10 N of force applied to the arms of the mesh (b). Contour plot depicting mesh burden with high mesh burden located in the areas where the pores of the mesh have collapsed (between the upper arms and within the lower arms of the mesh). Arrows depict the direction of tension. Block dots represent the location of fixation.

There is currently no established structural stiffness for synthetic meshes used in urogynecologic repairs. The uniaxial stiffness of these meshes is reported as low as 0.009 N/mm and as high as 1.66 N/mm [70,71]. Stiffness is a critical parameter given that a mesh that is not stiff enough can lead to recurrent prolapse and one that is too stiff can lead to a phenomenon referred to as stress shielding in which the stiffer material bears the majority of the load shielding
the less stiff surrounding tissues [123,124]. This can result in a maladaptive remodeling response that is characterized by degeneration and atrophy [127,128]. In a study conducted by Liang et al (2013), evaluating the impact of synthetic meshes (with various stiffness) on vaginal morphology and structural composition implanted in the nonhuman primate, stress shielding was shown to be the likely mechanism by which polypropylene meshes negatively impact the vagina. Thinning of the smooth muscle layer, increased apoptosis around mesh fibers, and decreases in collagen and elastin content were all negative responses to polypropylene prolapse mesh with the greatest negative response being observed with the stiffest mesh [131]. Thus, it is critical that the material used to manufacture newly designed meshes has a material stiffness that is comparable to the vagina. Although research has attempted to determine the stiffness of the vagina via uniaxial tests (6-14 MPa), the biaxial nature of this tissue necessitates more accurate data to be collected regarding the stiffness of vaginal tissue [142,143].

In vivo it is anticipated that prolapse meshes will be exposed to repetitive sub-failure loads due to activities that increase intra-abdominal pressure (e.g. coughing and squeezing). Jones et al 2009 showed that prolapse meshes undergo a significant amount of permanent deformation with cyclical loading [70]. This result is significant and suggests that mesh can potentially experience permanent alteration in length with consecutive increases in intra-abdominal pressure, especially during the postoperative period prior to the ingrowth of tissue. During this time, the mesh has the ability to move freely between host tissue and permanent changes in mesh geometry could potentially translate into complications due to pore collapse and failures due to mesh lengthening. Thus, it is important that synthetic meshes for prolapse repair exhibit elastic-like behavior. In other words, the mesh should be able to deform and return to its original configuration with increases (sudden and repetitive) in intra-abdominal loads. Previous studies have found that intra-abdominal
pressure ranges from 2 kPa to 15 kPa [144-148]. Although not absolute, this suggests that meshes must be able to support a load of at least 2 N to 3 N based on estimates of the intra-abdominal pressure reported with sitting and standing and on our estimates of the surface area of the anterior vagina using MRI measurements. However, intra-abdominal pressures increase with activities such as coughing, sneezing, and jumping; therefore, synthetic meshes must also be able to support forces higher than 3 N.

Currently there are no products available on the market that 1) have pores which expand in response to tension 2) while minimizing mesh burden, 3) have a material stiffness that is similar to vaginal tissue, and 4) do not experience permanent deformation with loading. Some companies are looking to alter their existing products by modifying the surface properties with collagen, biologic matrices, and inert substances such as silicone and immune modifying agents like chitosan to improve biocompatibility by decreasing the host foreign body response [149-151]. To date, however, these approaches have had limited success. We believe that the lack of success of these products is because they fail to address the fundamental problems associated with current meshes. Thus, we are proposing a novel approach in which we design and test a unique geometrical synthetic mesh with properties that specifically target the problems in current synthetic meshes (e.g. pore collapse, mesh bunching, mesh stiffness mismatch, permanent deformation). This new design will not only aim to meet the “ideal properties” of synthetic meshes (biocompatible, inert, non-carcinogenic, etc.), but in light of the problems with current meshes, newly designed synthetic meshes will also meet the following criteria:

1) The pores of the mesh will remain unchanged or expand in response to tension and the porosity of the mesh will at least be maintained and/or increased with tensioning.

2) Will have a material stiffness that is similar to vaginal tissue.
3) Will be able to withstand *in vivo* loads and deform without undergoing permanent deformation in response to these loads (both static and repetitive).

4) Will minimize mesh burden resulting in a minimal foreign body response.

Thus, the overall goal of this dissertation is to develop an initial prototype that will demonstrate the feasibility of creating auxetic meshes for prolapse repair and will address the outlined problems with current synthetic meshes. Additionally, the prototype meshes should follow the design criteria described previously. For our initial design, we focused on the design of prolapse meshes used in sacrocolpopexy repairs as the design of these meshes is technically easier than those used in transvaginal repairs. With sacrocolpopexy, forces are applied to the meshes from primarily one direction whereas with transvaginal meshes the forces are multi-axial. If able to successfully design a sacrocolpopexy mesh, we will be in a good position to modify this design to meet the needs of a transvaginal mesh.

Several steps were involved in the development of an auxetic mesh prototype and these steps comprised the specific aims of this dissertation. The specific aims were as follows:

**Specific Aim 1) Develop computational models and a protocol for assessing planar deformation of prolapse mesh models with standard and auxetic pores *in silico***

1a. Establish the design criteria for the creation of computational prolapse models with standard and auxetic pores and uniform discretization. The material properties for the computational models will be based on the structural properties (specifically load and elongation) of Restorelle (Coloplast) determined using an inverse finite element analysis.

1b. Develop a protocol for quantifying model and pore deformation *in silico* of computational prolapse models with standard and auxetic pores in response to tensile loading.
Specific Aim 2) Assess the behavior of CAD models with auxetic pores in response to tensile loading in silico

2a. Examine the overall model and pore deformation of CAD models with auxetic pores subjected to simulated uniaxial tensile tests via FEA and compare this response to CAD models with standard pores. Model behavior will be characterized via quantitative measurements of model deformation, changes in pore size, porosity, and effective porosity, as well as the overall expansion of the model.

2b. Determine the impact of the loading direction on model behavior of CAD models with auxetic pores. Model behavior will be quantified as described in Aim 2a.

Specific Aim 3) Manufacture and characterize auxetic meshes made from the elastomer polydimethylsiloxane (PDMS)

3a. Determine the mixing ratio (base to curing agent) and thickness of PDMS that will yield a material stiffness similar to vaginal tissue. Uniaxial mechanical properties of PDMS, dog-bone shaped samples will be obtained via uniaxial tensile tests.

3b. Manufacture auxetic meshes made from PDMS and assess the behavior of these meshes in response to tensile loading. Specifically, PDMS auxetic meshes will be made using the mixing ratio and thickness determined in Aim 3a. Mesh behavior will be assessed via quantitative measurements of pore deformation (changes in pore size, porosity, and effective porosity), relative elongation, mesh burden, and permanent elongation.
Specific Aim 4) Evaluate the host response to elastomeric meshes

4a. To provide a descriptive analysis of the impact of an elastomeric mesh with auxetic pores on the host response and to determine how pore size impacts this response. Elastomeric meshes, manufactured from PDMS, will be implanted into the abdomens of rats for 35 days. A qualitative description of the host cellular response, the presence/absence of bridging fibrosis, amount of encapsulation/fibrosis, and characterization of tissue between the pores will be performed. Additionally, comparisons will be made between the elastomeric meshes with pore sizes of 1.0 mm and 1.5 mm. When relevant, qualitative and quantitative comparisons will be made to Gynemesh PS.
2.0 DEVELOPMENT OF FINITE ELEMENT MESH MODELS

2.1 INTRODUCTION

Finite element analysis (FEA) (also referred to as the finite element method) is a powerful tool that researchers, scientists, and engineers use to model and understand complex problems that are difficult to investigate experimentally. Traditionally, FEA is used in industrial settings, particularly for product design and development, as the creation of prototypes is often expensive and time consuming. With FEA, computational models of prototypes are made and then assessed without the need to physically produce the product. Given the power of this technique, FEA has also played a vital role in modeling of disease and medical product development. For example, the cardiovascular field has utilized FEA for the design and analysis of stents and valves and to model cardiovascular blood flow [152-154]. FEA has also aided researchers in the orthopedic field with understanding bone remodeling for hip implant development and with understanding the knee joint and ligaments [123,124,155]. Compared to other fields, the use of computational modeling and FEA within the urogynecologic field is relatively new and is used primarily to establish models of the female pelvic anatomy and to simulate childbirth and pelvic floor disorders [156-160]. However, researchers are beginning to use modeling and FEA to predict the load-elongation behavior of synthetic mesh and to quantify mesh pore deformation [73,161,162]. As part of the FEA process, a finite element model (FEM) is often created. The development of a FEM includes
the following major steps: 1) creation of the solid model geometry (or geometries), 2) determination of material properties that will be assigned to the model(s), 3) discretization of the model(s), and 4) convergence testing.

Prolapse meshes are exposed to predominately tensile loading conditions in vivo causing the pores to collapse by 70-90% in response to loads within the physiologic range. When pore sizes decrease below 1 mm, bridging fibrosis occurs in which the foreign body response to one mesh fiber overlaps with that of a neighboring fiber effectively merging the response resulting in encapsulation. Additionally, pore collapse alters the mechanical behavior of the mesh causing it to bunch and behave stiffer in this region. We propose to design synthetic meshes with a macrostructure affording auxetic behavior; that is, the pores expand laterally, instead of contracting. Conceptually this idea makes sense; however, it has not been proven. Auxeticity can be achieved with a range of geometric structures. Thus instead of manufacturing multiple meshes, finite element analysis, specifically 3D quasi-static, large deformation FEA, was used to assess the behavior (i.e. model expansion or contraction, model elongation, changes in pore dimensions, porosity, and effective porosity) of computational models with varying pore geometries. As mentioned in the previous paragraph, a finite element model(s) is needed prior to conducting FEA. In the remaining sections of this Chapter, an introduction to auxetic materials and geometries will be given and the development of the finite element models used in the FEA in Chapter 3 will be discussed.

2.1.1 Auxetic Materials and Geometries

The term auxetic refers to materials that have a negative Poisson’s ratio and to structures that demonstrate behaviors such as lateral expansion when placed in tension. Poisson’s ratio describes
an intrinsic material property that defines the compressibility of a material. Specifically, it is a measure of the degree of contraction along one axis of a material relative to the degree of expansion along the orthogonal axis. Most materials have a positive Poisson’s ratio ranging from 0 to 0.5 where a material with a Poisson’s ratio closer to 0 would experience little to no lateral deformation from tension or compression whereas a material with a value closer to 0.5 would laterally deform, i.e. contract in the transverse direction, when stretched longitudinally. Although uncommon, there are materials that have a negative Poisson’s ratio, i.e. they expand laterally when deformed longitudinally. This counterintuitive behavior is largely attributed to the geometry of the material and to the deformation of the internal structure of the material [163]. Auxetic materials were first introduced in 1987 by Roderic Lakes who showed that open-cell foam could be manufactured to have a negative Poisson’s ratio [164]. Since this time, research on auxetic materials has increased and a variety of materials are fabricated to exhibit auxetic behavior including expanded polytetrafluoroethylene, polyurethane foam, and polypropylene [164-166]. Auxetic materials have an enhanced shear modulus and an increased indentation resistance relative to conventional materials, among other properties [163]. Currently, these materials are being used in a wide variety of applications including seat cushions, aircraft nose cones, and bulletproof vests [167].

Auxetic behavior is often a result of specific geometric shapes, whether they exist at the nano or micro scale of materials or in the macro pores of structures. When stretched, specific pores will undergo lateral expansion instead of the typical contraction in which most people are familiar [168]. Recognizing the innate auxetic behavior of blood vessels, researchers within the cardiovascular field have created an annuloplasty prosthesis with an auxetic shape for use in cardiac valve repair surgery [169]. Additionally, the use of auxetic structures was proposed in the design of artificial intervertebral disks, cushion pads, and knee prosthetics [170]. The geometry of
many of these auxetic shapes can be classified as either re-entrant or chiral, though there exist shapes that contain both geometries. For example, an auxetic structure with a re-entrant geometry will have inward pointing angles whereas ones with a chiral geometry will contain circles and/or consist of a circle-like repeating pattern (Figure 17).

![Chiral Structure](image1.png)  ![Re-entrant Structure](image2.png)

Figure 17: Example of two auxetic structures.

2.2 DESIGN OF STANDARD AND AUXETIC COMPUTATIONAL MESH MODELS

The pore geometry of synthetic meshes plays a major role in the mechanics of the mesh [73]. It was therefore important that in addition to creating computational models with auxetic pore geometries, models with pores that have shapes similar to current prolapse meshes were also created. Squares, diamonds, and hexagons are three common pore geometries of commercially available polypropylene meshes. Therefore, these shapes were used to construct the standard pore computer-aided design (CAD) mesh models investigated in this work (Figure 18). In the literature,
there are a number of auxetic geometries that have been identified and used in other applications; however, not all of these geometries are suitable for prolapse meshes (i.e. some geometries were not sufficiently porous enough or too complex for manufacturing purposes) [168,171,172]. Thus, only those auxetic geometries that were deemed potentially appropriate by our experts (Drs. Steven Abramowitch and Pamela Moalli) for prolapse mesh designs were utilized in the creation of the auxetic CAD mesh models.

Computational mesh models were generated using SolidWorks 2013 x64 Edition (Dassault Systèmes SOLIDWORKS Corporation, Waltham, Massachusetts). Various methods within this software including linear patterning and Boolean subtraction were utilized to construct models that were approximately 90 mm x 15 mm. Eleven mesh models with various pore geometries were created: 3 with standard pore geometries (collectively referred to as standard pore models) and 8 with auxetic pore geometries (referred to as auxetic models). The standard pore models had pore geometries which included 1) square (SQ), 2) diamond (D), and 3) hexagon_a (Hexagon a or Ha) (Figure 18), while the auxetic pore geometries included 1) bowtie (B), 2) spiral (S), 3) triangle (T), 4) square chiral_a (Square Chiral a or SCa), 5) chiral hexagon (CH), 6) square chiral_b (Square Chiral b or SCb), 7) hexagon_b (Hexagon b or Hb), and 8) square grid (SG) (Figure 19). Note: geometries containing the letter a or b at the end of their respective name include an underscore within the text of this document in order to ensure that the letter is not misunderstood for a typo. For figures and tables, the underscore was omitted.
Figure 18: Standard computational models (top images) with similar pore geometry and dimensions as the respective commercial synthetic mesh product (bottom images). The yellow shapes in the images of the commercial products demonstrate the geometry that was used to create the computational models.
Figure 19: Auxetic CAD models with the pores aligned along the longitudinal axis of the model.

The square and diamond pored models were simplified geometries modeled after Restorelle (Coloplast, Minneapolis, MN) prolapse mesh and the hexagon_a model was a simplified geometry of Gynemesh PS (Ethicon, Somerville, NJ, USA) prolapse mesh. Calipers were used to measure the fiber width (distance between two fibers), thickness, and the pore size of the commercial products. These dimensions were then used to guide the development of the standard models. To create a realistic model with auxetic pore geometries, the dimensions (i.e. fiber width, thickness, and pore size) of the auxetic model pores were modeled after Restorelle. The latter was chosen as the “model mesh” given the relative simplicity of the pore geometry (square pore geometry) and the ease of measuring the dimensions of these pores. It is important to note that the pore size of the auxetic pore geometries did not exactly match those of Restorelle due to the design/complexity
of these geometries. For all mesh designs, the volume of material was the same and the minimal pore dimension was at least 1 mm (i.e. the majority of the models had pore dimensions that were greater than 1 mm). Although, it should be noted that models containing angles less than 90° would have small regions of the pore where the fibers would be closer than 1 mm. To standardize this, the smallest allowable angle within a pore was 45°. For designs containing circles, the diameter of the circles was equal to 1 mm. Additionally, for all models, the width of the fibers equaled 0.30 mm. Figure 20 illustrates these design concepts. Lastly, it should be noted that the commercial meshes mentioned previously were used as a guide for representative pore geometries and dimensions to construct the standard pore models. These models were used to make comparisons between the standard pore and auxetic pore models based on pore shape and not to match the behavior of the mesh models created in this dissertation with actual (commercial) meshes.

Figure 20: Schematic illustrating the pore size, fiber width, and thickness of the mesh models (top left and right) and specific design criteria used in the construction of these models (bottom left and right).
The eleven models pictured in Figures 18 and 19 depict the geometry of the pores aligned along the longitudinal axis of these models. Collectively, they are referred to as the 0° models. Models were also created for the auxetic models with the geometry of the pores rotated 45° (Figure 21) and 90° (Figure 22) with respect to those in Figures 18 and 19, following the same design concepts as outlined previously. Consistent with the naming convention for the 0° models, these models will be referred to as 45° models and 90° models, respectively. It is important to note that the 90° models were not created for all auxetic models as a 90° rotation for some models (e.g. the square chiral_a) would result in the same pore configuration as the 0° model. Additionally, 45° and 90° models were also not created for the standard models, as they were tested in the configuration in which they are implanted and served as a comparison for the auxetic models.
Figure 21: Auxetic CAD models with the pores rotated 45° with respect to the longitudinal axis of the model.
2.3 DISCRETIZATION OF STANDARD AND AUXETIC CAD MESH MODELS

Discretization is a fundamental step in the finite element method. It is the process by which a computational model is divided into smaller units referred to as finite elements or simply elements. Connecting the elements together are nodes, which can be located in three different places depending on the type of elements: 1) along the boundary lines, 2) along the surfaces, and 3) at points where two or more elements meet. The concept of discretization in the FEM is similar to that of approximating the area under a curve using rectangles (Reimann Sum) with the general idea that the more rectangles used, the better the area approximation. In the case of the FEM, the computational model is the curve and the elements are the rectangles. An algebraic equation is formulated for each element (rather than differential equations) and the solution for each equation is then combined to obtain the solution for the model as a whole. Thus, discretization essentially
converts a calculus problem into an algebraic one. Once a model is discretized, the resulting collection of elements is referred to as a “mesh”. However, to prevent one from confusing “mesh” (when used for a discretized mesh) with an actual urogynecologic mesh product, the term discretized finite element model (or discretized model) will be utilized throughout this dissertation. Typically, a mesh-generating program or the FEA software with discretization capabilities is used to discretize a model.

Finite elements are categorized into three main groups: one-, two-, and three-dimensional elements. The choice of which element(s) to use is driven by the design of the model and by the behavior that the model is attempting to simulate (e.g. assessing stress in the walls of a blood vessel due to the flow of blood through the vessel). Three-dimensional finite elements are often used in the urogynecology field as well as other biomedical and non-biomedical fields for the discretization of solid model analysis. Tetrahedron (or tetrahedral), pentahedron (or pentahedral), and hexahedron (or hexahedral) elements are three types of three-dimensional elements (Figure 21). Of the three, tetrahedral elements are the simplest. They consist of four triangular sides. Pentahedral elements have five sides, two sides are triangles and the remaining three sides are quadrilaterals. Accordingly, hexahedral elements (also called brick elements) have six sides, all of which are quadrilaterals. In addition to being 3-D elements, tetrahedral, pentahedral, and hexahedral elements are also classified by the number of nodes, which defines the polynomial order of the approximation. For example, the interpolating function (also referred to as the shape function) for the 1st order element is linear and that for the 2nd order element is quadratic. In addition to the differing shape functions, the difference in the linear and quadratic elements can be visually observed by the absence or presence of “midside/midpoint” nodes, respectively. For example, the linear tetrahedral element (tet4) consists of four nodes located at the vertices of the
tetrahedron whereas the quadratic tetrahedral element (tet10) has an additional six nodes located at the midpoint of the lines connecting the vertices for a total of ten nodes (Figure 23 a and b). The linear pentahedral element (penta6) contains six nodes (at the vertices) and the quadratic counterpart (penta15) contains 15 nodes, 6 at the vertices and 8 at the midpoints (Figure 23 c and d). Similarly, the linear hexahedral element (hex8) has eight nodes at the vertices while the quadratic hexahedral element (hex20) consists of eight nodes (at the vertices) and fourteen midpoint nodes (Figure 23 e and f).

Figure 23: Common elements used in finite element analysis: (a) linear tetrahedral, (b) quadratic tetrahedral, (c) linear pentahedral, (d) quadratic pentahedral, (e) linear hexahedral, and (f) quadratic hexahedral. Blue (closed circles) and yellow (open circles) represent nodes.
The decision to use linear versus quadratic elements is based heavily on the study (and user preference). Quadratic elements can be more beneficial for capturing nonlinear behavior and they tend to result in smaller errors (more accurate solutions) relative to linear elements. However, quadratic elements are more complex, hence they increase computational cost. Accurate solutions can also be achieved with linear elements by increasing the number of elements; conversely, this too is associated with a computational expense. Irrespective of the type of elements, it is important that convergence testing is performed in order to verify that the discretization of the model is sufficient (See Section 2.5 for more details regarding convergence testing). In this study, linear elements were used.

For discretization, the standard and auxetic computational models were imported into Autodesk Simulation Mechanical 2015 (Autodesk, Inc., San Rafael, CA). The default settings of this software were used to discretize the models. Often the default settings did not yield a homogenous discretization (i.e. the number and type of elements were not evenly distributed across the model as much as possible). Thus, the mesh size was adjusted until a homogenous, discretized model (relatively coarse) was achieved. This model was then imported into GMsh (V2.11.0) [173] and further refined by splitting the elements in half twice, which exponentially increased the overall number of elements in the model. Elements were only split in half twice as a third split would result in a discretized model that contained too many elements for the FEA analysis software (FEBio software suite, University of Utah, MRL) to handle. Additionally, for many of the models, convergence of the elongation and pore size was not achieved, despite increasing the number of elements in GMsh (V2.11.0) [173] (to be discussed in Section 2.5). For these models, a finer discretized model was created in Autodesk Simulation Mechanical 2015 (Autodesk, Inc., San
Rafael, CA). In other words, those models were re-meshed and further discretized using GMsh (V2.11.0) [173] as previously described.

Discretized models with evenly distributed hexahedral elements was the goal; however, for many of the models, the use of only hexahedral elements was not possible without significant distortions of the surface geometry. This was particularly true for models containing circles. In these situations, a combination of elements (tetrahedral, pentahedral, and hexahedral elements) was used. Nonetheless, discretized models containing mainly hexahedral elements were chosen. Once all models were discretized, they were further refined by splitting the elements in half twice. All refinements consisted of 1st order elements and each refinement (i.e. discretized model) was exported for further analysis.

## 2.4 DETERMINATION OF MATERIAL PROPERTIES

Before finite element analysis is performed on any model, the material behavior must be defined. To define the material behavior, two relationships must be known: 1) the relationship between displacement and strain and 2) the relationship between stress and strain (termed the constitutive law). Along with these relationships are material properties that must be determined. Correctly defining these relationships, particularly the constitutive law, and material properties is essential for accurately predicting the behavior of the model. Previously in our lab, the Neo-Hookean model was shown to accurately predict the uniaxial load-elongation behavior of Restorelle (Coloplast, Minneapolis, MN), a commercially available synthetic mesh, with the knots and fibers modeled as independent entities (i.e. the knots and fibers had different material properties) [161]. The Neo-Hookean model was therefore utilized in this study; however, the material properties used were
different from those used previously. The CAD model created in this dissertation to resemble
Restorelle contained all fibers and no knots, in reality Restorelle contains knots and fibers. This
product was modeled that way because based on the new design criteria for synthetic meshes, the
pores of these meshes will be auxetic, in other words they do not collapse with loading. The models
with auxetic pore geometries did not contain knots; therefore, the knots of Restorelle were omitted.

The Neo-Hookean model is one of the simplest constitutive laws used to predict the
nonlinear stress-strain behavior of a hyperelastic material that undergoes large strains. For a
hyperelastic material, the stress and strain relationship is defined by a strain energy function, \( \Psi \),
(also referred to as a stored-energy function). The general form of the strain energy function for a
Neo-Hookean model (incompressible) is given by:

\[
\Psi = C_1(I_1 - 3)
\]

where \( C_1 = \mu/2 \) is a constant, \( \mu \) is the shear modulus, and \( I_1 \) is the first invariant of the right Cauchy-
Green tensor. For a compressible Neo-Hookean model the strain energy function is as follows:

\[
\Psi = \frac{\mu}{2} (I_1 - 3) - \mu \ln J + \frac{\lambda}{2} (\ln J)^2
\]

where \( \lambda \) is the stretch ratio and \( J \) is the Jacobian of the deformation gradient tensor [174].
Collectively, \( \mu \) and \( \lambda \) are referred to as the Lamé parameters and they are defined by the Young’s
Modulus \( (E) \) and Poisson’s ratio \( (\nu) \):

\[
\lambda = \frac{E \nu}{(1+\nu)(1-2\nu)}
\]

\[
\mu = \frac{E}{2(1+\nu)}
\]

with the Young’s Modulus representing the stiffness of a material and the Poisson’s ratio being a
measure of material compressibility. Thus, to define a material using a Neo-Hookean model, only
the Young’s Modulus and Poisson’s ratio (i.e. the parameters describing the mechanical
properties) are needed.
To obtain representative values for all simulations in this study, an inverse finite element analysis was performed. Specifically, this analysis was done in order to determine the material properties of the Neo-Hookean model by fitting experimental data to a computational simulation of Restorelle. More specifically, the experimental data (load and elongation) was obtained from uniaxial tensile tests of 90 mm x 15 mm strips of Restorelle (n=5) loaded longitudinally with the pores in the square configuration. Mesh samples were loaded to 3 N and the resulting load and elongation were averaged for the five samples. Three Newtons represents the minimal amount of force that a mesh must be able to withstand based on estimates of the intra-abdominal pressure reported with sitting and standing and on our estimates of the surface area of the anterior vagina using MRI measurements [144-148]. A computational simulation of this test was performed using FEBio software suite (University of Utah, MRL) and the square CAD model developed in Section 2.2, which was designed to match the pore dimensions and geometry of Restorelle. This model was discretized as described previously (Section 2.3) and imported into Preview (University of Utah, MRL). The discretized model consisted of 178,944 linear hexahedral elements. To simulate a uniaxial tensile test the following boundary conditions were prescribed (Figure 24). The bottom edge of each discretized model was fixed in translation and rotation while the top edge was fixed to a rigid body. Both the top edge of the discretized model and the rigid body were allowed to displace in the y-direction, along the length of the model. Additionally, all deformation was constrained to be in the plane of the model. A prescribed displacement of 7.63 mm was applied to the rigid body. This displacement corresponds to the average displacement of Restorelle at 3 N for the five mesh samples tested.
Figure 24: To simulate a uniaxial tensile test, the bottom edge of the Restorelle CAD model was fixed in translation and rotation while the top edge was fixed to a rigid body (green). The top edge of the model and rigid body were allowed to displace in the y-direction. All deformation was constrained to be in the plane of the model.

The inverse FE method was performed in FEBio using a Levenberg-Marquardt optimization method with the range for the Young’s Modulus restricted to 1-100 MPa and Poisson’s ratio restricted to 0.4-0.49. These ranges were chosen for the following reasons: 1) in preliminary studies, Young’s Moduli greater than 100 MPa yielded a model that was too stiff to replicate the non-linear load-elongation behavior of Restorelle and 2) to ensure that the Poisson’s ratio determined from the inverse FE method is a realistic value for an actual material. For the optimization, the sum of the squared difference between the experimentally obtained forces for Restorelle, uni-axially loaded, and the resulting reaction force of the computational simulation of Restorelle, uni-axially loaded, (with the “unknown” parameters) was minimized with a
convergence tolerance of 0.001. From the inverse FEA, the optimized value for the Young’s Modulus was 52.98 MPa and the Poisson’s ratio was 0.41. In order to check the accuracy of these parameters, a uniaxial test was simulated using the optimized parameters and the same model and boundary (and loading) conditions as outlined above. The results of this simulation were then post-processed in PostView (University of Utah, MRL). A load-elongation curve for this simulation was created by plotting the observed elongation of the model versus the reaction force of the rigid body (which is similar to the reading of the load cell experimentally). For comparison, the load-elongation data from the experimental testing of Restorelle was also plotted with the simulation data.

Overall, the Neo-Hookean model with the optimized parameters was able to accurately describe the non-linear behavior of Restorelle (Figure 25). However, the model did have some trouble with predicting the load response in the elongation range between approximately 3 mm and 7 mm. This likely is attributed to the differences in the actual mesh product and the computational model. Restorelle consists of knots and fibers. During the early phase of loading (i.e. small elongation), the knots of Restorelle act as pivot points allowing the fibers to rotate and align with the direction of loading. The computational model of Restorelle did not contain knots; therefore, this behavior of fiber rotation could not be captured. Despite this limitation, there was good agreement between the Neo-Hookean model and the experimental results. The optimized material properties were therefore used throughout the rest of this computational study.
Figure 25: Resulting load-elongation curves from experimental data of Restorelle (blue) and the FEA data of the Restorelle CAD model (yellow) uni-axially loaded to 3 N. The finite element model, with the Young’s Modulus and Poisson’s ratio determined via inverse FEA, closely captured the load-elongation behavior of Restorelle as indicated by the fit of the finite element data to the experimental data.

2.5 CONVERGENCE TESTING

During the discretization process, the question of how many elements are needed arises. This is an important question given that the premise of the finite element method is to break a larger more complex problem into a smaller more manageable problem via finite elements. To address this question, convergence testing is performed. Convergence testing is the iterative process by which a model is discretized multiple times and the FEA solutions obtained are compared. Each time this process is done, the model is discretized with smaller (i.e. more) elements until an increase in the number of elements results in a minimal change in the solution (i.e. convergence is reached). The discretization that results in the least amount of elements to obtain convergence is typically chosen,
since a larger amount of elements increases computational time and computer cost in terms of
memory without significantly improving the accuracy of the solution.

To examine model convergence, the 3 or 6 discretization’s of increasing element count was
created for all CAD models in the three loading directions as described in Section 2.3. Following
discretization, models were imported into PreView (University of Utah, MRL) and a simulated
uniaxial tensile test was performed using FEBio (University of Utah, MRL). The bottom edge of
the discretized model was fixed in translation and rotation while the top edge was fixed to a rigid
body. Both the top edge of the discretized model and the rigid body were allowed to displace in
the y-direction, along the length of the model. Additionally, all deformations were constrained to
be in the plane of the model. A load of 3 N was applied to the rigid body. The material for all
models was defined to be Neo-Hookean with a Young’s Modulus of 52.98 MPa and Poisson’s
ratio of 0.41, as determined by the inverse FEA performed in Section 2.4. The solution for all
discretized models was obtained using FEBio (University of Utah, MRL) and then post-processed
to examine the convergence of elongation (given solution) and the pore dimensions (calculated
width and length) in response to 3 N. Convergence was achieved when an increase in the number
of elements resulted in a less than 5% change in the parameter being analyzed.

2.5.1.1 Quantification of Pore Dimensions
Several image-processing steps were completed prior to calculating the width and length of the
pores. Screenshots of the un-deformed (0 N of applied force) and deformed (3 N of applied force)
models were taken at the same pixel resolution. These images were then imported into ImageJ
1.49v (National Institutes of Health, Bethesda, MD) and the dimensions of the images were
converted from pixel distance to physical distance (i.e. converted from pixels to mm). This was
done by determining the number of pixels within a known distance of the model pore in the un-
deformed state, referred to as the conversion factor. Since the un-deformed (0 N) and deformed (3 N) images had the same pixel resolution, the conversion factor determined for the un-deformed state was also used for the deformed state. Next, images were cropped such that a 30 mm x 12 mm image of the central region of the models was remaining. Care was taken to ensure that during cropping the bounding edges of the cropping box were within the width of the model. Note that for models that contracted, the length of the cropped images remained at 30 mm but the width was smaller than 12 mm, to stay within the bounds (i.e width) of the models. Cropped images were then imported into a custom Mathematica V10 script (Wolfram, Champaign, IL) originally developed by William R. Barone, PhD (graduate of Dr. Abramowitch’s lab) and were slightly modified for use in this dissertation (Appendix A). Briefly, images were binarized such that the pixels representing the fibers of the model are black and the pixels representing the void spaces (all non-model fibers) are white. Next, an algorithm was used to identify the pores of the model, clusters of white pixels surrounded by clusters of black pixels. The width and length of the pores were then calculated using an algorithm that approximates the pores with the best-fitting ellipse; the smallest axis of the ellipse corresponds to the width and the largest axis to the length. Using this method, the pore dimensions of some of the models were over-estimated, particularly for the un-deformed pores, and this is a result of the geometry of the pores not conforming to the shape of an ellipse. When this occurred, the percent that the ellipse over-estimated was determined and the respective pore dimension was adjusted according to this amount. For example, if the ellipse estimated the length of the pore to be 8 mm and the actual length was 4 mm, the reported pore length was adjusted by a factor of 2. Pores that were not complete, those along the edge of the image, were excluded from analysis. In the end, the minimal width and minimal length for all of the pores in the cropped image were reported, as well as the overall elongation of the model.
2.5.1.2 Convergence Results

Convergence was achieved for all models and all parameters analyzed in the 0° loading direction. Analyzing the models based on the type of pore (standard and auxetic), both the elongation of the model and the pore dimensions (width and length) converged within 5% for the models with non-auxetic pores (Figure 26). Specifically, the elongation of the square and diamond models increased slightly with the first increase in the number of elements, 3.3% and 3.6%, respectively. Increasing the amount of elements further resulted in very little change in the amount of elongation for these two models. For the hexagon_a model, the number of elements was increased by a factor of 86 before convergence of less than 5% was achieved. Analyzing the pore dimensions, convergence of the width and length of the square model was achieved relatively quickly, particularly for the length (Figure 26). A similar result for length was observed with the diamond and hexagon_a models; however, the pores of these models, especially the pores of the hexagon model, underwent a considerable amount of deformation requiring additional elements beyond the 64-fold increase to reach convergence. Similarly, convergence was achieved for all of the auxetic models (Figures 27 and 28). All of these models required additional elements beyond the 64-fold increase with the exception of the spiral and square grid models. The same number of elements required for convergence of elongation was also enough to achieve convergence of the pore width and pore length with the final convergence for these two parameters being less than 3% for all auxetic models.
Figure 26: Convergence testing results for the standard 0° models. Both the elongation and pore dimensions (length and width) converged within 5%.
Figure 27: Convergence testing results for the bowtie, spiral, triangle, and square chiral\_a 0° models.

Both the elongation and pore dimensions (length and width) converged within 5%.
Figure 28: Convergence testing results for the chiral hexagon, hexagon_b, square chiral_b, and square grid 0° models. Both the elongation and pore dimensions (length and width) converged within 5%.
All 45° models (8 models in total) achieved convergence (Figures 29 and 30). Of the eight models, a 64-fold increase in the number of elements was sufficient to achieve convergence of less than 5% for the square chiral_a 45° and square chiral_b 45° models, all other models required additional elements. The elongation of the bowtie 45°, spiral 45°, triangle 45°, hexagon_b 45°, and square gird 45° all converged within 1%. The elongation of the square chiral_a 45°, chiral hexagon 45°, and square chiral_b 45° also converged to less than 5% (2.4%, 4.3%, and 3.8%, respectively). Lastly, convergence of less than 4% for the width and length of the pores was achieved for all 45° models.
Figure 29: Convergence testing results for the bowtie, spiral, triangle, and square chiral a 45° models. Both the elongation and pore dimensions (length and width) converged within 5%.
Figure 30: Convergence testing results for the chiral hexagon, hexagon_b, square chiral_b, and square grid 45° models. Both the elongation and pore dimensions (length and width) converged within 5%.
Similar to the 0° and 45° models, convergence of less than 5% was also achieved for the elongation and pore dimensions of the 90° auxetic models (Figure 31 and 32). Interestingly, a 64-fold increase in the number of elements was not enough for the elongation of all of the models to achieve convergence; therefore, additional elements were incorporated. With the addition of these elements, a change in elongation of less than 4% was observed for all models, thus achieving convergence. Additionally, the width and length for all 90° auxetic models converged to less than 2% with the final increase in the number of elements.

![Graphs showing convergence testing results for hexagon_b and square chiral_b 90° models.](image)

Figure 31: Convergence testing results for the hexagon_b and square chiral_b 90° models. Both the elongation and pore dimensions (length and width) converged within 5%.
Figure 32: Convergence testing results for the bowtie, triangle and chiral hexagon 90° models. Both the elongation and pore dimensions (length and width) converged within 5%.
Ensuring that the discretized mesh contains enough elements to achieve convergence of a solution is an important step in the FEA process. For this particular study, it was important that convergence of the elongation and the dimensions (width and length) of the pores be achieved since these parameters serve as a basis for the other parameters that were analyzed in this study (e.g. pore area and porosity). The results from convergence testing revealed that the elongation, pore width, and the pore length all converged to less than 5% with the final discretized models (i.e. the discretized models with the most elements) for all models. It was therefore determined that these discretized models contained a sufficient number of elements. For specific details regarding the type and number of elements for each mesh model, see Tables 3-5.

**Table 3: Composition of discretized computational 0° models. Numbers represent the number of elements for each element type listed in the heading.**

<table>
<thead>
<tr>
<th></th>
<th>Tetrahedral</th>
<th>Pentahedral</th>
<th>Hexahedral</th>
<th>Total Number of Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Square</td>
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<td>0</td>
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<td>178944</td>
</tr>
<tr>
<td>Diamond</td>
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<td>0</td>
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<td>228480</td>
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<td>39744</td>
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<td>Bowtie</td>
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<td>181184</td>
<td>265472</td>
<td>705088</td>
</tr>
<tr>
<td>Chiral Hexagon</td>
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<td>204352</td>
<td>242944</td>
<td>614592</td>
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<tr>
<td>Spiral</td>
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<td>0</td>
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<td>224768</td>
</tr>
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<td>Square Chiral a</td>
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<td>177024</td>
<td>286656</td>
<td>676480</td>
</tr>
<tr>
<td>Triangle</td>
<td>218240</td>
<td>105152</td>
<td>300224</td>
<td>623616</td>
</tr>
<tr>
<td>Square Chiral b</td>
<td>63680</td>
<td>74752</td>
<td>206656</td>
<td>345088</td>
</tr>
<tr>
<td>Hexagon b</td>
<td>162432</td>
<td>167872</td>
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<tr>
<td>Square Grid</td>
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Table 4: Composition of discretized 45° models. Numbers represent the number of elements for each element type listed in the heading.

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<th>Hexahedral</th>
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</tr>
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<td>72512</td>
<td>106816</td>
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<tr>
<td>(45°)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiral (45°)</td>
<td>201792</td>
<td>153032</td>
<td>272216</td>
<td>627040</td>
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<td>Square Chiral a</td>
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<td>572032</td>
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<tr>
<td>(45°)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triangle (45°)</td>
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<td>278656</td>
<td>524544</td>
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<td>Square Chiral b</td>
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<td>49728</td>
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<td>(45°)</td>
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<td>Hexagon b (45°)</td>
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<td>Square Grid (45°)</td>
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Table 5: Composition of discretized 90° models. Numbers represent the number of elements for each element type listed in the heading.

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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triangle (90°)</td>
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<tr>
<td>(90°)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexagon b (90°)</td>
<td>72512</td>
<td>122560</td>
<td>346560</td>
<td>541632</td>
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</tbody>
</table>
3.0 PREVENTING MESH PORE COLLAPSE WITH AUXETIC GEOMETRIES: A COMPUTATIONAL ANALYSIS

3.1 INTRODUCTION

Synthetic meshes are commonly used in the repair of pelvic organ prolapse and can be placed via transabdominal or transvaginal routes. In spite of good anatomic success rates, transvaginal prolapse meshes have been hampered by complications, prompting the Food and Drug Administration to issue two public health notifications (2008 and 2011) and to up-classify transvaginal meshes from level II to level III devices. The most common complications are mesh exposure through the vaginal epithelium and pain, but they also include infection, mesh shrinkage/contraction, erosion into an adjacent structure, bleeding, dyspareunia, and urinary dysfunction [60]. Unfortunately, complications have led to some patients having to undergo multiple revision surgeries, and for some, relief of the symptoms associated with these complications is not attained despite treatment efforts. It is therefore not surprising that numerous lawsuits were filed and continue to be filed resulting in millions of dollars being awarded to patients causing some companies to discontinue their products.

All current prolapse meshes are hernia meshes simply remarked for a different indication. Given that hernia meshes were in use prior to the 1976 Medical Device Amendments Act, companies were able to remarket their mesh products for prolapse repair without having to undergo
rigorous scientific testing and clinical trials. This resulted in the use of products that were not tailored for the unique tissues and loading conditions within the female pelvis. The abdomen and female pelvis drastically differ mechanically and physiologically; and based on this alone, the observance of complications with hernia meshes being used as prolapse repair devices is not unfathomable. The pathogenesis of prolapse mesh complications is currently not clear; however, recent research suggests that pore collapse may be a contributing factor.

Most prolapse meshes are knitted, lightweight (<45 g/m²), and have wide pores (>1 mm) with a porosity that is greater than 55%. However, these characteristics describe the mesh prior to implantation and in its unloaded state. Once implanted and loaded, the geometry of the mesh may be altered, particularly when loading occurs prior to tissue incorporation. *In vivo* prolapse meshes are exposed to primarily tensile forces [77]. Indeed, in prior studies, the pores of most prolapse meshes (both transvaginal and sacrocolpopexy) collapsed below the critical diameter of 1 mm in response to loads that are well within the physiologic range [76,161]. This is particularly problematic given that data from hernia meshes indicate that for polypropylene, pores should be at least 1 mm for tissue incorporation and improved biocompatibility. These data also show that meshes with larger pores yield better tissue integration with increased collagen deposition between pores and decreased inflammation and fibrosis relative to meshes with smaller pores [79,119-121]. Pore size was also shown to be inversely proportional to bridging fibrosis (overlapping of the foreign body response between neighboring fibers), a process that can lead to encapsulation and pain [79,119]. Clinically, mesh contraction (i.e. pores collapsing) is associated with vaginal pain [82] and interestingly, problematic areas for patients experiencing mesh complications are often located in areas where the pores of a mesh have collapsed from over tensioning or overloading. Collectively, these findings demonstrate the importance of pore size and the need for a mesh that
maintains large pores with loading. To overcome the problem of pore collapse, we proposed to design prolapse meshes with auxetic pore geometries. Auxetic geometries and materials expand, instead of contracting, when loaded or deformed. For an in-depth background on auxetic materials and geometries, the interested reader should refer to Section 2.1.1.

The ability of pores designed with auxetic shapes to open in response to a mechanical stimulus has the potential to be highly beneficial in biomedical applications, especially for urogynecologic mesh. Given that pore size, porosity, and effective porosity are key design criteria for the biocompatibility of synthetic urogenital meshes and knowing that a small pore size (due to design and/or collapsing under mechanical loading) can lead to fibrotic encapsulation of the mesh and increase the potential for complications, an auxetically designed synthetic mesh has the potential to significantly improve the current state of the art of prolapse meshes. We hypothesized that a prolapse mesh with auxetic properties would allow for increased pore size with loading, thereby overcoming the problem of pore collapse. Thus, the objective of this study was to assess the behavior of meshes with auxetic pore shapes. To achieve our objective, we constructed computational models of 11 meshes (8 with auxetic pore shapes and 3 with standard pore shapes for comparison) and subjected them to simulated uniaxial tensile tests via 3D quasi-static, large deformation finite element analysis. The pores of the models were aligned along the longitudinal axis of the model, i.e. the longest axis of the model (in the direction of loading), or were rotated 45° and 90° with respect to this axis in order to determine if the behavior of the model (i.e. pore expansion) is directionally independent. This information is critical for understanding how these meshes may respond to multi-axial loading and for design consideration in terms of implantation direction. The resulting model behavior was characterized via quantitative measurements of model deformation, changes in pore size, porosity, and effective porosity, as well as the overall expansion.
of the model. It is anticipated that increases in pore size, porosity, and effective porosity as well as the overall expansion of the model (i.e. the prevention of pore collapse) will all depend on the orientation of the pore with respect to the loading direction and this will be particularly true for pores that are rotated 45° from the axis of loading.

3.2 METHODS

3.2.1 Computational Analysis

The discretized models and parameters developed in Chapter 2 were used in this finite element analysis. In total there were eleven 0° models (pores aligned along the longest axis of the model, with the loading direction, Figures 18 and 19), eight 45° models (pores rotated 45° with respect to the loading direction, Figure 21), and five 90° models (pores rotated 90° with respect to the loading direction, Figure 22). The 0° models consisted of three models with standard pore geometries (square, diamond, and hexagon_a) and eight models with auxetic pore geometries (bowtie, spiral, triangle, square chiral_a, chiral hexagon, square chiral_b, hexagon_b, and square grid). The 45° models included all of the auxetic pore geometries whereas the 90° models included the bowtie, triangle, chiral hexagon, hexagon_b, and square chiral_b geometries. The average length for all models was 85.22 ± 4.26 mm while the average width was 14.92 ± 0.78 mm. All models had an aspect ratio (length to width) of at least 5. Additionally, the volume of all models average 91.18 ± 0.70 mm³. For the dimensions and volume of each model, see Tables 6-8. Lastly, the initial minimal pore size for all models was at least 1 mm.
Table 6: Length, width, and volume of discretized computational 0° models.

<table>
<thead>
<tr>
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<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Volume (mm³)</th>
</tr>
</thead>
<tbody>
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<td>Hexagon a</td>
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<td>92.60</td>
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<td>92.15</td>
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<tr>
<td>Square Grid</td>
<td>94.60</td>
<td>15.90</td>
<td>91.87</td>
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</tbody>
</table>

Table 7: Length, width, and volume of discretized computational 45° models.

<table>
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<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Volume (mm³)</th>
</tr>
</thead>
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<td>Square Grid (45°)</td>
<td>93.09</td>
<td>16.05</td>
<td>91.90</td>
</tr>
</tbody>
</table>

Table 8: Length, width, and volume of discretized computational 90° models.

<table>
<thead>
<tr>
<th></th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Volume (mm³)</th>
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<td>Square Chiral b (90°)</td>
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<td>Hexagon b (90°)</td>
<td>88.85</td>
<td>15.77</td>
<td>90.74</td>
</tr>
</tbody>
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Models were discretized and refined using Autodesk Simulation Mechanical (Autodesk, Inc., San Rafael, CA) and GMsh (V2.11.0), respectively. Discretized finite element models (FEMs) consisted of a combination of tetrahedral, pentahedral, and hexahedral elements (Section 2.6, Tables 3-5). Convergence testing was conducted and the overall elongation of the mesh model and the pore dimensions, length and width, all converged to less than 5% (Section 2.5). A Neo-Hookean material model with a Young’s Modulus of 52.98 MPa and Poisson’s ratio of 0.41 was used for all mesh models as described in Chapter 2. Simulated uniaxial tensile tests were performed via 3D quasi-static, large deformation FEA using identical methods as described previously (Section 2.5). Briefly, one end of the mesh was fixed while the opposing end was attached to a rigid body and allowed to displace in the y-direction. All elements were restricted from displacement in the z-direction and rotation was constrained in the x and y directions. Three Newtons of force was applied along the longitudinal axis of the model. This force represents the minimal amount of force that a mesh must be able to withstand based on estimates of the intra-abdominal pressure reported with sitting and standing and on our estimates of the surface area of the anterior vagina using MRI measurements [144-148]. The resulting relative elongation, minimal pore width, effective pore area, porosity, effective porosity, and relative lateral contraction were quantified.

3.2.2 Quantification of Parameters

Relative elongation is the normalized version of elongation and was calculated by dividing the overall elongation of the mesh models by the initial length of the model. The minimal pore width, effective pore area, porosity, effective porosity, and relative lateral contraction were all quantified.
using custom Mathematica V10 (Wolfram, Champaign, IL) scripts (Appendix A and B). These parameters were calculated for the pores within the central region of the mesh models using the image processing techniques described previously (Section 2.5.1.1). Briefly, screenshots of the central region of the models in the un-deformed (0 N) and deformed (3 N) states were taken and imported into Mathematica. Images were then binarized and an edge detection algorithm was used to identify the fibers of the model (black pixels) and the pores (white pixels). The minimal width of the pores was determined using an algorithm that approximates the pores with the best-fitting ellipse (the smallest axis of the ellipse corresponding to the width). Using this method, the pore dimensions of some of the models were over-estimated, particularly for the un-deformed pores, and this is a result of the geometry of the pores not conforming to the shape of an ellipse. When this occurred, the percent that the ellipse over-estimated was determined and the respective pore dimension was adjusted according to this amount. For example, if the ellipse estimated the length of the pore to be 8 mm and the actual length was 4 mm, the reported pore length was adjusted by a factor of 2. Next, an algorithm was used to approximate the area of each pore. The effective pore area represents the area of the pores with widths that are greater than 1 mm, and was calculated by summing up the area of the pores greater than 1 mm and dividing this value by the total pore area. Porosity is a measure of the amount of void space within a given material, in this case mesh; it is also viewed as the amount of mesh material per unit area. Thus, a mesh that has no void spaces/pores (i.e. it is a completely solid mesh) will have a porosity of 0 (or 0%) and a porosity of 1 (or 100%) would indicate no mesh. The following formula was used to calculate porosity:

\[ \text{Porosity} = \frac{\text{Area of white pixels}}{\text{Total pixel area in the image}} \]

Effective porosity is calculated in a similar manner as porosity; however, only the areas of the pores with widths that are greater than 1 mm were included in this calculation (Effective Porosity = Area of white pixels with pore widths
Unlike the previous parameters, the relative lateral contraction was calculated for the mesh models using images of the entire mesh. The un-deformed (0 N) and deformed model images (0.6 N, 1.5 N, 2.4 N, 3 N) were imported into Mathematica and the pores were identified using algorithms as previously described. Next, the center of mass (i.e. the centroid) was located for each pore and the coordinate position of these centroids, in the undeformed and deformed states, was exported. These positions were then used to calculate the relative lateral contraction as follows: Relative lateral contraction = -(\text{relative elongation}_{\text{transverse}} / \text{relative elongation}_{\text{longitudinal}}), where relative elongation is defined as the change in length divided by the initial length. This parameter is representative of the degree of contraction of the model with a positive value indicating contraction of the model (i.e. pore collapse) and a negative value indicating expansion (i.e. pores remaining open/enlarging).

3.2.3 Model Assessment Criteria

Analyzing the pore and overall model deformation of the FE models with auxetic pore geometries, the following criteria was used to define what would be clinically considered a positive mesh response to uniaxial loading \textit{in vivo}.

1) Pore deformation – the geometry of the pores should be stable with loading. In other words, the pores can expand but the overall geometry of the pore should not be significantly altered nor undergo excessive movement in response to loading. This criterion stems from the literature in which micro-motion can negatively impact the host response to an implant. For example, micro-motion at the skin-device interface of percutaneous implants interrupts the skin seal that forms around these implants and this consequently leads to chronic wounding and infection.
Additionally, excessive micro-motion at the dental implant-bone interface can lead to fibrous tissue encapsulation, implant loosening, and it can also inhibit osseointegration [177-179]. Given the negative impact that micro-motion can have on the host response to a device, auxetic geometries which undergo a significant degree of rotation in response to loading will not be considered as appropriate pore geometries for prolapse meshes intended for uniaxial applications (i.e. abdominal sacrocolpopexy).

2) Pore size, porosity, effective porosity, and effective pore area – the size of the pores as well as the overall porosity should be maintained or increased with loading. In both the abdominal hernia and urogynecology literature, large pore, high porosity meshes yield better tissue integration with increased collagen deposition between pores and decreased inflammation and fibrous relative to meshes with small pores and low porosity [79,119-121]. For polypropylene meshes, 1 mm is identified as the optimal minimal pore diameter needed to allow for tissue ingrowth and for the prevention of bridging fibrous [79]. Thus in this study, the minimal pore diameter should be at least 1 mm and the effective pore area as well as the porosity and effective porosity should be maintained following the application of load.

3) Expansion – the pores of the mesh as well as the mesh overall should expand in response to load or deformation. Arguably expansion of mesh pores is most critical at the time of implantation and prior to tissue incorporation within the mesh. It is during these times that the mesh can freely deform without restrictions. Thus, any tension or deformation applied to the mesh whether it is applied by the surgeon when fixing the mesh in place or through changes in intra-abdominal pressure,
could result in pore contraction or even pore collapse, which increases the chances of bridging fibrosis, a phenomenon associated with mesh contraction and pain [119]. Additionally, contraction of prolapse meshes is associated clinically with vaginal pain, dyspareunia (pain with sexual intercourse), and tenderness upon palpation of the contracted portion of the mesh [82]. It is therefore critical that pore contraction and collapse during the early stages of mesh implantation be prevented. With this in mind, the auxetic geometry or geometries that result in a negative relative lateral contraction in response to load will be considered beneficial for prolapse repair.

4) Mesh elongation – overall the amount of mesh elongation should be minimal to provide maximal stiffness of the mesh with a minimal amount of material. In other words, the mesh should be as stiff as possible using the least amount of material. Thus in terms of the pore geometry, the auxetic geometry or geometries that would require a stiffer material relative to the vagina should be avoided for prolapse meshes given that materials that are too stiff can lead to stress shielding, a phenomenon associated with a maladaptive and degenerative remodeling response [123-131]. Similarly, auxetic geometries that may require more material to increase the structural stiffness of mesh should also be avoided as lightweight meshes are more favorable than heavyweight meshes [79,81,122,134-136].
3.3 RESULTS

3.3.1.1 0° Models Finite Element Analysis

In total, the behavior of 11 computational models, three with standard pores and eight with auxetic pores, in response to 3 N of force was assessed via a simulated uniaxial tensile test. Qualitatively analyzing the FEA results via visual inspection for the three standard pore models, the shape of the pores of the square model stayed relatively the same whereas the pores of the diamond and hexagon_a models contracted (Figure 33).
Figure 33: Finite element results of the square (SQ), diamond (D), and hexagon_a (Ha) at 0 N and 3 N. Overall, the amount of elongation differs for each model. The pores of the diamond and hexagon_a model collapsed, resulting in model contraction. However, the pores of the square model remained relatively open.

The scale bar is representative of elongation.
For the auxetic models, pore expansion was visibly apparent for all with the exception of the triangle model and the models containing circles (Figures 34-35).

Figure 34: Finite element results of the bowtie (B), spiral (S), triangle (T), and square chiral_a (SCa) 0° models at 0 N and 3 N. Overall, the amount of elongation differs for each model. All models appear to expand with the exception of the triangle model, which contracted. The scale bar is representative of elongation.
Figure 35: Finite element results of the chiral hexagon (CH), square chiral_b (SCb), hexagon_b (Hb), and square grid (SG) 0° models at 0 N and 3 N. Overall, the amount of elongation differs for each model.

Expansion of the square grid model was clearly observed.

The pores of the triangle model and the circles within the chiral hexagon, square chiral_a, and square chiral_b models all contracted. Overall, the most substantial change in pore shape was observed with the square grid geometry. In response to 3 N of force, the pores of the square grid model significantly rotated changing the initial pore shape from a collection of rectangles to a collection of large squares (Figure 35). Analyzing the overall shape of the models, expansion of
the bowtie, square chiral_a, and square grid models was observed throughout loading unlike the
diamond and hexagon_a models in which contraction was observed. Initially expansion of the
triangle and hexagon_b models was visibly apparent; however, with the application of additional
force, model contraction was observed. The subtle changes in the deformation of the square, spiral,
chiral hexagon, and square chiral_b models made it difficult to qualitatively determine whether
these models were undergoing expansion, contraction, or both throughout loading.

Figure 36: Minimal pore diameter of the 0° models at 0 N (blue) and 3 N (orange). The minimal pore
diameter of all models was greater than 1 mm with the exception of the diamond, square chiral_a, chiral
hexagon, and square chiral_b models.
Quantitatively assessing the deformation of the pores at 3 N, the minimal pore diameter of the bowtie, hexagon_b, and square grid models all increased while the minimal pore diameter of all other models decreased (Figure 36). Notably, the pores of the diamond, square chiral_a, chiral hexagon, and square chiral_b models all decreased below 1 mm. This decrease in the minimal pore diameter of the previously mentioned models also translated to a decrease in the effective pore area. None of the pores of the diamond model had minimal diameters greater than 1 mm resulting in a 100% decrease in the effective pore area. On the other hand, the effective pore area for the square chiral_a, chiral hexagon, and square chiral_b models decreased by 13.0%, 10.7%, and 12.3%, respectively. The effective pore area was maintained at 100% for all other models (square, hexagon_a, bowtie, spiral, triangle, hexagon_b, square grid) (Figure 37).

![Graph showing effective pore area for 0° models in response to 3 N of force. Initially, the effective pore area was 100% for all models (0 N, red). However, the effective pore area decreased for the diamond, square chiral_a, chiral hexagon, and square chiral_b models at 3 N. The effective pore area was maintained at 100% for all other models at 3 N.](image_url)
Assessing the expansion of the models via quantification of the relative lateral contraction, interesting results were observed (Figure 38). As expected, the relative lateral contraction for the standard pore models was positive signifying lateral contraction of the model. In terms of the auxetic models, initially the relative lateral contraction for all of these models was negative indicating lateral expansion, which is consistent with auxetic behavior. However, at 1.5 N and 2.4 N, the triangle and chiral hexagon models contracted and the relative lateral contraction became (and remained) positive for the remainder of loading. For all other models (the bowtie, spiral, square chiral_a, square chiral_b, hexagon_b, and square grid) the relative lateral contraction remained negative throughout loading signifying expansion with the bowtie experiencing the greatest amount of expansion.

![Figure 38: Change in relative lateral contraction with loading for the 0° models. As expected, the relative lateral contraction was positive for the non-auxetic models throughout loading. Initially, the relative lateral contraction was negative for all auxetic models. However, at 1.5 N and 2.4 N, the relative lateral contraction was positive (and remained positive) for the triangle and chiral hexagon models, respectively. A positive value indicates model contraction and a negative value expansion.](image-url)
The porosity of the standard pore square and all of the auxetic models increased in response to 3 N of force (Figure 39, top). However, a decrease in the effective porosity was observed for the diamond, hexagon_a, and chiral hexagon models (Figure 39, bottom). There was no change in the effective porosity for the square chiral_a and square chiral_b models with loading. Overall, the elongation of all models differed. The square and hexagon_a models deformed the least with relative elongations of 9.3% and 16.2%, respectively while the square grid model deformed the most, elongating 112.1% more than its initial length. The relative elongation for the remaining models from least to greatest was as follows: chiral hexagon 23.7%, square chiral_b 27.2%, bowtie 37.3%, diamond 41.8%, spiral 42.4%, square chiral_a 43.1%, hexagon_b 52.5%, and triangle 55.9%.
Figure 39: Porosity and effective porosity before (0 N, blue) and after (3 N, yellow) loading for 0°
models. The porosity increased for all models with the exception of the diamond and hexagon_a models.
Additionally, the effective porosity was maintained and/or increased for all models excluding the diamond,
hexagon_a, and chiral hexagon models.
3.3.1.2 45° Models Finite Element Analysis

The behavior of the eight auxetic models with the pores rotated 45° with respect to the axis of loading was assessed via a simulated uniaxial tensile test. Qualitatively analyzing the FEA results, loading the models with the pores rotated 45° gravely impacted the deformation of the pores with the exception of the chiral hexagon model pores (Figures 40 and 41). In response to 3 N of force, the pore geometry of the bowtie 45°, spiral 45°, and square grid 45° models was substantially altered resulting in pore collapse and consequently model contraction. For the triangle 45°, square chiral_a 45°, hexagon_b 45°, and square chiral_b 45° models, the degree of pore collapse was not as drastic as the three previously mentioned models. However, contraction of these models was noticeable. Of all of the 45° auxetic models, the pore geometry of the chiral hexagon 45° model was the most stable with loading.
Figure 40: Finite element results of the bowtie 45°, spiral 45°, triangle 45°, and square chiral_a 45° models at 0 N and 3 N. Overall, the amount of elongation differs for each model. The pores of the triangle 45° (T) and square chiral_a 45° (SCa) models remained opened when loaded to 3 N, whereas significant pore collapse was observed with the bowtie 45° (B) and spiral 45° (S) models. Contraction was observed for all models in this figure.
Figure 41: Finite element results of the chiral hexagon 45°, hexagon_b 45°, square chiral_b 45°, and square grid 45° models at 0 N and 3 N. Overall, the amount of elongation differs for each model. The pores of the chiral hexagon 45° (CH) and square chiral_b 45° (SCb) models remained open at 3 N. Additionally, the pores of the hexagon_b 45° (Hb) model slightly collapsed and those for the square grid (SG) significantly collapsed. Model contraction was noticeably observed for all models pictured with the exception of the chiral hexagon model.
In response to 3 N of force, the minimal pore diameter of all of the 45° auxetic models decreased (Figure 42). Specifically, the minimal pore diameter was drastically reduced by 82.2% and 71.8% for the spiral 45° and square grid 45° models, respectively. For triangle 45° and hexagon 45°, the minimal pore diameter decreased 10% and 1.39%, respectively. However, despite this decrease, triangle 45° and hexagon_b 45° were the only two models in which the minimal pore diameter was greater than 1 mm following the application of 3 N of force.

Figure 42: Minimal pore diameter of 45° models at 0 N (blue) and 3 N (orange). The minimal pore diameter decreased for all models with the greatest decreases observed with spiral 45° and square grid 45°. Only the triangle 45° and hexagon_b 45° models had a minimal pore diameter that was greater than 1 mm in response to 3 N of force.
Initially the effective pore area for all models was 100%. However, in response to the applied load, the effective pore area for the bowtie 45°, spiral 45°, square chiral_a 45°, chiral hexagon 45°, and square grid 45° models decreased (Figure 43). Specifically, there was a complete loss in the effective porosity for the bowtie 45°, spiral 45°, and square grid 45° models. The effective pore area decreased by 18%, 11%, and 16.7% for square chiral_a 45°, chiral hexagon, and square chiral_b 45°, respectively. For the remaining two models, triangle 45° and hexagon_b 45°, the effective pore area was maintained at 100% in response to 3 N of force.

Figure 43: Effective pore area for the 45° models in response to 3 N of force. Initially, the effective pore area was 100% for all models (0 N, red). However, the effective pore area was maintained at 100% for only the triangle 45° and hexagon_b 45° models. All other models experienced a decrease or complete loss in the effective pore area.
Quantifying the expansion (or lack thereof) of the models via calculation of the relative lateral contraction confirmed that the models contracted. At 3 N, the relative lateral contraction was positive for all models analyzed (Figure 44). However, initially the chiral hexagon 45° model expanded as indicated by the negative relative lateral contraction at 0.6 N. Expansion of this model was only temporary as the addition of load beyond 0.6 N resulted in model contraction. This behavior was not observed with the remaining seven 45° models; the relative lateral contraction was positive (model contraction) early in the loading process and remained positive (contracted) throughout loading.

Figure 44: Change in relative lateral contraction with loading for the 45° models. Initially, the chiral hexagon 45° model expanded, hence the negative relative lateral contraction. However, by 3 N all models contracted. A positive value indicates model contraction and a negative value model expansion.
Overall, the porosity decreased for all of the 45° models with the exception of the triangle 45° and chiral hexagon 45° models (Figure 45). The greatest decrease in porosity was observed with bowtie 45° and spiral 45° with decreases of 36.1% and 32.8%, respectively. A 14.0% increase in the porosity was observed with triangle 45° model and a 10.3% increase for chiral hexagon 45°. Analyzing the effective porosity, a similar trend was observed as the porosity. A decrease in the effective porosity was observed for all 45° models with the exception of triangle 45°. The effective porosity for triangle 45° increased by 14.0%. Lastly, assessing the relative elongation of these models, square grid 45° (154.7%) deformed the most followed by bowtie 45° (83.5%) and spiral 45° (81.3%) with chiral hexagon 45° (27.8%) deforming the least. The relative elongation for the remaining models from least to greatest was as follows: triangle 45° 43.6%, square chiral_b 45° 49.9%, hexagon_b 45° 54.4%, and square chiral_a 45° 57.9%.
Figure 45: Porosity and effective porosity before (0 N, blue) and after (3 N, yellow) loading for the 45° models. The porosity increased for only triangle 45° and chiral hexagon 45° while all other 45° models experienced a decrease in porosity. Additionally, the effective porosity was only increased for triangle 45° and decreased for all other 45° models.
3.3.1.3 90° Models Finite Element Analysis

Simulated uniaxial tensile tests were conducted on five auxetic 90° models (bowtie 90°, triangle 90°, chiral hexagon 90°, hexagon_b 90°, and square chiral_b 90°) in order to assess the behavior of these models in response to 3 N of force. Qualitatively assessing the deformation of the pores, the pores of the bowtie 90° and triangle 90° models expanded (Figure 46). The circles of the chiral hexagon 90° and square chiral_b 90° models appeared to elongate and contract whereas the polygons in these models (triangles for the chiral hexagon 90° and quadrilaterals for the square chiral_b 90°) expanded. For hexagon_b 90°, the pores also appeared to elongate and contract. Overall, model expansion was clearly evident for bowtie 90° and triangle 90°.
Figure 46: Finite element results of the auxetic 90° models at 0 N and 3 N. Overall, the amount of elongation differs for each model. The pores of all of the 90° models remained open in response to 3 N of force. Model expansion was visibly apparent for the bowtie 90° (B) and triangle 90° (T) models. CH = Chiral Hexagon, Hb = Hexagon_b, Square Chiral_b = SCb.

Following the application of 3 N of force, a decrease in the minimal pore diameter, less than 1 mm, was observed for the chiral hexagon 90° (15% decrease) and square chiral_b 90° (34% decrease) models (Figure 47). For the remaining three 90° models, the minimal pore diameter increased, increasing by 19.0% for triangle 90°, 45.2% for hexagon_b 90°, and 260% for bowtie 90°, with all diameters being greater than 1 mm.
Figure 47: Minimal pore diameter of the 90° models at 0 N (blue) and 3 N (orange). The minimal pore diameter was greater than 1 mm for the bowtie 90°, triangle 90°, and hexagon_b 90° models, and less than 1 mm for the chiral hexagon 90° and square chiral_b 90° models.

Similar to the results of the minimal pore diameter, the effective pore area also decreased for the chiral hexagon 90° (9.0% decrease) and square chiral_b 90° (12.0% decrease) models (Figure 48). However, the effective pore area was maintained at 100% for the bowtie 90°, triangle 90°, and hexagon_b 90° models.
Figure 48: Effective pore area for the 90° models in response to 3 N of force. Initially, the effective pore area was 100% for all models (0 N, red). However, the effective pore area decreased for the chiral hexagon 90° and square chiral_b 90° models. The effective pore area was maintained at 100% for all other models.

Analyzing the overall expansion (or lack thereof) of the 90° models, bowtie 90°, triangle 90°, and square chiral_b 90° all expanded with loading, although at 3 N the relative lateral contraction for square chiral_b 90° was barely negative with a value of -0.01 (Figure 49). Initially the relative lateral contraction for chiral hexagon 90° was negative signifying model expansion. However, at 2.4 N the model contracted resulting in a positive relative lateral contraction. Hexagon_b 90° was the only model that contracted throughout loading. Overall, bowtie 90° expanded the most followed by triangle 90°.
Figure 49: Change in relative lateral contraction with loading for the 90° models. Initially (0.6 N and 1.5 N), the relative lateral contraction was negative for all models excluding hexagon_b 90°. At 2.4 N, the relative lateral contraction for chiral hexagon 90° was positive. Of the five models, bowtie 90° expanded the most followed by triangle 90°. A positive value indicates model contraction and a negative value model expansion.

Overall, the porosity of all of the 90° models increased with bowtie 90° increasing the most, by 27.1%, and square chiral_b 90° increasing the least, by only 8.1% (Figure 50, top). The effective porosity increased for all 90° models with the exception of square chiral_b 90° (Figure 50, bottom). Again, the effective porosity increased the most for bowtie 90° (27.1% increase) whereas chiral hexagon 90° increased the least (1.7% increase). In terms of the relative elongation, bowtie 90° elongated the most (59.1%), followed by hexagon_b (37.4%), square chiral_b 90° (33.2%), chiral hexagon 90° (23.9%), and triangle 90° (21.0%).
Figure 50: Porosity and effective porosity before (0 N, blue) and after (3 N, yellow) loading for 90° models. The porosity increased for all 90° models while the effective porosity increased for all models with the exception of square chiral_b 90°.
3.4 DISCUSSION

In this study, a computational modeling approach was utilized to assess the behavior (i.e. pore deformation and the overall expansion or contraction) of synthetic mesh models with auxetic pore geometries in response to 3 N of force. This behavior was evaluated with the pore geometries rotated 0°, 45°, and 90° with respect to the longitudinal axis of the models. For comparison, the behavior of models with standard pore geometries was also analyzed. As anticipated, the pores of the models with standard geometries contracted or collapsed with loading and this result is analogous to *ex vivo* testing of commercial synthetic mesh products with similar pore geometries [77]. Congruent with our hypothesis, models with auxetic pore geometries prevented the pores of the model from collapsing when the pores were rotated 0° and 90°. However, this was not the case for all models when the pores were rotated 45°. A 45° rotation of the pores resulted in a decrease in pore size and model contraction for all auxetic models. This result is not surprising given that a 45° rotation of the pores aligned the 45° angle of most pore geometries with the axis of loading which favors pore collapse. Additionally, one important caveat to note is that using auxetic geometries as pore does not guarantee that the pores will remain open and that the model as a whole will expand indefinitely. This was observed with some models in which the relative lateral contraction of the model was negative initially (for example at 0.6 N) but by 3 N the relative lateral contraction was positive signifying model contraction. Collectively, these results demonstrate that the auxetic behavior is not maintained for all auxetic geometries with loading and that this behavior is directionally and load dependent. Thus, when designing a mesh for prolapse repair with auxetic pore geometries it is important that the geometry of the pores be orientated in such a way that the auxetic behavior will be maximized.
Analyzing the behavior of the models with auxetic pore geometries, the question of which auxetic geometry is best for abdominal sacrocolpopexy meshes arises. To address this question, the 0° mesh models were assessed using the model assessment criteria outlined in Section 3.2.3. At first glance, the square grid geometry may appear to be the optimal choice for a mesh pore given the significant expansion of the pores with this geometry. This expansion was quantitatively confirmed; the size of the pores, the porosity, and the effective porosity all increased the most for the square grid relative to the other auxetic geometries. However, this expansion of the pores was at the expense of significant pore deformation. Specifically, the pores underwent a large degree of rotation in order to expand. This degree of pore rotation could potentially pose a problem clinically when the mesh is integrated with tissue, causing tissue damage related to micro-motion of the mesh fibers. Additionally, the square grid model deformed the most overall (more than doubling in length), which would necessitate the use of more material or a stiffer material to achieve the smaller deformed lengths of the other meshes. Based on the large degree of rotation and model elongation, it was determined that the square grid geometry would not be appropriate for abdominal sacrocolpopexy mesh pores. The chiral auxetic shapes (chiral hexagon, square chiral_a, and square chiral_b) also had drawbacks largely due to contraction of its circles with loading causing the minimal diameter of the pores to be less than 1 mm, ultimately decreasing the effective porosity and effective pore area. This may be overcome by increasing the initial diameter of the circles; however, the circles will still contract and this is likely to negatively impact elongation of this design. Analysis of the four remaining auxetic geometries (bowtie, spiral, triangle, and hexagon_b) showed that the relative lateral contraction was negative for all models with the exception of the triangle, which was positive but then became negative with additional loading, signifying model contraction. Of the 3 remaining, the bowtie stood out as the most favorable
geometry due to its increasing porosity and greater effective porosity with loading. Compared to the spiral and hexagon_b models, the bowtie model deformed the least while concomitantly having the greatest increase in the porosity and effective porosity. Additionally, the relative lateral contraction was the most negative for the bowtie model compared to all other models in this study. Thus, the bowtie configuration shows the most potential for uniaxial applications and likely warrants focus of additional investigations. It is important to note however that the bowtie auxetic behavior, and that for all other geometries analyzed in this study, is specific to the direction in which it was loaded. Indeed, rotation of the bowtie pores by 45° with respect to the loading axis results in destabilization of the pores with pore collapse and a decrease in the effective porosity. Thus, this design may be best suited for uniaxial applications, i.e. sacrocolpopexy repairs.

The benefits of an auxetic geometry can be somewhat masked when compared to the standard pore geometries, particularly the square pores. In fact, from a modeling perspective, it can be argued that the square geometry performed just as well as the best auxetic geometry, the bowtie. The pores of the square model remained open, the square model deformed the least overall, and the minimal diameter of the pores was greater than 1 mm following the applied load of 3 N. Additionally, the effective pore area was maintained at 100% and the porosity and effective porosity increased. However, the relative lateral contraction of the square model was positive implying that the model contracted. Not only did the model as a whole contract, but the individual pores contracted as well. The contractile nature of the square pore geometry restricts the dimensions of the pore to be great enough such that contraction of the pore does not result in a dimension that is less than 1 mm when loaded or deformed. Outside of the results in this study, one may also argue based on in vivo studies by Feola et al (2013) and Liang et al (2013) that a square pore mesh performs better in terms of its impact on vaginal smooth muscle function and
morphology compared to other common shapes for prolapse meshes [129,131]. However, it is important to understand that not only were the pore shapes of the meshes in the previously mentioned studies different, but so were the structural stiffness and weight of each mesh, two factors that impact the host response to mesh (see Sections 1.4.4 and 1.4.5). It therefore cannot be definitive stated that the square pore shape is preferred over the other pore shapes. Nevertheless, with pore size being a key factor in mesh biocompatibility, the ability of auxetic pore geometries to expand provides an added benefit that standard pore geometries cannot.

The way in which the models were designed and evaluated is a major strength of this study. All models were designed such that the aspect ratio (length to width), fiber width, and the amount of material (the volume) were the same. Additionally, for any models containing circles and/or acute angles, the diameter of the circles and the degree of the acute angulation were the same. In terms of model evaluation (i.e. uniaxial tensile testing via FEA), the analysis and aspect ratio of all models as well as the material model used was consistent. By designing and testing the models in this way, the impact of the pore geometry on the overall behavior of the model could be evaluated and compared as the dependent variable. However, FEA is limiting in that the results obtained are theoretical predictions and they must be validated. Thus, future studies involving the manufacturing and *ex vivo* mechanical characterization of the models in this study are needed to verify that these findings are absolute. Additionally, it is important for the reader to keep in mind that the dimensions of the geometries have not been optimized and that the results obtained are specific to the dimensions of the pore geometries and model parameters (e.g. stiffness) analyzed in this study. Changing these parameters (e.g. increasing and decreasing angles, thickness, stiffness, etc.) will produce numerical values, including the Poisson’s ratio, that are different from the ones reported in this study [180-182]. For example, Whitty et al (2002) determined that
decreasing the thickness of the vertical ribs (i.e. vertical or horizontal fibers of the bowtie geometry in this study) while keeping the thickness of the diagonal ribs (i.e. the diagonal fibers of the bowtie geometry in this study) constant reduces the magnitude of Poisson’s ratio [181]. The opposite affect was observed when the thickness of the diagonal ribs is reduced; the Poisson’s ratio increases with decreasing diagonal rib thickness. Future studies will therefore aim to optimize the auxetic behavior of the bowtie geometry per the criteria described above for urogynecologic mesh via parametric analysis. Although the bowtie geometry was identified as the best geometry for uniaxial applications in this study, future optimization studies with other geometries, for example the hexagon_b geometry which was a close second to the bowtie geometry, may be worth pursuing given that changes in the dimensions can impact the Poisson’s ratio. Similar to dimensional changes, preliminary, parametric studies conducted in our lab determined that changing the material properties, for example, the material stiffness of the model, also affects pore deformation (including pore expansion) and model elongation. Specifically, decreasing the Young’s modulus of the Neo-Hookean material resulted in increasing model elongation and pore deformation. With this in mind, it would be interesting to see how sensitive relative lateral contraction is to changes in material properties and to what extent does changing these properties affect when the model, in the case of FEA, goes from expanding to contracting. One obvious solution to preventing this transition from expanding to contracting is to make the material stiffer. Although this solution may work, one of the design criteria for new synthetic meshes is that the material stiffness of the mesh be similar to vaginal tissue. Thus, there is a limit to material stiffness that one must keep in mind. Additionally, it is also important to note that the Neo-Hookean material model and properties used in this study were derived from the load-elongation behavior of a polypropylene mesh; however, this material model will likely not be able to accurately capture the deformation of the pores and
model overall of mesh manufactured from an elastomeric material, which is not as stiff as polypropylene. Thus, it is important that the material model and properties used to define the finite element models intended for FEA studies reflect the material from which the actual mesh will be manufactured in order to obtain an accurate representation of the actual mesh pore deformation and mesh elongation and expansion \textit{ex vivo}. Another limitation of this study is that the auxetic behavior of the models was assessed for one loading (3 N applied along the longitudinal axis of the model) and boundary condition (both the entire top and bottom edges of the models were constrained). \textit{In vivo} surgeons use sutures to attach mesh to the vagina and other anchoring structures. These suture attachments create point loads, which result in non-uniform loading and deformation of mesh. Barone et al (2015) demonstrated that increasing the number of point loads applied to commercial synthetic meshes subjected to tensile loading resulted in a significant increase in out-of-plane deformation, observed as mesh wrinkling/bunching (also referred to as folding) \cite{75}. Clinically, mesh wrinkling/bunching is observed and these areas where mesh wrinkling/bunching has occurred are identified as problematic areas for patients \cite{82-85}. In addition to expansion, auxetic materials and structures also have the added benefit of demonstrating synclastic or double curvature (i.e. they become dome shaped) in response to out-of-plane bending compared to non-auxetic materials and structures which display anticlastic curvature (i.e. they become saddle shaped) \cite{183,184}. This property may be beneficial in terms of reducing the amount of out-of-plane deformation that synthetic meshes experience in response to point loading. Specifically, it is hypothesized that an opposite result will be observed from Barone et al (2015). The amount of out-of-plane deformation with increasing point loads will be minimal for meshes containing auxetic pores relative to meshes with non-auxetic pores. Future studies using FEA and \textit{ex vivo} mechanical testing of models (FEA) and meshes (mechanical testing) with
auxetic pores subjected to tensile loading with various boundary conditions (i.e. point loads) will be able to address this hypothesis. In addition to applying point loads, loading the models in this study from multiple directions simultaneously would also help to determine which auxetic geometry (or geometries) may be most appropriate for transvaginal meshes, the loading conditions for transvaginal meshes is multi-directional. It would particularly be interesting to see how well the chiral hexagon model will respond to multi-directional loading given that this geometry was the most consistent in terms of pore deformation and model elongation and expansion with changes in pore orientation. Lastly, contact was not applied in the FEA performed in this study; however, this was only problematic for the bowtie 45° model as fibers of this FE model could be seen penetrating through other fibers which is not realistic. Despite this limitation, the overall result of pores collapsing and model contracting with the bowtie 45° model would still remain the same.

As a final note, the term “mesh” is typically used to describe a textile that is knitted or woven. The models evaluated in this study are more appropriately described as mesh analogues since behaviors of knots and other factors (knit/weave patterns, etc.) were not simulated.

The benefit of expanding pores is not just exclusive to the urogynecologic field but other fields within the biomedical community can also benefit from auxetics. For example, the models developed in this study can be used to develop meshes for abdominal hernia repairs, which like prolapse meshes are experiencing complications [185,186]. Additionally, the concept of an auxetic can also be beneficial to the tissue engineering and drug delivery fields. For example, instead of using the auxetic geometries to construct meshes for structural support, they can be used to construct scaffolds that delivery a therapeutic drug in response to a specific stimulus. These ideas will be further explored in Chapter 6.
3.5 CONCLUSION

Overall this work provides an initial proof of concept that constructing meshes with auxetic pore geometries can prevent pore collapse and mesh contraction. However, not all auxetic geometries can produce such behavior indefinitely and under all conditions, i.e. the ability of the auxetic structure to expand is directionally and load dependent. Understanding these limitations will aid in the design of synthetic meshes with auxetic pore geometries that have stable pores, which have the potential to expand. Based on previous research highlighting the importance of pore size, this is likely to afford better ingrowth of host tissue into the pores, host integration of the mesh, and also decrease the likelihood of bridging fibrosis. Successfully designing an auxetic mesh as described, may significantly reduce the occurrence of major mesh related complications.
4.0 MANUFACTURING AND CHARACTERIZATION OF AUXETIC MESHES

4.1 OVERVIEW

In the previous chapter (Chapter 3), the concept that auxetic geometries can be used as pore shapes for prolapse mesh to prevent pore collapse was demonstrated using finite element analysis (FEA). However, the expanding behavior of auxetically designed pores was found to be directionally and load dependent. For example, an auxetic pore may expand when oriented in one direction and collapse (non-auxetic behavior) when oriented in another. This particular behavior was demonstrated for the majority of the auxetic pores when they were rotated 45° with respect to the axis of loading. Similarly, pore expansion was also load dependent. For some auxetic pore models, the pores expanded at 0.6 N and remained expanded throughout loading whereas for other models the pores may have initially expanded at 0.6 N and by 3 N, the pores had collapsed. From the FEA, we were also able to gain a better understanding of the auxetic geometry (or geometries) that would be most appropriate for prolapse meshes. Collectively, this information will be used to guide the design of an innovative mesh for pelvic organ prolapse repair. However, given that the above information was based on FEA, which is merely a prediction, the next step in the design process is to confirm the behavior of meshes designed with auxetic pores. This can be accomplished through manufacturing and experimentally testing auxetic meshes, which will be the focus of this Chapter.
To recall, there were four main design criteria established in Chapter 1 for novel synthetic meshes in this dissertation.

1) The pores of the mesh will remain unchanged or expand in response to tension and the porosity of the mesh will at least be maintained and/or increased with tensioning.

2) Synthetic meshes will have a material stiffness that is similar to vaginal tissue.

3) Synthetic meshes will be able to withstand *in vivo* loads and deform without undergoing permanent deformation in response to these loads (both static and repetitive).

4) Synthetic meshes will minimize mesh burden resulting in a minimal foreign body response.

In terms of manufacturing auxetic meshes, one of the first steps is to identify a material that will satisfy criteria 2. Once this material is identified, meshes can then be manufactured and experimentally tested using criteria 1, 3, and 4 as a guide. The first part of this Chapter will focus on the identification of a material while the second part will be devoted to discussing the manufacturing and *ex vivo* characterization of meshes with auxetic pores via mechanical testing. Finally, this chapter will conclude with an overall summary of the results.
4.2 CHARACTERIZATION OF THE UNIAXIAL MECHANICAL PROPERTIES OF PDMS

4.2.1 Introduction

The majority of current synthetic meshes used in the surgical repair of pelvic organ prolapse (prolapse) are composed mainly of polypropylene. Polypropylene was chosen as the material for these meshes not because research was done to establish polypropylene as the optimal material, but rather to take advantage of the 510k process. Prolapse meshes are simply hernia meshes remarke ted for the indication of prolapse repair, meaning no rigorous scientific testing or research was done (or required with the 510k process) to design meshes specifically for the tissues and loading conditions within the female pelvis. Since polypropylene was approved for hernia meshes by the FDA, it was grandfathered in as the material of choice for prolapse meshes. This in turn made polypropylene a convenient material for prolapse meshes, but it is not necessarily the most ideal.

The material stiffness of a single fiber of polypropylene mesh is orders of magnitude stiffer than that of vaginal tissue, 1.5 GPa - 2.0 GPa versus 6.0 MPa – 14 MPa, respectively [142,143]. In order to reduce the structural stiffness of a device made with this material, polypropylene fibers are knitted together. Despite knitting, the structural stiffness of prolapse meshes overall remains high. Specifically, the uniaxial stiffness of prolapse meshes is reported as low as 0.009 N/mm and as high as 1.66 N/mm for a sample the size of 15 cm x 5 cm or 90 mm x 15 mm [70,71]. Structural stiffness is a critical parameter given that a mesh that is not stiff enough can lead to recurrent prolapse and one that is too stiff can lead to a phenomenon referred to as stress shielding in which the stiffer material bears the majority of the load shielding the less stiff surrounding tissues.
This can result in a maladaptive remodeling response that is characterized by degeneration and atrophy. In a study conducted by Liang et al (2013), stress shielding was shown to be a potential concern for synthetic meshes. Thinning of the vaginal smooth muscle layer, increased apoptosis around mesh fibers, and decreases in collagen and elastin content in the vagina were all negative responses to synthetic mesh with the greatest negative response being observed with the stiffest mesh [131]. In addition to stress shielding, the local mechanical environment can also lead to micro-injury at the device-tissue interface and to changes in the cellular response. For example, shear stresses that develop at the mesh-tissue interface causes micro-trauma to the tissue surrounding percutaneous implants, which ultimately results in chronic wounding at the implant site [175,176]. Using a computational modeling approach, Subbaroyan et al (2005) showed that probes produced from softer substrates reduced the strain at the probe-tissue interface relative to probes produced from stiffer substrates [187]. This result is believed to translate clinically to a reduction in the inflammatory response due to motion-damaged tissue; however, more research is needed to substantiate this claim [188]. The material stiffness of an implant also impacts the cellular response. Studies conducted by Blakney et al (2012) and Irwin et al (2012) revealed that stiffer substrates are associated with increased macrophage activation and decreased macrophage adhesion relative to softer substrates [189,190]. Collectively these studies demonstrate the importance of matching the mechanical properties of an implant to that of the tissue in which the device will be implanted. It is therefore critical that newly designed meshes must have a material stiffness that is comparable to the vagina. It is also important that prolapse meshes be able to withstand changes in intra-abdominal pressures, both sudden and repetitive without permanently deforming. Current synthetic meshes do not have this ability; in fact, they permanently deform in response to loading and this occurs at loads that are well within the
physiologic range, 0.5 N to 15 N [70,71]. Permanent deformation of mesh can lead to prolapse reoccurrence, and this is particularly concerning prior to tissue incorporation within the pores of the mesh. It is doing this time that prolapse mesh is at an increased risk for permanently elongating.

Manufacturing meshes from an elastomeric material will overcome the issues of material stiffness mismatch and permanent deformation as described in the previous two paragraphs. Elastomeric materials are advantageous for prolapse meshes since the material stiffness of elastomers can be tuned to that of the vagina. Additionally, vaginal tissue like many tissues within the body, contain elastin, which gives these tissues the ability to recoil back to their original shape when stretched or loaded (as long as stretching or loading does not exceed the elastic limit which will induce permanent deformation/damage or injury). Given that synthetic meshes will be in direct contact with the vagina, it is important that the mesh not hinder the ability of the vagina to be able to deform and recoil back to its original shape, hence prolapse meshes should also have this ability. Constructing meshes from an elastomeric material, which has shape memory, would allow a mesh to return to its original configuration in response to sudden and repetitive changes in force.

To manufacture our initial auxetic mesh prototypes, we wanted to use an elastomer that is biocompatible, relatively inexpensive, easy to manufacture, and one that has mechanical properties which can be tuned to that of the vagina. Polydimethylsiloxane, PDMS, is one such elastomer that meets the criteria outlined above. It is a biocompatible elastomer that can be manufactured easily (by combining a base and curing agent) and is relatively inexpensive [191,192]. Additionally, it has a material stiffness that is approximately on the same order of magnitude as vaginal tissue (PDMS stiffness = 0.7 MPa – 1.5 MPa versus vaginal stiffness = 6 MPa – 14 MPa) [142,143,193,194]. However, the stiffness of PDMS can be altered during the manufacturing
process, which allows for greater control to tune the mechanical properties of PDMS to that of vaginal tissue. For example, studies have shown that the mixing ratio, base to curing agent, can impact the mechanical properties and behavior of PDMS [195, 196]. Studies have also shown that PDMS cured at high temperatures results in a lower ultimate tensile stress relative to PDMS cured at a lower temperature. Additionally, at low temperatures, the mechanical properties of PDMS are reported to be independent of heating time [193]. However, this is not the case for thickness. The ultimate tensile stress and Young’s Modulus of PDMS membranes were found to be dependent on the thickness of the membrane; thinner members resulted in a higher ultimate tensile stress and Young’s Modulus [194]. With the variability in the mechanical properties that can be achieved by altering the manufacturing of PDMS, it was first important to explore the mechanical properties of PDMS for applications in synthetic mesh for prolapse repair. However, it is important to note that, PDMS is brittle and therefore it is likely not optimal for humans; but, it was a good starting material for the purposes of this dissertation. Thus, the objective of this study was to determine the mixing ratio (base to curing agent) and thickness of PDMS that will yield a material stiffness that is similar to vaginal tissue. To accomplish our objective, PDMS dog-bone shaped samples, manufactured with a mixing ratio of 10:1 and 5:1 and thickness of 1.0 mm, 1.5 mm, and 2.0 mm were created. We hypothesized that the 1.0 mm thickness and 5:1 mixing ratio will yield a material stiffness that matches vaginal tissue.

4.2.2 Methods

4.2.2.1 Creation of PDMS Dog Bones

PDMS dog bones were manufactured from 184 Silicone Elastomer Kit (Dow Corning, Midland, MI), which contains a base and curing agent, using a mold-fill process. First, a dog bone
mold was created using SolidWorks 2013 x64 Edition (Dassault Systèmes SOLIDWORKS Corporation, Waltham, Massachusetts). The dimensions of the dog bone shape were based on those of the ASTM D412 test standard for vulcanized rubber and thermoplastic elastomers - tension with a gauge length of 33.0 mm, width of 3.0 mm, and thickness of 1.0 mm, 1.5 mm, or 2.0 mm. Next, the mold was cut from aluminum by the Machine Shop at the Swanson Center for Product Innovation, University of Pittsburgh. In total, the mold contained three different dog bones, all having the same gauge length and width but differing thicknesses (either 1.0 mm, 1.5 mm, or 2.0 mm) (Figure 51).

![Dog bone mold with three different thicknesses](image)

*Figure 51: Top view of dog-bone mold with three different thicknesses, from left to right: 1.0 mm, 1.5 mm, and 2.0 mm.*

The base and curing agent were combined in a 10:1 ratio (manufacturer’s recommended ratio) and 5:1 ratio by weight. The mixture was then manually mixed for 10 minutes and degassed.
in a vacuum oven at approximately 74 cmHg for 20 minutes in order to remove any air bubbles that were created during mixing. Following degassing, PDMS was carefully poured into the molds being cautious not to introduce new air bubbles. The mold with PDMS mixture was then placed in an oven and the PDMS mixture was heat cured for approximately 30 minutes at 90°C. At the conclusion of curing, the PDMS dog bones were carefully removed from the mold. See Figure 52 for a schematic of this process.
Sylgard 184 Silicone Elastomer (Dow Corning)

10:1 & 5:1 (Base:Curing Agent)

10 minute mix

Degas for 20 minutes

Pour PDMS onto molds

Heat cure for 30 minutes at 90°C

Remove PDMS dog bone from mold

PDMS dog bone removed from mold

Figure 52: Schematic of manufacturing process for PDMS dog bones.
4.2.2.2 Experimental Testing

To obtain the mechanical properties of PDMS, uniaxial load to failure tests were performed on the PDMS dog bones created in the previous section. In total 30 dog bones were tested with the following mixing ratios and thicknesses: 10:1 and 1.0 mm (n=5), 10:1 and 1.5 mm (n=5), 10:1 and 2.0 mm (n=5), 5:1 and 1.0 mm (n=5), 5:1 and 1.5 mm (n=5), and 5:1 and 2.0 mm (n=5). Prior to mechanical testing, two contrast markers were placed in the narrow region of the dog bones. A Cannon Rebel EOS T3 camera was used to track the markers throughout testing and a custom Mathematica (V9; Wolfram, Champaign, IL, USA) script was used to calculate strain. Custom clamps were applied to the opposing ends of the dog bone. One end of the clamped dog bone was attached to the base of the Instron™ 4502 (Instron, Norwood, MA, USA) while the other was attached to the crosshead of the testing machine in series with a 50-lb load cell (Model 31; Honeywell, Morristown, NJ, USA), with a resolution of 0.1 N. Dog bones were then submerged in a 37°C water bath, in a slack position, and allowed to equilibrate for 10 minutes. Following the equilibrium period, a preload of 0.1 N was applied at a rate of 10 mm/min in order to establish an initial clamp-to-clamp distance and an initial inter-marker distance. Dog bones were then loaded to failure at a rate of 50 mm/min. The resulting load and elongation were recorded and used to generate a load-elongation curve. This curve was then used to calculate a stress-strain curve. Stress was calculated by dividing the measured force by the initial cross-sectional area and strain was based on the change in the inter-marker distance divided by the initial inter-marker distance. From the stress-strain curves, the Young’s modulus, tensile strength, ultimate strain, and strain energy density were calculated.
4.2.2.3 Statistics

Based on the ASTM D412 test standard for vulcanized rubber and thermoplastic elastomers – tension, five dog-bones per group were created. Kolmogorov-Smirnov tests determined that the data was normally distributed. The mechanical properties were compared using two-way independent MANOVAs, for ratio and thickness, followed by univariate ANOVAs with a Bonferroni correction (p-value < .0125 for significance) for Young’s modulus, tensile strength, ultimate strain, and strain energy density. Additionally, Independent Samples T-Tests or Mann-Whitney U Tests with a Bonferroni correction (p-value < .017 for significance) were used to compare the differences in the mechanical properties with respect to the mixing ratio. Statistical analysis was performed using SPSS 24 (IBM, Armonk, NY, USA).

4.2.3 Results

Loading the PDMS dog bones to failure resulted in similar stress-strain curves for all dog bones tested irrespective of the mixing ratio and thickness (Figure 53). Specifically, all stress-strain curves were roughly bilinear with the majority of the curves overlapping throughout loading, deviating slightly in the second linear region prior to failure. The first linear region of the 10:1 dog bones was longer than that of the 5:1 ratio while the slope of the 5:1 ratio curves appeared steeper. All failures occurred between the strain markers allowing for the tensile strength, ultimate strain, and strain energy density to be reported along with the Young’s modulus.
Figure 53: Average stress-strain curves from PDMS dog-bone samples uniaxially loaded to failure.

Overall, there was not much difference in the stress-strain curves for each ratio. The initial linear region of the 10:1 ratio samples was longer than that of the 5:1 ratio samples whereas the slopes of the 5:1 ratio samples appeared steeper than that of the 10:1 ratio samples.

Assessing the 10:1 ratio PDMS samples, very little difference was observed in the mechanical properties between the 1.0 mm, 1.5 mm, and 2.0 mm thicknesses (Figure 54). Specifically, the difference in the highest and lowest values for the Young’s modulus, tensile strength, and ultimate strain was less than 12% and for the strain energy density this difference was approximately 22%. A similar result was observed with the mechanical properties for the 5:1 ratio samples (Figure 54). The difference between the highest and lowest values was the greatest for the strain energy density at 18% and lowest for the ultimate strain at 8%. Given the similarities in the mechanical properties across thicknesses, it was not surprising that thickness did not significantly impact the mechanical properties of PDMS, $V = 0.377$, $F(8,44) = 1.277$, p-value = .280 (Pillai’s Trace). Similar results were obtained with univariate ANOVAs assessing the mechanical properties individually. The Young’s modulus $F(2,24) = 1.628$, p-value = .217, tensile
strength $F(2,24) = 1.371$, p-value = .273, ultimate strain $F(2,24) = 1.236$, p-value = .308, and strain energy density $F(2,24) = 1.406$, p-value = .265 all were not significantly different for thickness. See Table 6 for a summary of these results.

Unlike thickness, significant differences in the mechanical properties were observed based on ratio, $V = 0.743$, $F(4,21) = 15.192$, p-value < .001 (Pillai’s Trace). However, the results from univariate ANOVAs revealed that significant differences in the mechanical properties were only observed for the Young’s modulus, $F(1,24) = 40.973$, p-value < .001, and ultimate strain, $F(1,24) = 17.535$, p-value < .001. In general, the Young’s modulus was higher for the 5:1 ratio samples compared to the 10:1 ratio samples, with a significant difference observed between the 1.0 mm samples. Specifically, the Young’s modulus for the 5:1 ratio 1.0 mm samples was 41.6% higher than that of the 10:1 ratio 1.0 mm samples, p-value < .001. For the remaining thicknesses, the Young’s modulus was 18.5% (for 1.5 mm samples) and 27.3% (for 2.0 mm samples) higher than that of the respective 10:1 ratio samples. Overall, the ultimate strain for the 10:1 ratio samples was higher than that of the 5:1 ratio samples, with a significant difference observed between the 1.0 mm samples. Specifically, the ultimate strain for the 10:1 ratio 1.0 mm samples was 26.7% higher than that of the 5:1 ratio samples, p-value < .001. Similarly, the ultimate strain was 15.7% (for 1.5 mm samples) and 12.7% higher for the 10:1 ratio samples relative to the respective 5:1 ratio samples. Lastly, univariate ANOVAs analyzing the effect of ratio revealed that the tensile strength was not significantly different, $F(1,24) = 0.300$, p-value = .589, nor was the strain energy density, $F(1,24) = 1.261$, p-value = .273. See Table 9 for a summary of these results.
Figure 54: Uniaxial mechanical properties of PDMS following a load to failure test. Overall, the mechanical properties of PDMS were similar for the 1.0 mm, 1.5 mm, and 2.0 mm samples. However, the Young’s modulus was significantly higher for the 5:1 ratio 1.0 mm samples relative to the 10:1 ratio samples. Additionally, the 10:1 ratio 1.0 mm samples deformed significantly more than that of the 5:1 ratio 1.0 mm samples.
Table 9: Uniaxial mechanical properties of PDMS. Data represented as mean ± standard deviation. P-values obtained from Two-way Independent ANOVA\textsuperscript{a} and Independent Samples T-Tests\textsuperscript{b} with Bonferroni corrections.

<table>
<thead>
<tr>
<th></th>
<th>Young’s Modulus (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Ultimate Strain (mm/mm)</th>
<th>Strain Energy Density (MPa)</th>
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</thead>
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<tr>
<td>10:1 Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mm</td>
<td>6.99 ± 0.57</td>
<td>4.79 ± 0.55</td>
<td>1.33 ± 0.10</td>
<td>2.24 ± 0.43</td>
</tr>
<tr>
<td>1.5 mm</td>
<td>7.40 ± 0.85</td>
<td>4.37 ± 0.75</td>
<td>1.18 ± 0.13</td>
<td>1.80 ± 0.45</td>
</tr>
<tr>
<td>2.0 mm</td>
<td>6.81 ± 0.64</td>
<td>4.60 ± 0.43</td>
<td>1.24 ± 0.06</td>
<td>2.04 ± 0.29</td>
</tr>
<tr>
<td>5:1 Ratio</td>
<td></td>
<td></td>
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<tr>
<td>1.0 mm</td>
<td>9.90 ± 0.37</td>
<td>5.18 ± 0.84</td>
<td>1.05 ± 0.10</td>
<td>1.92 ± 0.60</td>
</tr>
<tr>
<td>1.5 mm</td>
<td>8.77 ± 0.90</td>
<td>4.42 ± 1.13</td>
<td>1.02 ± 0.17</td>
<td>1.60 ± 0.61</td>
</tr>
<tr>
<td>2.0 mm</td>
<td>8.67 ± 1.49</td>
<td>4.64 ± 0.94</td>
<td>1.10 ± 0.16</td>
<td>1.92 ± 0.68</td>
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<tr>
<td>Effect of Thickness</td>
<td></td>
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<tr>
<td></td>
<td>.217\textsuperscript{a}</td>
<td>.273\textsuperscript{a}</td>
<td>.308\textsuperscript{a}</td>
<td>.265\textsuperscript{a}</td>
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<tr>
<td>Effect of Ratio</td>
<td></td>
<td></td>
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<tr>
<td>10:1 vs 5:1</td>
<td>&lt; .001\textsuperscript{a}</td>
<td>.589\textsuperscript{a}</td>
<td>&lt; .001\textsuperscript{a}</td>
<td>.273\textsuperscript{a}</td>
</tr>
<tr>
<td>1.0 mm</td>
<td>&lt; .001\textsuperscript{b}</td>
<td>N/A</td>
<td>.002\textsuperscript{b}</td>
<td>N/A</td>
</tr>
<tr>
<td>1.5 mm</td>
<td>.038\textsuperscript{b}</td>
<td>N/A</td>
<td>.136\textsuperscript{b}</td>
<td>N/A</td>
</tr>
<tr>
<td>2.0 mm</td>
<td>.239\textsuperscript{b}</td>
<td>N/A</td>
<td>.175\textsuperscript{c}</td>
<td>N/A</td>
</tr>
</tbody>
</table>

4.2.4 Discussion

The primary objective of this study was to determine the mixing ratio and thickness of PDMS that will yield a material stiffness that is similar to vaginal tissue. To accomplish this objective, the uniaxial mechanical properties of PDMS, dog-bone shaped samples manufactured from two different mixing ratios (10:1 and 5:1) and three different thicknesses (1.0 mm, 1.5 mm, 2.0 mm)
were explored. Congruent with our hypothesis, PDMS manufactured at a 5:1 mixing ratio and 1.0 mm thickness yielded a material stiffness that is on the same order of magnitude as vaginal tissue, 9.90 MPa (PDMS) versus 6 MPa – 14 MPa (vaginal tissue).

Secondary to our primary objective, the results of this study also revealed that mixing ratio significantly impacts the mechanical properties of PDMS. Overall, the 5:1 ratio samples were stiffer and deformed less compared to the 10:1 ratio samples. This result was anticipated given that increasing the amount of curing agent increases the number of cross-links, essentially stiffening the PDMS. Unlike ratio, thickness did not impact the mechanical properties of PDMS. This result is opposite to that found by Liu et al 2009, in which the Young’s modulus and tensile strength (referred to as ultimate tensile or mechanical strength in the paper) were shown to be thickness dependent [194]. In our study, the thickness of PDMS samples ranged from 1.0 mm – 2.0 mm, whereas in the Liu et al 2009 study, the thickness ranged from 0.030 mm – 0.350 mm. It is possible that at higher thicknesses, the mechanical properties are a reflection of the bulk material and independent of thickness relative to thinner samples. This indeed was the case for the thicker PDMS samples tested in the Liu et al 2009 study. The stress-strain curves were similar for the thicker samples and decreasing the thickness, below 0.020 mm, resulted in thickness dependent mechanical properties.

In addition to having a similar material stiffness as vaginal tissue, the tensile strength of PDMS was comparable to animal and human vaginal tissue. The tensile strength of PDMS in this study ranged from 4.37 MPa to 5.18 MPa. These values are slightly higher than animal studies which report the tensile strength of vaginal tissue to range between 2.1 MPa to 4.4 MPa for the nulliparous vagina and between 0.96 MPa and 3.1 MPa for the parous vagina [197]. Few human studies have reported the tensile strength of vaginal tissue. However, Rubod et al (2008), report
that the tensile strength of human vaginal tissue from a patient without prolapse is approximately 1.48 MPa and for a patient with prolapse it is roughly 3.82 MPa [198]. Different from vaginal tissue, the ultimate strain of PDMS is an order of magnitude greater than that of both human and animal vaginal tissue, 102% - 133% (PDMS), 14% - 32% (animal), and 19% - 46% (human) [197,198]. Similarly, the strain energy density is one to two orders of magnitude greater than animal vaginal tissue, 1.60 MPa – 2.24 MPa (PDMS) versus 0.07 MPa – 0.25 MPa [197]. Strain energy density is not reported for humans in the literature. These data suggest that the material itself, PDMS, is just as strong as vaginal tissue, deforms more, and requires more energy to reach failure (i.e. is tougher) than the vagina. Clinically, the benefits (or lack thereof) of implanting a mesh manufactured from a material that has the previously mentioned properties is not clear since these data are reflective of the material properties of PDMS. Once a material is manufactured into a mesh, it is more relevant to assess the structural properties of the mesh in order to understand how these properties, particularly stiffness, may impact the performance of the device in vivo.

One major limitation of this study is that the mixing ratio (5:1) and thickness (1.0 mm) combination which yielded a PDMS stiffness that is similar to that of vaginal tissue is specific to the mixing ratio and thicknesses evaluated in this study. It is possible that a different mixing ratio and thickness combination(s) could produce similar results. Thus, more studies are needed to further investigate the mechanical properties of PDMS obtained from a variety of mixing ratio and thickness combinations. These studies will be particularly beneficial for evaluating the potential of PDMS as an alternative material to polypropylene for urogynecologic mesh.
4.3 EX VIVO CHARACTERIZATION OF AUXETIC MESHES

4.3.1 Introduction

In Chapter 3, finite element analysis (FEA) was used to assess the behavior of meshes with auxetic pore geometries. The results of this study revealed that auxetic pores will afford mesh the ability to expand rather than contract in response to uniaxial loading. However, an important caveat to this behavior is that the expansion of pores is largely dependent on the orientation of the pores with respect to the loading direction. As was seen with the bowtie model, when the pores are aligned with (bowtie 0°) or rotated 90° from (bowtie 90°) the axis of loading, pore and model expansion occurs. Conversely, rotating the pores of this model 45° (bowtie 45°) resulted in pore collapse and model contraction. Despite this limitation, the bowtie geometry was shown to have the most potential as a pore shape for synthetic meshes used in abdominal sacral colpopexy meshes because of the uniaxial loading that occurs. These results are significant and will be crucial from a design standpoint; however, as with any type of FEA results, they must be validated. Thus, the purpose of this study was to assess the behavior of auxetic meshes manufactured from PDMS. One re-entrant structure, the bowtie, and one chiral structure, the chiral hexagon, were chosen as the pore geometries for the PDMS auxetic meshes. We chose the bowtie geometry because it stood out among all of the auxetic geometries in its 0° and 90° configurations. The chiral hexagon geometry was chosen as it had the most stable response irrespective of the orientation of the pores with respect to the loading direction and therefore may be a good design option for transvaginal applications.

Auxetic meshes were created, as described in the next section, with the pores aligned at 0°, 45°, and 90°. Three Newtons of force was applied along the longitudinal axis of the meshes and
pore deformation was assessed via quantification of the pore diameter, porosity, and effective porosity. In addition to pore deformation, mesh burden and the relative elongation of the meshes were also quantified. Based on the FEA of the bowtie 0°, 45°, 90°, we hypothesized that 1) the pores of the bowtie 0° and 90° meshes would remain open/expand in response to uniaxial loading while the pores of the bowtie 45° mesh would contract/collapse. For the chiral hexagon meshes, we anticipated that pore deformation would be similar for all of the chiral hexagon meshes irrespective of the orientation of the pores with respect to the loading direction. Specifically, the pores of the chiral hexagon mesh would elongate and contract, and this is based on the FEA chiral hexagon 0°, 45°, 90° models results presented in Chapter 3. The pores of the chiral hexagon models contracted at 3 N despite chiral hexagon being an auxetic geometry. We also hypothesized that for meshes in which the pores expand an associated increase in the porosity and effective porosity coupled with a decrease in mesh burden would be observed. However, a decrease in porosity and effective porosity paired with an increase in mesh burden would be observed for meshes in which the pores contracted. Similar to pore deformation and mesh burden, we expected that the relative elongation of the bowtie and chiral hexagon meshes would follow a similar trend as the FEA results. Specifically, the 0° configurations for both the bowtie and chiral hexagon meshes would deform the least while overall the bowtie 45° meshes would deform the most and the chiral hexagon 0° meshes would deform the least. In addition to the previously mentioned parameters, the amount of permanent deformation that the auxetic meshes experienced in response to cyclic loading was also assessed. This assessment was important because in vivo it is anticipated that synthetic meshes experience repetitive sub-failures loads which can lead to permanent elongation of the mesh and possibly complications. Given that the auxetic meshes were manufactured from
PDMS, which is an elastomeric material, we anticipated that there would be no permanent elongation of the meshes in response to cyclic loading.

4.3.2 Methods

4.3.2.1 Manufacturing of Auxetic Meshes

Bowtie and chiral hexagon meshes with the pores rotated 0°, 45°, and 90° with respect to the longitudinal axis of the mesh were created from poly(dimethylsiloxane), PDMS, using a mold-fill process. Molds of the meshes were first created in SolidWorks 2013 x64 Edition (Dassault Systèmes SOLIDWORKS Corporation, Waltham, Massachusetts). To create the molds, first bowtie 0°, 45°, 90° and chiral hexagon 0°, 45°, 90° meshes were created using various techniques within SolidWorks including linear patterning and Boolean subtraction. The following design criteria were utilized during the creation of these meshes.

1) The minimal pore dimension was at least 1 mm. Although, it should be noted that models containing angles less than 90° had small regions of the pore where the fibers were closer than 1 mm.

2) The smallest allowable angle within a pore is 45°.

3) For the chiral hexagon meshes, the diameter of the circles was 1 mm.

4) The width of the fibers for both the bowtie and chiral hexagon meshes equaled 0.75 mm.

5) The thickness of the meshes was 1 mm.

The width of the fibers and thickness of the meshes were chosen so that the mesh can be removed from the mold without breaking and to make sure that the meshes could withstand 3 N of force. After the meshes were created, molds were constructed by performing a Boolean subtraction with
the meshes and a solid body (i.e. subtracting the solid volume of the meshes from a solid body) (Figures 55 and 56).

Figure 55: Bowtie 0º (a), 45º (b), and 90º (c) mesh computer-aided design molds.

Figure 56: Chiral hexagon 0º (d), 45º (e), and 90º (f) mesh computer-aided design molds.
Initially, mesh molds were 3D printed using fusion deposition modeling printers in our laboratory, MakerBot Replicator Desktop 3D Printer 5th Generation Model (MakerBot, Brooklyn, NY) and Printrbot Simple (Lincoln, California). However, the layer resolution of these printers was too large such that a smooth, detailed, and watertight mold could not be created with these 3D printers. Thus, printing of molds was outsourced to Shapeways, Inc (New York, NY). These molds were printed using multijet modeling and were made from a UV cured acrylic that had a lower layer resolution than the 3D printers in our lab, which allowed for the creation a smooth, detailed, and watertight model.

PDMS meshes were manufactured from Sylgard 184 Silicone Elastomer (Dow Corning, Midland, MI) using a similar process described previously, Section 4.2.2.1. Briefly, the base and curing agent were combined in a 5:1 ratio by weight and mixed for 10 minutes. To remove the air bubbles created during mixing, the mixture was degassed in a vacuum oven at approximately 74 cmHg for 20 minutes. Following degassing, PDMS was carefully poured into the molds. Prior to pouring, the molds were sprayed with a mold release spray (Mann Release Technologies Ease Release) in order to facilitate removal of the mesh. The molds were then placed in an oven and heat cured for approximately 90 minutes at 90°C. At the conclusion of curing, the PDMS was carefully removed from the mold and a porous construct (i.e. mesh) remained. See Figure 57 for a schematic of this process.
Figure 57: Schematic of auxetic mesh manufacturing process.
4.3.2.2 Quantification of Pore Deformation, Mesh Burden, and Relative Elongation

The ability of auxetic mesh pores to expand when loaded was assessed via uniaxial tensile testing. Strips of mesh approximately 90 mm x 15 mm were cut along the longitudinal axis of the bowtie and chiral hexagon meshes manufactured in Section 4.3.2.1. Five mesh samples for each pore orientation (0°, 45°, 90°) per mesh (bowtie and chiral hexagon) were tested for a total of 30 meshes. Meshes were clamped on opposing ends using custom clamps. An aspect ratio of at least 5 was maintained for all meshes with clamping. One clamp was rigidly attached to the base of an Instron™ 4502 (Instron, Norwood, MA, USA) while the other was attached to the crosshead of the testing machine in series with a 50-lb load cell (Model 31; Honeywell, Morristown, NJ, USA), with a resolution of 0.1 N. A preload of 0.1 N was applied at an elongation rate of 10 mm/min to remove slack from the meshes. Following the application of the preload, the distance between the clamps was recorded (clamp-to-clamp distance) and then the meshes were loaded to 3 N at a rate of 50 mm/min in increments of 0.5 N. It is important to note that the bowtie 45° meshes were only loaded to 2.5 N, as any additional load would result in failure. Pictures of the central portion of the mesh were taken at 0.1 N, 0.5 N, 1 N, 2 N, and 3 N (or 2.5 N in the case of the bowtie 45° meshes) using a digital SLR camera (Canon, EOS Rebel T3, Melville, NY) with a 60 mm macro lens (Canon, EFS f/2.8, Melville, NY). Mesh samples were imaged using the same camera setup and image settings (F2.8 and ISO 100). To assess the geometry of the pores with loading, images were cropped to 10 mm x 30 mm using ImageJ (NIH, Bethesda, MD). For meshes that experienced contraction during loading, images were cropped to the width of the mesh while the length was cropped to 30 mm. Lastly, during the manufacturing process of the mesh, remnants of PDMS cured in between some pores around the edges. Depending on the amount of PDMS, these pieces could pose problems for the Mathematica code utilized to assess pore geometry potentially identifying
remnants as pores. To eliminate this risk as much as possible, PDMS remnants were carefully removed from the pores without distorting the mesh using Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, California).

A custom Mathematica V10 (Wolfram, Champaign, IL) script was used to analyze the geometry of the pores as described previously (Section 2.5.1.1). Images were binarized and an edge detection algorithm was utilized to detect the pores of the mesh. Given the somewhat transparent nature of the PDMS mesh, the threshold used in this image processing analysis was slightly higher than the one used for the computational models. The minimal width of the pores was determined using an algorithm that approximates the pores with the best-fitting ellipse (the smallest axis of the ellipse corresponding to the width). Using this method, the pore dimensions of some of the models were over-estimated, particularly for the un-deformed pores, and this is a result of the geometry of the pores not conforming to the shape of an ellipse. When this occurred, the percent that the ellipse over-estimated was determined and the respective pore dimension was adjusted according to this amount. For example, if the ellipse estimated the length of the pore to be 8 mm and the actual length was 4 mm, the reported pore length was adjusted by a factor of 2.

Next, an algorithm was used to approximate the area of each pore. Pores along the edge of the image were excluded from the analysis. In the end, the minimal width and minimal length for all the pores in the cropped image was determined. The effective pore area was calculated by summing up the area of the pores greater than 1 mm and dividing this value by the total pore area. Additionally, the porosity and effective porosity were calculated as follows: Porosity = Area of white pixels / Total pixel area in the image and Effective Porosity = Area of white pixels with pores > 1 mm / Total pixel area in the image. Mesh burden was quantified by normalizing the area
of black pixels (mesh pixels) by the total pixel area. Lastly, the relative elongation was calculated as the amount of elongation divided by the clamp-to-clamp distance.

4.3.2.3 Quantification of Permanent Elongation

To quantify the amount of permanent elongation that the PDMS auxetic meshes experienced in response to repetitive loading, a cyclic loading protocol was performed on the bowtie 0° (n=4) and chiral hexagon 0° (n=4) meshes. Only the 0° meshes were chosen for this analysis as the 45° and 90° meshes are the same as the 0° meshes with the exception that the pores are orientated differently. Given this small difference, it was anticipated that if the 0° meshes did not experience permanent deformation than the 45° and 90° meshes would not as well. Indeed, this was verified experimentally as permanent deformation was not observed for the bowtie and chiral hexagon 45° and 90° meshes (data not shown). Additionally, one could argue that the assessment of permanent deformation was only needed for one of the auxetic meshes given that they were all made from an elastomeric material, PDMS, which by definition does not experience permanent deformation. If the meshes were manufactured from different non-elastomeric materials, this would not be the case and the assessment of permanent deformation would have to be examined for all meshes. Nevertheless, to air on the side of caution, permanent deformation was analyzed for both the bowtie 0° and chiral hexagon 0° meshes.

The testing setup used to assess permanent deformation was similar to that used to assess pore deformation. Briefly, mesh strips, approximately 90 mm x 15 mm, were clamped on opposing ends using custom clamps and then loaded onto the Instron 4502™ testing machine. An aspect ratio of at least 5 was maintained for all meshes with clamping. A similar cyclic loading protocol was utilized as previously described by Shepherd et al (2012) [71]. However, the loads applied to the auxetic meshes in this study were less than those applied to the polypropylene meshes in the
Shepherd et al (2012) study. This is largely because the auxetic meshes were manufactured from PDMS and they were not strong enough to withstand loads greater than 5 N. It is important for the reader to keep in mind, that the load limit for the auxetic meshes in this study is specific to these meshes. Auxetic meshes manufactured with different dimensions (e.g. a different thickness or mixing ratio) will probably have a different load limit. The cyclic loading protocol was as follows, a preload of 0.1 N was applied at a rate of 10 mm/min in order to remove slack from the meshes. Next, the distance between the clamps was noted (referred to as the clamp-to-clamp distance) and served as a reference point for the initial length of the mesh. The meshes were then cyclically loaded using three separate cycling regimens (Figure 58). During cycle 1, meshes were loaded from 0.5 N to 1.5 N at a rate of 50 mm/min for 10 cycles. For the second and third cycles, meshes were loaded from 0.5 N to 4.5 N and 0.5 N to 1.5 N, respectively, at the same elongation rate and number of cycles as cycle 1. In between each cycling regimen, the preload of 0.1 N was reapplied and the clamp-to-clamp distance was recorded and normalized by the initial clamp-to-clamp distance in order to determine the amount of permanent deformation that the meshes experienced.
Figure 58: Cyclic loading protocol used to assess permanent deformation of the auxetic meshes. C1: 0.5 N – 1.5 N, C2: 0.5 N – 4.5 N, C3: 0.5 N – 1.5 N.

4.3.2.4 Statistics

One-way ANOVAs with a bonferroni correction (p-value < .017 for significance) were used to compare the relative elongation between the bowtie meshes, chiral meshes, and to compare the overall elongation of all meshes. Significant ANOVAs were followed with Tukey’s post-hoc tests. Additionally, an Independent Samples T-test was used to compare the relative elongation between the bowtie 0° and 90° meshes. Prior to conducting these analyses, Kolmogorov-Smirnov tests were used to determine whether the data was normally distributed. Statistical analysis was performed using SPSS 24 (IBM, Armonk, NY, USA).
4.3.3 Results

4.3.3.1 Bowtie Mesh Pore Deformation Results

Overall, the pores of the bowtie 0° meshes expanded in response to force applied along the longitudinal axis of the meshes. Qualitatively assessing the behavior of these meshes when progressively loaded to 3 N, an increase in the length of the pores was observed while the width of the pores remained relatively constant (Figure 59, top). These observations were confirmed quantitatively, with the width of the pores increasing by only 13.6% (1.05 mm to 1.22 mm) and the length nearly doubling from 1.84 mm to 3.37 mm (45.4% increase) (Figure 59, graph). Consistent with an increase in the dimensions of the pores, the porosity and effective porosity both increased by 33.3% (Figure 60). The effective pore area was maintained at 100% throughout loading and this result is expected given that both the width and length of the pores remained above 1 mm.
Figure 59: Representative images of the bowtie 0° mesh central region loaded longitudinally from 0.1 N to 3 N (top). As seen qualitatively (top) and quantitatively (graph), increasing the amount of load resulted in an overall increase in the length of the pores while the width of the pores remained relatively constant.
Figure 60: Both the porosity and effective porosity increased with loading for the bowtie 0° meshes.

Note: The diameter of all of the pores analyzed at each load was greater than 1 mm; therefore, the porosity and effective porosity were the same.

Unlike the bowtie 0° meshes, the pores of the bowtie 45° meshes contracted (i.e. collapsed) when loaded along the long axis of the mesh. Though subtle, collapse of the pores was observed early during loading, after the 0.1 N preload was applied (Figure 61, a). Additionally, pore collapse was also associated with the creation of tiny spaces that were typically less than 0.10 mm (Figure 61, b). These spaces are similar to the interstices of knitted, synthetic meshes and were difficult to measure; thus, they were not considered in the calculation of the pore dimensions. Overall, increasing the load applied from 0.1 N to 2.5 N resulted in a 49.0% decrease in the width of the pores (1.02 mm to 0.52 mm) and a 38.1% increase in the length (1.94 mm to 3.14 mm) (Figure 61, graph).
Figure 61: Representative images of the bowtie 45° mesh central region when loaded longitudinally from 0.1 N to 2.5 N. As seen qualitatively (a) and quantitatively (graph), increasing the amount of load resulted in an increase in the length of the pores and a decrease in the width reflective of pore collapse. As depicted in image b, pore collapse was also associated with the creation of tiny spaces that were typically less than 0.10 mm (yellow arrows). These spaces were not included in the calculation of the width of the pores.
Both the porosity and effective porosity decreased with loading for the bowtie 45° meshes with a complete loss in the effective porosity at forces greater than or equal to 1 N (Figure 62). Similar results were observed for the effective pore area. Initially the effective pore area was 100% at 0.1 N. However, at 0.5 N, an 83.5% decrease was observed in the effective pore area and with subsequent loading (at forces greater than or equal to 1.0 N) 0% of the pore area was effective.

Figure 62: Overall, the porosity and effective porosity decreased with loading for the bowtie 45° meshes with a complete loss in the effective porosity observed at forces greater than and equal to 1 N.
As anticipated, the pores of the bowtie 90° meshes expanded in response to 3 N of force applied along the longitudinal axis of the mesh (Figure 63, top). The changes observed with the pore dimensions of the bowtie 90° meshes were similar to that of the bowtie 0° meshes, but in reverse. For instance, the width remained relatively constant for the bowtie 0° meshes while the length increased. Yet for the bowtie 90° meshes, the width increased by 72.5% (1.15 mm to 4.19 mm) and very little increase was observed in the length, 5.2% increase from 1.77 mm to 1.87 mm (Figure 63, graph). It is important to note that although the overall length of the bowtie 90° meshes increased, from 0.1 N to 3 N, contraction was observed. Initially the length of the bowtie 90° meshes increased and reached a peak of 1.93 mm at 1 N. With the application of additional force, a decrease in the length of the pores was observed signifying contraction. Despite the observed contraction, the length of the pore remained above 1 mm. Consistent with the observed increases in the length and width of the pores, the porosity and effective porosity also increased, increasing by 33.6% for both parameters (Figure 64). With increases in the pore dimensions, the effective pore area was maintained at 100% throughout loading.
Figure 63: Representative images of the bowtie 90° mesh central region when loaded longitudinally from 0.1 N to 3 N. As seen qualitatively (top) and quantitatively (graph), increasing the amount of load resulted in an overall increase in the width of the pores while the length of the pores remained relatively constant.
Both the porosity and effective porosity increased with loading for the bowtie 90° meshes.

Note: The diameter of all of the pores analyzed at each load was greater than 1 mm; therefore, the porosity and effective porosity were the same.
4.3.3.2 Chiral Hexagon Mesh Pore Deformation Results

Overall, pore deformation of the chiral hexagon 0°, 45°, and 90° meshes when subjected to a longitudinal force of 3 N was similar. This is particularly true for the chiral hexagon 0° and 45° meshes. As load was applied to the latter two meshes, the pores appeared to elongate and contract with the circular pores deforming into an oval shape and the triangular pores into a tear drop shape (Figures 65 and 66).

![Chiral Hexagon 0° Mesh](image)

Figure 65: Representative images of the chiral hexagon 0° mesh central region when loaded longitudinally from 0.1 N to 3 N. The circles and triangles appear to elongate and contract.
Consistent with these observations, quantitatively the width of the chiral hexagon 0° and 45° pores decreased while the length increased (Figure 67). Specifically, applying 3 N of force resulted in a 28.8% and 26.4% reduction in the width of the circles for the chiral hexagon 0° and 45° meshes, respectively, with the length of these pores doubling (increasing by 103% and 103.9% for the chiral hexagon 0° and 45° meshes, respectively). Similar results were observed for the triangular pores (Figure 68). The width decreased by 18.4% and the length increased by 94.8% for the triangular pores of the chiral hexagon 0° meshes. Additionally, the width of the triangular pores for the chiral hexagon 45° meshes decreased by 20.7% while the length increased by 97.3%.
Figure 67: Resulting minimal pore diameter for the width and length of the circles within the chiral hexagon 0° and 45° meshes in response to 3 N of force. The width of the circles decreased with loading while the length increased.
Figure 68: Resulting minimal pore diameters for the width and length of the triangles within the chiral hexagon 0° and 45° meshes in response to 3 N of force. The width of the triangles decreased with loading while the length increased.
Loading the chiral hexagon meshes to 3 N with the pores rotated 90° resulted in slightly different pore shapes compared to when the pores were rotated 0° and 45°. The circular pores deformed into elongated ovals whereas the triangular pores elongated into a lemon-slice shape (Figure 69). As observed, the width of the circles decreased by 16.6% and the length increased by 96.4% (Figure 70, top graph). For the triangular pores, a 96.8% increase in the width was observed while the length of these pores decreased by 13.5% (Figure 70, bottom graph).

Figure 69: Representative images of the chiral hexagon 90° mesh central region when loaded longitudinally from 0.1 N to 3 N. The circles and triangles appear to elongate and contract.
Figure 70: Resulting minimal pore diameters for the width and length of the circles and triangles within the chiral hexagon 90° mesh in response to 3 N of force. The width of the circles decreased while the length increased with loading. For the triangles, the width increased with loading while the length decreased.
Overall, there was a gradual increase in the porosity throughout loading for the chiral hexagon meshes (Figure 71). Specifically, the porosity of the chiral hexagon 0° meshes increased by 37.6% from 19.1% at 0.1 N to 26.3% at 3 N. Similarly, a 38.1% and 40.6% increase was observed in the porosity for the chiral hexagon 45° and 90° meshes, respectively. Unlike the bowtie meshes, the effective porosity was not calculated for the chiral hexagon meshes and this is a result of the dimensions of the circular pores. The diameter of the circular pores was designed to be 1 mm; however, once the mesh was removed from the mold, the diameter was less than 1 mm. This may be a consequence of small PDMS remnants inside the circular pores thus decreasing the overall diameter of the circles. With the initial diameters (length and width) being less than 1 mm prior to loading, the initial effective porosity and effective pore area would not be 100% for the chiral hexagon meshes as originally designed. Thus, the effective porosity and effective pore area were not calculated for the chiral hexagon meshes.
4.3.3.3 Relative Elongation Results

Although the bowtie 0° and 90° meshes were able to withstand 3.0 N of force, the bowtie 45° meshes could not. Thus, the relative elongation of the bowtie meshes were compared at 2.5 N and a separate comparison between the bowtie 0° and 90° meshes was performed at 3.0 N. Analogous to the trends observed for the bowtie FE mesh models, the bowtie 0° meshes deformed significantly less than the 45° and 90° meshes, p-value < .001. Specifically, at 2.5 N the relative elongation for the bowtie 0° meshes was 33.3% and 32.0% less than that of the bowtie 45° and 90° meshes, respectively, p-value for both < .001. There was no significant difference observed in the relative elongation between the bowtie 45° and 90° meshes, p-value = .903, at 2.5 N. Additionally, at 3.0 N, the bowtie 90° meshes deformed significantly more than the bowtie 0° meshes, p-value < .001. See Table 10 for a summary of these results.
Table 10: Relative elongation of bowtie meshes at 2.5 N and 3.0 N. Data represented as mean ± standard deviation. P-value obtained from One-way Anova\textsuperscript{a} with a Tukey’s post-hoc test\textsuperscript{b} and Independent Samples T-Test\textsuperscript{c}.

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Relative Elongation (mm/mm) at 2.5 N</th>
<th>Relative Elongation (mm/mm) at 3.0 N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowtie 0°</td>
<td>0.50 ± 0.04</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>Bowtie 45°</td>
<td>0.75 ± 0.06</td>
<td>N/A</td>
</tr>
<tr>
<td>Bowtie 90°</td>
<td>0.74 ± 0.05</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>Overall p-value</td>
<td>&lt; .001\textsuperscript{a}</td>
<td>N/A</td>
</tr>
<tr>
<td>Bowtie 0° vs 45°</td>
<td>&lt; .001\textsuperscript{b}</td>
<td>N/A</td>
</tr>
<tr>
<td>Bowtie 0° vs 90°</td>
<td>&lt; .001\textsuperscript{b}</td>
<td>&lt; .001\textsuperscript{c}</td>
</tr>
<tr>
<td>Bowtie 45° vs 90°</td>
<td>.903\textsuperscript{b}</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Overall, there was a significant difference observed in the relative elongation of the chiral hexagon meshes at 3 N, p-value < .01 (Figure 72). Specifically, the chiral hexagon 90° meshes deformed 15.6% and 15.3% less than the 45° and 90° meshes, respectively, p-value for both < .01. There was only a slight difference, 0.004%, in the relative elongation of the chiral hexagon 0° and 45° meshes, p-value = .995.
Figure 72: The chiral hexagon 0° and 45° meshes deformed significantly more than the chiral hexagon 90° meshes in response to 3 N of force. *Comparisons made relative to chiral hexagon 90°, p-value < .01.

Comparing the relative elongation for all meshes in response to 2.5 N, the chiral hexagon 90° meshes deformed the least followed by the bowtie 0° meshes, 44.34% and 50.12%, p-value = .335. Overall, the bowtie 45° and bowtie 90° meshes deformed the most. Specifically, the relative elongation of the bowtie 45° and 90° meshes was significantly higher relative to all of the chiral hexagon meshes, p-values < .001 (Figure 73).
4.3.3.4 Mesh Burden Results

Overall, mesh burden results for the bowtie and chiral hexagon meshes were congruent with our hypothesis. As the porosity of the mesh increased, mesh burden decreased and vice versa. Specifically, mesh burden decreased by 25.5% and 30.9% for the bowtie 0° and 90° meshes, respectively, whereas a 14.4% increase in mesh burden was observed for the bowtie 45° meshes (Table 11). This result was not surprising for the bowtie 45° meshes given that a collapse in the pores of these meshes led to localized bunching of the mesh. For all of the chiral hexagon meshes, mesh burden decreased. Particularly, mesh burden was reduced by 9.6%, 10.5%, and 11.8% for the chiral hexagon 0°, 45°, and 90° meshes, respectively.
Table 11: Mesh burden results for bowtie 0°, 45°, and 90° meshes and chiral hexagon 0°, 45°, and 90° meshes.

<table>
<thead>
<tr>
<th>Synthetic Mesh</th>
<th>Mesh Burden at 0.1 N (mm²/mm²)</th>
<th>Mesh Burden at Max Load 2.5 N or 3.0 N (mm²/mm²)</th>
<th>Percent Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowtie 0°</td>
<td>0.66</td>
<td>0.49⁢b</td>
<td>↓25.5%</td>
</tr>
<tr>
<td>Bowtie 45°</td>
<td>0.67</td>
<td>0.76⁢a</td>
<td>↑14.4%</td>
</tr>
<tr>
<td>Bowtie 90°</td>
<td>0.63</td>
<td>0.44⁢b</td>
<td>↓30.9%</td>
</tr>
<tr>
<td>Chiral Hexagon 0°</td>
<td>0.77</td>
<td>0.70⁢b</td>
<td>↓9.6%</td>
</tr>
<tr>
<td>Chiral Hexagon 45°</td>
<td>0.76</td>
<td>0.68⁢b</td>
<td>↓10.5%</td>
</tr>
<tr>
<td>Chiral Hexagon 90°</td>
<td>0.78</td>
<td>0.69⁢b</td>
<td>↓11.8%</td>
</tr>
</tbody>
</table>

4.3.3.5 Permanent Deformation Results

As anticipated, cyclically loading the bowtie 0° and chiral hexagon 0° meshes resulted in no permanent deformation of these meshes, despite the meshes being stretched between 20% and 70% of their initial length. After each loading regimen the clamp-to-clamp distance was the same as the initial clamp-to-clamp distance, which indicated that permanent deformation did not occur. Additionally, the peaks and valleys for each cycle regimen overlapped which also suggested no permanent deformation (Figure 72). If permanent deformation had occurred, the peaks and valleys of the loading and unloading curves would shift to the right.
Figure 74: Representative curves for cyclic loading of bowtie 0° mesh and chiral hexagon 0° mesh at C1 (10 cycles from 0.5 N to 1.5 N), C2 (10 cycles from 0.5 N to 4.5 N), and C3 (10 cycles from 0.5 N to 1.5 N). Cyclic loading of the meshes resulted in no permanent deformation as evidenced by the overlapping of the curves for C1, C2, and C3. If permanent deformation were to have occurred, there would be a shift to the right of the curves with each cycle.
4.3.4 Discussion

In this study, bowtie 0°, 45°, 90° and chiral hexagon 0°, 45°, 90° meshes were manufactured from PDMS and the deformation of the pores as well as mesh burden were assessed in response to uniaxial loading. Additionally, the relative elongation in response to 3 N applied along the longitudinal axes of the meshes and the amount of permanent elongation that the bowtie and chiral hexagon meshes experienced in response to cyclic loading were evaluated. Consistent with our hypothesis, the pores of the bowtie 0° and 90° meshes remained open throughout loading. Specifically, both the width and length of the bowtie 0° and 90° mesh pores increased indicating an overall expansion of these pores. Along with an increase in the pore dimensions, the porosity and effective porosity also increased while mesh burden for the bowtie 0° and 90° meshes decreased. Although the length of the pores for the bowtie 45° meshes increased, the width of these pores were significantly reduced resulting in pore collapse, a decrease in the porosity of the mesh, and an increase in mesh burden. Also consistent with our hypothesis, pore deformation for the chiral hexagon 0°, 45°, and 90° meshes was similar; the pores of these meshes elongated and contracted. Despite contraction of the pores, the porosity of the chiral hexagon 0°, 45°, and 90° meshes increased and mesh burden decreased. Overall, the 0° configurations for both the bowtie and chiral hexagon meshes resulted in the least amount of deformation for these meshes while overall the bowtie 45° and 90° meshes deformed the most. Lastly, cyclically loading the bowtie and chiral hexagon meshes resulted in no permanent deformation and this result was also congruent with our hypothesis. Manufacturing the auxetic meshes from PDMS, which has elastic properties, allowed the meshes to undergo a large degree of deformation, repetitively, without deforming, giving the mesh shape memory (i.e. the mesh returned to its original shape).
From a clinical perspective, the expansion of pores is more favorable than the contraction and collapse of pores given that larger pore and high porosity meshes yield better tissue integration with increased collagen deposition between pores and decreased inflammation and fibrous relative to meshes with small pores and low porosity [79,119-121]. Thus, in terms of mesh design, the bowtie pore geometry with the pores in the 0° or 90° configuration is best suited for uniaxial applications, i.e. for abdominal sacrocolpopexy meshes. One may consider manufacturing a transvaginal mesh with the body of the mesh consisting of the bowtie pores in the 0° configuration and the arms of the mesh in the 90° configuration. This design may work in terms of keeping the pores of the arms open, which often collapse in vivo. However, the pores of the mesh would have to be perfectly aligned with the direction of loading otherwise pore collapse will occur, as observed with the bowtie pores in the 45° configuration. Not only is a decreasing pore size unfavorable clinically, but when the pores of the bowtie 45° meshes collapsed, tiny spaces analogous to interstices of knitted, synthetic meshes, were created and these interstices have the potential to harbor bacteria which can lead to infection, as seen with multifilament meshes [88-93]. It is for the reasons stated previously that the bowtie geometry not be recommended for multi-axial loading, i.e. not for transvaginal meshes. Unlike the bowtie geometry, pore deformation for the chiral hexagon geometry was less impacted by the orientation of the pore with respect to the loading direction. This in turn may translate clinically to the chiral hexagon geometry being more suitable for transvaginal meshes. In fact, loading the chiral hexagon pore from multiple directions, simultaneously may actually facilitate expansion of the pore rather than contraction as seen with the chiral hexagon meshes in this study. Future in silico and ex vivo mechanical testing studies of the chiral hexagon mesh loaded simultaneously will help to refute or corroborate this claim. In addition to pore expansion, the absence of permanent deformation with cyclic loading is another
clinical benefit of the meshes developed in this dissertation for prolapse. Immediately after a synthetic mesh is implanted into the body and prior to tissue ingrowth within the mesh pores, the mesh is able to freely deform with changes in intra-abdominal pressure (e.g. increasing pressure with coughing). Both sudden and repetitive increases in pressure applied to the mesh could lead to lengthening of the mesh, which can potentially result in re-current prolapse. However, by manufacturing the meshes in this dissertation from an elastomeric material, the meshes did not permanently deform with repetitive loading. This in turn could translate to a decrease in the chance of re-current prolapse due to mesh lengthening.

Comparing the pore deformation results of the bowtie 0° and 90° pores in this study to pore deformation of commercial synthetic mesh products, the most striking difference between the two is the ability of the bowtie pores to remain open/expand and the porosity as well as the effective porosity to increase with loading. These results are opposite to those observed by Barone et al (2016) in which tensile loading of commercial mesh products resulted in a progressive decrease in the minimal pore diameter and for some meshes a complete collapse of the pores [77]. Additionally, the decrease in pore size of the commercial synthetic meshes was also associated with a decrease in the overall porosity of these meshes. The differences observed in pore deformation between the bowtie pores and commercial mesh pores (e.g. squares, diamonds, and hexagons) are largely attributed to the respective geometry of the pores. The bowtie pore contains inward pointing angles; thus, when these angles are aligned with the direction of loading, they enlarge which leads to pore opening (Figure 75a). This is in contrast to commercial mesh pores, which do not contain these angles, hence loading favors pore contraction and/or pore collapse (Figure 75b-d).
Figure 75: The bowtie mesh (a) contains inward pointing angles (*) which allows pore opening, in the 0° configuration (picture) and the 90° configuration (not pictured). Commercial meshes with square (b), diamond (c), and hexagon (d) shaped-pores do not have inward pointing angles, thus pore contraction and/or collapse is more likely.

However, similar to the study by Barone et al (2016), the orientation of the pores with respect to the loading direction also impacts whether an auxetic pore will contract and/or collapse. Specifically, rotating the pores by 45° for the bowtie mesh resulted in collapse of the pores associated with a decrease in the porosity and effective porosity with tensile loading (Figure 76). Similarly, rotating the pores of Restorelle (Coloplast, Minneapolis, MN), a square pore mesh, 5° offset to the square configuration resulted in pore contraction whereas a complete collapse of the pores was observed with the pores oriented 45° offset to the square configuration (Figure 76). In
both the bowtie 45° and Restorelle 45° offset meshes, the pores were oriented such that an acute angle was aligned with the direction of loading which resulted in mesh contraction and collapse.

Figure 76: Pore orientation with respect to the loading direction impacts whether pores contract and/or collapse with loading for a representative commercial mesh, Restorelle, and for the bowtie mesh with the pores in the 45° configuration. Yellow lines demonstrate alignment of the angle with respect to the loading direction, which leads to pore contraction and collapse.
Unlike the bowtie and commercial meshes, the orientation of the pores with respect to the loading direction did not significantly impact pore deformation for the chiral hexagon mesh. This result is likely attributed to rotational symmetry. In a study conducted by Shan et al. (2015), the Poisson’s ratio for patterns with three-fold and six-fold rotational symmetry was the same irrespective of the loading direction [199]. This isotropic behavior was not observed for patterns with two- and four-fold rotational symmetry. The chiral hexagon geometry can be rotated six times and still maintain its geometrical orientation in contrast to the bowtie and square pore configurations which have two- and four-fold rotational symmetry, respectively (Figure 77).

Figure 77: The chiral hexagon geometry (top) has six-fold rotational symmetry whereas the bowtie (middle) and square (bottom) geometries have two-fold and four-fold rotational symmetry, respectively.
Compared to commercial mesh pores, auxetic pores have the advantage of being able to prevent pore collapse through pore expansion. However, like the commercial mesh pores auxetic pores are also directionally dependent, meaning they will contract and/or collapse depending on the orientation of the pore with respective to the loading direction. To increase the applicability of auxetic meshes for prolapse repair, the development of an auxetic geometry that is directionally independent is a must. The chiral hexagon geometry shows promise towards developing an isotropic mesh and optimizing the design of a mesh with chiral hexagon pores, or other geometries with three- and six-fold rotational symmetry, is worth exploring further.

In addition to assessing the pore deformation of the bowtie and chiral hexagon meshes, the relative elongation of these meshes was also assessed. However, unlike studies in the literature that evaluated the relative elongation at failure of commercial synthetic meshes, relative elongation was assessed up to 3 N in this study. Three Newtons was chosen because it reflects the minimal amount of force that a mesh must be able to withstand based on estimates of the intra-abdominal pressure reported with sitting and standing and on our estimates of the surface area of the anterior vagina using MRI measurements [144-148]. This decision limits our ability to make one-to-one comparisons between the relative elongation of commercial synthetic meshes and the meshes developed in this dissertation. Nevertheless, the relative elongation of commercial synthetic meshes was used to determine whether the relative elongation of the bowtie and chiral hexagon meshes in this study was reasonable, given that there is no standard elongation established for prolapse meshes. Studies in the literature report the relative elongation at failure of prolapse meshes ranges from approximately 40% to 100% with failure loads ranging from roughly 7 N to 68 N [70,71]. For meshes in which the failure loads were closest to 3 N, the relative elongations were 87.9% (failure load of 7.83 N) and 61.7% (failure load of 9.62 N). The relative elongations
of the meshes in this study were between 50.8% and 81.8% at 3 N. Compared to the commercial meshes, the bowtie and chiral hexagon meshes deformed more in 3 N than many of the commercial meshes did prior to failure. This result is somewhat concerning given that the purpose of prolapse mesh is to provide structural support, and a mesh that is elongating too much may not provide the structural support needed to correct prolapse. This limitation of the bowtie and chiral hexagon meshes however can be overcome by increasing the structural stiffness of these meshes. One way that this can be done is to manufacture the bowtie and chiral hexagon meshes from a stiffer material than the PDMS in this study.

Previously in Chapter 3, FEA was utilized to assess changes in pore size, effective pore area, porosity, and effective porosity of finite element models (FEMs), with auxetic pores in response to 3 N of force applied along the longitudinal axis of the model. Additionally, the pores of the models were rotated 0°, 45°, and 90° with respect to the axis of loading. Overall, the bowtie and chiral hexagon meshes displayed the behavior (i.e. whether the pores would open, contract, or collapse) that was predicted by the FEA in Chapter 3. Specifically, the FEA predicted that the pores of the bowtie 0° and 90° meshes would expand in response to 3 N of force and indeed they did. Similarly, the pores of the bowtie 45° finite element model collapsed and this same result was observed with the actual bowtie 45° meshes. However, the degree of expansion or contraction of the FE model pores differed from the mesh pores (Table 12). For example, the bowtie 0° finite element model predicted that the width and length of the pores would increase by 53.1% and 40.0%, respectively. An opposite result was observed for the actual bowtie 0° meshes. The length of the bowtie 0° meshes increased more than the width, 83.2% versus 15.7%, respectively. Additionally, the bowtie 0° finite element model also under-predicted the increases in the porosity and effective porosity, 25.9% FE model versus 50.0% meshes. Similarly, the bowtie 45° FE model
under-predicted the changes in the width and length and over-predicted the change in porosity (Table 12). The opposite affect was observed for the bowtie 90° meshes, in which the bowtie 90° FE model over-predicted the width and length and under-predicted the change in the porosity and effective porosity (Table 12). Similar to the bowtie meshes, the pores of the chiral hexagon meshes elongated and contracted as predicted by the chiral hexagon FE models (Table 13). Specifically, the chiral hexagon FE models predicted the change in the width of the circles pretty well relative to the length and porosity in which the FE models under-predicted these parameters, especially the length. Similar results were observed for the triangles; the chiral hexagon FE models under-predicted the elongation and contraction of the triangles. Overall, the relative elongations of all the bowtie and chiral hexagon FE models under-predicted the actual relative elongation of these meshes (Table 14). Given these differences between the model and FEA results, the results of this study were only able to validate the behavior of the FEA meshes in terms of how the pores opened or collapsed with uniaxial loading. An absolute one-to-one comparison could not be made.
Table 12: Percent change in pore size (width and length), effective pore area, porosity, and effective porosity in response to 3 N of force applied along the longitudinal axis of the bowtie FE models and meshes.

<table>
<thead>
<tr>
<th>Bowtie</th>
<th>Width</th>
<th>Length</th>
<th>Effective Pore Area</th>
<th>Porosity</th>
<th>Effective Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0° Model</td>
<td>↑53.1%</td>
<td>↑40.0%</td>
<td>No change</td>
<td>↑25.9%</td>
<td>↑25.9%</td>
</tr>
<tr>
<td>0° Mesh</td>
<td>↑15.7%</td>
<td>↑83.2%</td>
<td>No change</td>
<td>↑50.0%</td>
<td>↑50.0%</td>
</tr>
<tr>
<td>45° Model</td>
<td>↓37.0%</td>
<td>↑47.5%</td>
<td>↓100.0%</td>
<td>↓36.1%</td>
<td>↓100.0%</td>
</tr>
<tr>
<td>45° Mesh</td>
<td>↓49.1%</td>
<td>↑61.6%</td>
<td>↓100.0%</td>
<td>↓27.4%</td>
<td>↓100.0%</td>
</tr>
<tr>
<td>90° Model</td>
<td>↑153.5%</td>
<td>↑21.7%</td>
<td>No change</td>
<td>↑27.1%</td>
<td>↑27.1%</td>
</tr>
<tr>
<td>90° Mesh</td>
<td>↑72.5%</td>
<td>↑5.2%</td>
<td>No change</td>
<td>↑33.6%</td>
<td>↑33.6%</td>
</tr>
</tbody>
</table>

Table 13: Percent change in pore size (width and length), and porosity in response to 3 N of force applied along the longitudinal axis of the chiral hexagon FE models and meshes.

<table>
<thead>
<tr>
<th>Chiral Hexagon</th>
<th>Circle Width</th>
<th>Circle Length</th>
<th>Triangle Width</th>
<th>Triangle Length</th>
<th>Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0° Model</td>
<td>↓18.9%</td>
<td>↑28.6%</td>
<td>↑8.2%</td>
<td>↑28.6%</td>
<td>↑6.9%</td>
</tr>
<tr>
<td>0° Mesh</td>
<td>↓22.4%</td>
<td>↑103.0%</td>
<td>↓18.4%</td>
<td>↑94.8%</td>
<td>↑37.6%</td>
</tr>
<tr>
<td>45° Model</td>
<td>↓25.7%</td>
<td>↑32.4%</td>
<td>↓12.2%</td>
<td>↑33.3%</td>
<td>↑10.3%</td>
</tr>
<tr>
<td>45° Mesh</td>
<td>↓20.9%</td>
<td>↑103.9%</td>
<td>↓20.7%</td>
<td>↑97.3%</td>
<td>↑38.1%</td>
</tr>
<tr>
<td>90° Model</td>
<td>↓15.1%</td>
<td>↑25.2%</td>
<td>↑27.5%</td>
<td>↓4.2%</td>
<td>↑13.6%</td>
</tr>
<tr>
<td>90° Mesh</td>
<td>↓16.6%</td>
<td>↑96.4%</td>
<td>↑96.8%</td>
<td>↓13.5%</td>
<td>↑27.1%</td>
</tr>
</tbody>
</table>
Table 14: Relative elongation of the bowtie and chiral hexagon FE models and meshes at 3 N. The bowtie 45° failed prior to 3 N; therefore, the relative elongation is not reported for this mesh.

<table>
<thead>
<tr>
<th></th>
<th>Relative Elongation Model</th>
<th>Relative Elongation Mesh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowtie 0°</td>
<td>37.3%</td>
<td>56.6%</td>
</tr>
<tr>
<td>Bowtie 45°</td>
<td>83.5%</td>
<td>N/A</td>
</tr>
<tr>
<td>Bowtie 90°</td>
<td>59.1%</td>
<td>81.8%</td>
</tr>
<tr>
<td>Chiral Hexagon 0°</td>
<td>23.7%</td>
<td>60.3%</td>
</tr>
<tr>
<td>Chiral Hexagon 45°</td>
<td>27.8%</td>
<td>60.0%</td>
</tr>
<tr>
<td>Chiral Hexagon 90°</td>
<td>23.9%</td>
<td>50.8%</td>
</tr>
</tbody>
</table>

Initially, the observed differences between the results of the computational and experimental analyses were believed to be a result of the differences in the dimensions of the bowtie and chiral hexagon FE models and meshes. Specifically, the fiber width and overall thickness of the bowtie and chiral hexagon meshes were thicker than the FE models. A mold-fill process was used to manufacture the bowtie and chiral hexagon meshes in this study. Using this process, meshes that were the same dimensions as the FE models, would often break when being removed from the mold, and if they could be removed from the molds without breaking, they were not able to withstand 3 N. Thus, to overcome these issues, the bowtie and chiral hexagon meshes were made thicker. Given these differences in dimensions, a new bowtie 0° FE model that has the same dimensions as the bowtie 0° mesh was created and the same boundary and loading conditions and material model (the Neo-Hookean) were applied to this new model as were done in Chapter 3. The results of this preliminary study revealed that applying the Neo-Hookean material model to a thicker computational model actually restricted deformation of the model. Specifically, the model only elongated by 0.86 mm and there was hardly any noticeable deformation in the pores. Parametric analyses adjusting the Young’s modulus and Poisson’s ratio had a similar effect; the
FEM models were still not able to capture the pore deformation observed experimentally with the bowtie $0^\circ$ mesh. Recalling that the Neo-Hookean material model, and subsequent properties, was used to model a polypropylene mesh in Chapter 3, this result makes sense. The meshes developed in this study were manufactured from PDMS, which is silicone, a material that is not as stiff as polypropylene. The deformation that the auxetic meshes experience in response to tensile loading requires a more advanced model than the Neo-Hookean material model. Thus, future studies will aim to determine an appropriate material model for PDMS meshes that can be used to computationally model the auxetic meshes in this study. This model could then be used to validate the FEA predictions of the PDMS meshes and can also be used to model auxetic meshes in response to various loading and boundary conditions.

In addition to some of the limitations mentioned previously, there are a few others that must be addressed. First, the relative lateral contraction could not be calculated for the bowtie chiral hexagon meshes due to limitations of the experimental setup. This parameter is a measure of mesh expansion or contraction; therefore, it cannot be definitively stated whether the meshes are auxetic or not. However, for the meshes in which pore expansion was observed, the statement that these meshes exhibited auxetic-like behavior is more appropriate. Secondly, the pore deformation results are limited to the specific designs of the meshes used in this study. Changing the dimensions, thickness, geometry, etc of the meshes would produce different results; however, it is anticipated that the overall behavior of the meshes would remain the same. Another limitation of this study is that the diameter of the circles within the chiral hexagon meshes was less than 1 mm. This may be a result of residual amounts of PDMS within the circles after the mesh was removed from the mold. Additionally, the diameter of the circles in the SolidWorks files of the molds was set to be 1 mm. It is possible that during the printing process, the diameters were printed
less than 1 mm in the actual mold. Regardless of the cause, the effective porosity and effective pore area could not be calculated for the chiral hexagon meshes. This limitation does not negate the fact that the diameter of the circles contracted; therefore, the effective porosity and effective pore area would decrease no matter the initial pore diameter.

### 4.4 Conclusion

Using computational modeling in Chapter 3, we were able to demonstrate that auxetic geometries when used as pores for synthetic meshes will expand with uniaxial loading. However, it was also determined that the orientation of the pores with respect to the loading direction will dictate whether the pores expand (auxetic behavior) or contract (non-auxetic behavior). This was especially true for when the auxetic pores were rotated 45° with respect to the axis of loading; instead of expanding, the pores contracted for the majority of the mesh models. These results are promising and showed the potential as well as the limitations of using auxetic geometries for pores. However, the behavior of these models was based on predictions and must be validated. Thus, the purpose of this study was to assess the behavior of auxetic meshes manufactured from PDMS in response to uniaxial loading via *ex vivo* mechanical testing. In other words, uniaxial tensile tests were performed on the bowtie and chiral hexagon 0°, 45°, and 90° meshes and the resulting pore deformation was analyzed. Indeed, applying 3 N of force along the longitudinal axis of the bowtie 0° and 90° meshes resulted in pore expansion whereas the pores of the bowtie 45° meshes significantly contracted/collapsed. For the chiral hexagon 0°, 45°, and 90° meshes, pore deformation was similar for all of these meshes with the circles and triangles elongating and contracting. These observed behaviors were also congruent with the predictions of the finite
element simulations in Chapter 3. Additionally, the quantitative results of this study paralleled those of the finite element simulations. Pore expansion was associated with an increase in the porosity and effective porosity while pore contraction resulted in a decrease in the porosity and effect porosity. Overall, the 0° configurations for both the bowtie and chiral hexagon meshes resulted in the least amount of deformation for these meshes while overall the bowtie 45° and 90° meshes deformed the most. Secondary to our primary objective, the results of the experiments in this study also revealed that 1) mesh burden was decreased with pore expansion and increased with pore contraction and 2) auxetic meshes manufactured from PDMS, an elastomer, do not experience permanent deformation in response to cyclic loading. Overall, the results of this study demonstrated the feasibility of creating a synthetic mesh that meets the design criteria for novel meshes as outlined in this dissertation.
5.0 IN VIVO EVALUATION OF THE HOST RESPONSE TO AN ELASTOMERIC MESH

Since the inception of synthetic meshes for treatment of pelvic organ prolapse, not much has changed in regards to the design of these products. Over the years, manufacturers have learned that knitted, lightweight, and larger pore meshes are more favorable (associated with less complications) relative to woven, heavyweight, and smaller pore meshes [51,79,95,119-121]. Meshes were used by surgeons to improve upon the durability of native tissue repairs, which have high failure rates. Unfortunately, prolapse and incontinence meshes were 510k devices and could be introduced into the market based on similarity to a predicate device with little to no preclinical testing. As synthetic meshes were shown to be safe and effective in the repair of abdominal hernias, it was assumed that they would be safe and effective for urogynecologic repairs. While similar types of complications occurred in these two surgeries, the rate of complications was higher in urogenital applications. In addition, women who experienced complications often required surgical removal of mesh in one or more surgeries. As the number of complications reported in the MAUDE database increased, the FDA released two public health notifications (PHNs); the first in 2008 and a second in 2011. Not surprisingly, the PHNs prompted multiple lawsuits and eventually a multi-district litigation forcing many companies to abandon their products based on financial decisions and not on science. More recently, the FDA up-classified transvaginal prolapse meshes from Class II to Class III devices, requiring that small clinical trials meet premarket
approval guidelines. Thus, the current state of affairs provides an unprecedented opportunity for companies/manufactures, even researchers, to design a mesh that is specifically tailored for prolapse repairs, which is the overarching aim of this dissertation. Up until this point, the mechanical benefits of pore expansion with an associated decrease in mesh burden were demonstrated with auxetic pores. Additionally, an elastomeric material (polydimethylsiloxane, PDMS) afforded the creation of a mesh that 1) has a material stiffness that is similar to that of the vagina, 2) has shape memory, and 3) does not permanently deform with repetitive loading. Thus, an initial prototype mesh has been developed that overcomes the current problems with prolapse meshes. However, the host response to an elastomeric auxetic mesh has not been defined; therefore, the primary focus of this chapter is to understand the host response to an elastomeric mesh and to determine how this response compares to the host response of a commonly used polypropylene prolapse mesh.

5.1 INTRODUCTION

Expanded polytetrafluoroethylene (ePTFE), polyethylene terephthalate, and polypropylene are some of the materials that have been or are currently utilized in the construction of urogynecologic meshes. However, in response to the high complication rates observed with ePTFE and polyethylene terephthalate, polypropylene has been the primary material utilized for prolapse meshes. Despite a lower complication rate, polypropylene mesh is not without complications. Pain and mesh exposure are the two most commonly reported complications [60]. Others include mesh shrinkage/contraction, erosion into an adjacent structure, recurrent prolapse, infection, and dyspareunia [82-84,112-117,200,201]. Although the exact mechanism(s) by which mesh
complications occur is not known, pore collapse and bridging fibrosis have been identified as potential pathways leading to mesh shrinkage/contraction and pain. Assuming a mesh is placed flat, prior to the integration of tissue, “early” mesh shrinkage is likely a result of pore collapse due to the application of tension and \textit{in vivo} loads. A decrease in pore size is detrimental to the host response given that smaller pores are associated with poor tissue integration, increased inflammation, and increased fibrotic response relative to larger pores [79,119-121]. For polypropylene hernia meshes, the optimal pore size has been shown to be 1 mm. When pore sizes decrease below 1 mm, the chances of bridging fibrosis increases [79]. Bridging fibrosis is a consequence of the merging of the foreign body response around neighboring mesh fibers, which results in fibrous encapsulation of the mesh. It is believed that contraction of the myofibroblasts within the fibrous capsule generates tension on the mesh, which can also lead to mesh contraction and pain [202-206].

From a mechanics perspective, polypropylene is not the ideal material for prolapse meshes. First, the stiffness of polypropylene is orders of magnitude greater than that of vaginal tissue (GPa vs MPa, respectively). This difference predisposes polypropylene mesh to cause stress shielding, a phenomenon associated with a maladaptive remodeling response characterized by tissue degeneration and atrophy [123-128]. Indeed, deterioration in vaginal structure and function associated with degradation of collagen and elastin and atrophy of smooth muscle was observed when polypropylene prolapse mesh was implanted into the vagina of non-human primates [129,131]. Such a negative tissue response likely increases the risk of mesh exposure. Secondly, polypropylene meshes permanently deform in response to loading [70,71]. This behavior is in direct contrast to the vagina, and most tissues within the body, which have the ability to recoil due to their elastin content (i.e. they can withstand \textit{in vivo} loads and deform without undergoing
permanent deformation). Given these two limitations, manufacturing mesh from an elastomeric polymer is more applicable for prolapse applications. The mechanical properties, specifically the material stiffness, of elastomeric materials could be tuned to that of vaginal tissue. Additionally, the shape memory of these materials would allow a mesh to return to its original configuration in response to sudden and repetitive changes in force. The two mechanical benefits mentioned previously were confirmed in Chapter 3 of this dissertation through the development and mechanical characterization of an elastomeric mesh manufactured from PDMS. However, these benefits are meaningless if an elastomeric mesh is not well tolerated within the body. Thus, the primary objective of this study was to evaluate the overall host response to an elastomeric mesh. Given the importance of pore size, it was also important to determine the impact of pore size on the host response to an elastomeric material. To accomplish these objectives, the following two elastomeric meshes (manufactured from PDMS, a soft elastomer) with differing auxetic pore sizes were implanted into the abdomen of rats for 35 days: Bowtie 0° 1.0 mm (pore size = 1.0 mm) and Bowtie 0° 1.5 mm (pore size = 1.5 mm). At 35 days, the host response was compared between the two meshes using hematoxylin and eosin (H&E), Masson’s trichrome, and picrosirius red staining. It is hypothesized that there will be a more favorable host response characterized by a decrease in inflammation, decreased fibrosis, and improved quality of tissue between pores for the Bowtie 0° 1.5 mm meshes relative to the Bowtie 0° 1.0 mm meshes. Specifically, cellularity and vascularity were proposed to increase with thinner collagen fibers present between the pores with the larger pore mesh. This is in contrast with what we typically see for commercially available polypropylene meshes, which tend to be encapsulated by thick highly aligned collagen fibers with minimal collagen or tissue between fibers. Thus, for comparison, Gynemesh PS (Ethicon, Somerville, NJ, USA), the prototype prolapse mesh, was also implanted into the abdomens of rats for 35 days and
similar histologic analyses were performed as described for the elastomeric meshes. Relative to both elastomeric meshes, it was anticipated that the fibrotic response to Gynemesh PS would increase. Specifically, bridging fibrosis and encapsulation would be present around the fibers of Gynemesh PS, particularly in the case of knots, but this would not occur with the elastomeric mesh. Thick, densely packed, highly aligned collagen fibers would be interpreted as evidence of fibrosis and encapsulation.

5.2 METHODS

5.2.1 Animals

Twenty-four female Long-Evans rats approximately four to nine months old were utilized in this study in accordance with the University of Pittsburgh Animal Care and Use Committee (IACUC #15117003). Animals were housed with a 12-h alternating light-dark cycle and were allowed access to food and water ad libitum.

5.2.2 Mesh In vivo Implantation

To assess the host response to elastomeric meshes, Bowtie 0° meshes with two different pore sizes were implanted into the abdominal wall of rats: Bowtie 0° 1.0 mm and Bowtie 0° 1.5 mm. The width and thickness of the fibers was the same for both meshes while the pore size differed between the two with Bowtie 0° 1.0 mm having a pore size of 1.0 mm and Bowtie 0° 1.5 mm a pore size of 1.5 mm (Figure 78).
Figure 78: CAD model of a single pore from the Bowtie 0° mesh illustrating pore size. Bowtie 0° meshes were manufactured with pore sizes of 1.0 mm or 1.5 mm.

The same methods used to manufacture the elastomeric meshes with auxetic pores from PDMS for mechanical testing in Section 4.3.2.1 were also used to manufacture the meshes for this study. In addition to implanting the Bowtie 0° meshes, Gynemesh PS (Ethicon, Somerville, NJ, USA) was implanted into the abdomen of rats for qualitative (and quantitative when appropriate) comparison. Prior to implantation, all meshes were sterilized with ethylene oxide. In total, n=8 rats per mesh were implanted.

On the day of surgery, rats were anesthetized with isoflurane and the surgical site (abdomen) was shaved and scrubbed with povidone-iodine. Sterile drapes were placed on the rats leaving the surgical site exposed. A midline incision was made in the skin from the xyphoid superiorly to the pubic symphysis inferiorly. Next, the underlying subcutaneous fat was carefully dissected away exposing the anterior rectus fascia and two pieces of mesh (1.5 cm x 1.5 cm) were
implanted (two per side) away from midline leaving the contralateral side as sham (dissected but no mesh implanted) or control (no dissection and no mesh implanted) (Figure 79).

![Figure 79: Image of Bowtie 0° mesh and Gynemesh PS prior to implantation (a). Actual image of the Bowtie 0° mesh taken immediately after the mesh was implanted on the abdominal wall of a rat prior to closing (b). Two pieces of mesh were implanted, one proximal to the other.]

Meshes were then secured with resorbable suture (4-0 Vicryl, Ethicon, Sommerville, NJ) at the four corners or with a continuous suture along two opposing sides of the mesh. Following implantation, the skin was closed with wound clips or a combination of continuous sutures and wound clips. At the conclusion of 35 days, mesh-tissue complexes were excised, fixed in formalin, embedded in paraffin, and sectioned at 7 μm for histologic analysis.
5.2.3 Host Response to Synthetic Meshes

The host response was evaluated via histological staining with H&E, in conjunction with the Histology Core at Magee Womens Research Institute, Pittsburgh, PA. Briefly, slides were deparaffinized, hydrated in water, and then stained with SelecTech Hematoxylin 560 (Leica Biosystems, Buffalo Grove, IL) for 5 minutes. Slides were then rinsed in running tap water, placed in SelecTech Define MX-aq (Leica Biosystems, Buffalo Grove, IL), and rinsed again with each step performed for 1 minute. Next, slides were stained with SelecTech Blue Buffer 8 (Leica Biosystems, Buffalo Grove, IL) for 1 minute and rinsed with running tap water for 1 minute. Finally, slides were dipped 3 times in 70% ethanol, counterstained in Eosin with Phloxine for 30 seconds, dehydrated, cleared, and then mounted. For the elastomeric meshes, 20X images of the mesh pores were imaged using a Nikon Eclipse 90i imaging microscope (Melville, NY, USA). The analysis of these images focused on the host response to the fiber and the tissue within the pores (between fibers). Specifically, the pore area (i.e. the area of the tissue between the pores, which is essentially a measure of the amount of tissue ingrowth) and pore width (i.e. the perpendicular distance between two adjacent fibers, measured from the midpoint of one fiber to the next) were measured using ImageJ 1.49v (National Institutes of Health, Bethesda, MD). The number of blood vessels and foreign body giant cells was quantified via manual counting using a blinded-investigator. Only blood vessels with a defined lumen were counted. Cellularity was quantified using ImageJ plug-in Colour Deconvolution and the Analyze Particle function. Images of the tissue between the knots and fibers of Gynemesh PS meshes were also acquired at 20X.
5.2.4 Qualitative Assessment of Bridging Fibrosis and Encapsulation

Overall connective tissue deposition and the fibrotic response were qualitatively assessed using Masson’s trichrome staining. Slides were first deparaffinized with xylene and then exposed to a series of ethanol solutions with decreasing concentration, from 100% to 70%. Following deparaffinization, slides were placed in 1X PBS followed by 95% ethanol for 10 minutes each. Slides were then air dried at room temperature for 1 hour. After drying, slides were stained with warm Bouin’s Solution (Sigma-Aldrich, St. Louis, MO) for 1 minute, rinsed, and then stained with Hematoxilin (Sigma-Aldrich, St. Louis, MO) for 5 minutes. Slides again were rinsed, stained with Trichrome Stain AB Solution (Sigma-Aldrich, St. Louis, MO) for 2 minutes, and then rinsed. Lastly, slides were dehydrated in ethanol (95%-100%), placed in xylene, and then were cover slipped using xylene based mounting media. For the Bowtie 0° meshes, 20X images of the tissue within the pores were imaged using a Nikon Eclipse 90i imaging microscope (Melville, NY, USA). Images (20X) were also taken of the tissue between the knots and fibers for Gynemesh PS. For this portion of the experiment, bridging fibrosis was defined as an overlap of the foreign body capsule of one fiber with that of a neighboring fiber. A mesh was considered to be encapsulated if bridging fibrosis was present in >75% of the fibers analyzed (1 to 4 fields were analyzed per an image).

5.2.5 Quantification of the Fibrotic Response – Collagen Fiber Thickness

The total amount of collagen and thickness of collagen fibers was assessed using picrosirius red staining. Prior to staining, slides were first deparaffinized with xylenes and then exposed to a series of ethanol solutions with decreasing concentration, from 100% to 70%. Slides were then stained
with Picro-Sirius Red Solution (0.1 g Direct Red 80, Sigma-Aldrich, St. Louis, MO and 500 mL Saturated Aqueous Picric Acid, Arlington, TX) for 1 hour at room temperature. After staining, slides were decolorized in acetic acid water (35% acetic acid) for 10 seconds and then exposed to a series of ethanols from 95%-100% and xylene. Finally, Permount (Electron Microscopy Sciences, Hatfield, PA) was applied and the slides were cover slipped. For the Bowtie 0° meshes and Gynemesh PS, 20X images of the tissue within the pores were imaged using polarized light imaged with a Nikon Eclipse 90i imaging microscope (Melville, NY, USA). A custom Matlab script (The Mathworks, Natick, MA, USA), written by Christopher A, Carruthers (PhD graduate of the University of Pittsburgh, Bioengineering Department), was used to separate the collagen fibers based on hue (green, yellow, orange, and red). Each hue is representative of fiber thickness in which the thinnest fibers are green, and the remaining fibers in order of increasing thickness are yellow, orange, and red. From this analysis, the total amount of collagen and the percent of red, orange, yellow, and green fibers as well as the ratio of red to green fibers were quantified.

5.2.6 Statistics

Based on a study by Wolf et al (2013), it was estimated that a sample size of at least four is needed to detect significant differences in the total amount and fiber size of collagen deposited between mesh fibers [63]. Kolmogorov-Smirnov tests were used to determine whether the data was normally distributed. The following parameters were compared between the Bowtie 0° 1.0 mm and Bowtie 0° 1.5 mm meshes using a one-way independent MANOVA followed by univariate ANOVAs with a Bonferroni correction (p-value < .008 for significance): pore width, total amount of collagen, percent of red, orange, and yellow fibers, and the ratio of red to green fibers. Non-parametric Kruskal Wallis tests with a Bonferroni correction (p-value < .0125 for significance)
were used to compare the pore area, cellularity, vascularity, and the percent of green fibers between
the Bowtie 0° 1.0 mm and Bowtie 0° 1.5 mm meshes. Additionally, collagen was also compared
between Bowtie 0° 1.0 mm, Bowtie 0° 1.5 mm, and Gynemesh PS. Specifically, the total amount
of collagen (normalized to pore area), and the percent of red and yellow fibers were compared
using one-way independent MANOVA followed by univariate ANOVAs with a Bonferroni
correction (p-value < .017 for significance) and a Gabriel’s pairwise post-hoc test (when
appropriate). The ratio of red to green fibers and the percent of orange and green fibers were
compared between the three meshes using Kruskal Wallis tests with a Bonferroni correction (p-
value < .017 for significance). All statistical analyses were performed using SPSS 24 (IBM,
Armonk, NY, USA).

5.3 RESULTS

Mesh samples were implanted in the abdomens of rats for approximately 35 days. Initially, n = 8
rats per group for a total of 24 rats (Gynemesh PS n=8, Bowtie 0° 1.0 mm n=8, and Bowtie 0° 1.5
mm n=8). However, after implantation, the wounds of some rats opened (dehisced), and as a result,
these rats were euthanized early. Additionally, one rat developed a seroma and was excluded from
analysis. Thus, at the time of explant, n=6 for Bowtie 0° 1.0 mm, n=7 for Bowtie 0° 1.5 mm, and
n=7 for Gynemesh PS.

For inclusion in our in vivo analysis, we required the following: pores with defined fiber
borders and tissue without PDMS debris were included. This criterion was set as it was determined
that loose pieces of PDMS (resulting from cutting the mesh or removing the mesh from the mold)
within the pores resulted in the histologic appearance of a hole in the tissue, and induced an
independent foreign body response that we thought should not be included in the analysis. Secondly, although we attempted to imbed and cut meshes so that the fibers were perpendicular to the cut edges, some sections contained 1 or 2 pores while others had more. Thus, to get an overall idea of the host response to the Bowtie 0° meshes, the response for each pore, per tissue section per animal was averaged.

5.3.1 Comparison of Pore Area and Pore Width: Bowtie 0° Meshes

Overall, there was no statistically significant difference between the pore area (i.e. the amount of tissue between the pores) for the Bowtie 0° 1.0 mm and Bowtie 0° 1.5 mm meshes, 1.05 (0.98) mm² and 1.13 (0.37) mm², respectively, p-value = .886. There was a 6.0% difference between the pore widths for the two meshes, 1.14 ± 0.54 mm (1.0 mm meshes) and 1.21 ± 0.75 mm (1.5 mm meshes); however, this difference was not statistically significant, p-value = .862. This result may be somewhat surprising given that one would expect a greater difference between the pore area and pore width for the two different Bowtie 0° meshes. The lack of such difference is largely due to the way in which the mesh-tissue explant was cut. Depending on where the mesh-tissue explant section was cut in reference to the bowtie, the distance between adjacent fibers varied and resulted in some 1.0 mm mesh pores having a comparable or greater pore area and pore width than that of the 1.5 mm mesh pores (Figure 80). This indeed was the case for many of the pores.
Figure 80: CAD model of a Bowtie 0° mesh, single pore demonstrating how the distance between adjacent fibers can vary depending on where along the pore the tissue section was cut (dashed-yellow lines).

In this image, the distance between adjacent fibers is greatest for cut 2 and the least for cut 3.

5.3.2 Macroscopic and Microscopic Appearance of Mesh-Tissue Explants

Mesh samples (Bowtie 0° 1.0 mm, Bowtie 0° 1.5 mm, and Gynemesh PS) were all well incorporated into the host tissue and laid flat against the abdomen (Figure 81a and 81b). Gynemesh PS, however, appeared to be encapsulated based on gross inspection. These observations were confirmed microscopically as the deposition of loose host tissue between the pores of the Bowtie 0° meshes was evident following Masson’s trichrome staining (81d). Surrounding and between the mesh fibers of Gynemesh PS was dense connective tissue (Figure 81c). Additionally, there was no evidence of mesh contraction for any of the Bowtie 0° meshes (measurements of mesh border dimensions at the time of implant and explant, data not shown, verified the absence of mesh contraction). Similarly, the measurements of Gynemesh PS at the time of implant and explant were
the same (data not shown), indicating absence of mesh contraction. The absence of mesh contraction was expected given that the meshes were implanted abdominally with no tension applied.

Figure 81: Gynemesh PS (a) and Bowtie 0° mesh (b) were well incorporated into the abdominal wall of the rat after 35 days of implantation. Representative Masson’s trichrome sections of Gynemesh PS (c) demonstrating the deposition of dense connective tissue between mesh fibers (MFs) and of Bowtie 0° mesh (d) demonstrating the deposition of loose connective tissue between the MFs.
5.3.3 Characterization of the Host Response to Synthetic Meshes

The host response to Bowtie 0° meshes (1.0 mm and 1.5 mm) was evaluated using H&E and Masson’s trichrome staining focusing on the mesh-tissue interface and the tissue between the fibers (i.e. newly deposited tissue within the pores). Overall, the host responses between the 1.0 mm and 1.5 mm meshes were similar and characteristic of the default host response to a foreign material. At the mesh-tissue interface, the host cellular response consisted of approximately 1 to 5 cell layers of primarily rounded mononuclear (likely macrophages) and spindle-shaped cells (likely myofibroblasts) with only a few foreign body giant cells present (Figure 82). The vascular response was minimal with a small number of blood vessels present at the surface. Dense connective tissue was observed along the mesh-tissue interface while loose connective tissue was observed within the pores of the mesh (Figure 83).
Figure 82: The host cellular response consisted of predominantly rounded mononuclear and spindle-shaped cells with only a few foreign body giant cells and blood vessels at the mesh-tissue interface. Representative H&E images of the mesh-tissue interface for Bowtie 0° 1.0 mm (left) and 1.5 mm (right).

Figure 83: Dense connective tissue was observed along the mesh-tissue interface whereas loose connective tissue was deposited between the pores of the Bowtie 0° 1.0 mm (a) and 1.5 mm (b) meshes.
Newly incorporated tissue was present within the pores of the Bowtie 0° meshes with a similar appearance to control and sham tissue (Figure 84). Unlike the response at the surface of the mesh, newly formed blood vessels were observed between the pores of the meshes (Figure 84a and 84b). Additionally, foreign body giant cells were scarce within the pores of either mesh. The cellular response consisted of mainly mononuclear cells with adipose present between some but not all of the mesh pores (Figure 84a and 84b).

Figure 84: Newly incorporated tissue within the pores of the Bowtie 0° meshes was similar to that of control and sham tissue. A prominent vascular response was also observed. Mainly mononuclear cells were present within the pores with some pores containing adipose. Representative H&E tissue sections of Bowtie 0° 1.0 mm (a), 1.5 mm (b), 1.0 mm – Sham (c), and Bowtie 1.5 mm – Control (d).
There was no significant difference in the number of cells and blood vessels present within the pores of the two Bowtie 0° meshes. Specifically, the number of cells for the 1.0 mm meshes was 6042.50 (8392.88) compared to the 1.5 mm meshes with 5752.00 (2347.00) cells, p-value = .886. Additionally, the 1.0 mm meshes had 91.22 ± 22.49 blood vessels relative to the 85.14 ± 64.24 blood vessels contained within the 1.5 mm meshes, p-value = .475.

5.3.4 Bridging Fibrosis and Encapsulation

Masson’s trichrome and picrosirius red staining were used to evaluate the fibrotic response to the Bowtie 0° meshes. Overall, collagen consisting of a mixture of thick and thin fibers was incorporated throughout the pores of the Bowtie 0° 1.0 mm and Bowtie 0° 1.5 mm meshes (Figure 85). Additionally, a collagen capsule immediately adjacent to the mesh fibers was visibly apparent for a portion of the PDMS meshes (Figure 85a and 85b). As expected, this capsule consisted of mainly thick collagen fibers, evident by the red staining adjacent to the mesh fibers (Figure 85c and 85d). Similar results were observed for Gynemesh PS, in which collagen, consisting of thick and thin fibers, was incorporated between the fibers of the mesh with a dense collagen capsule surrounding the mesh knots (Figure 86).
Figure 85: Representative images of Bowtie 0° 1.0 mm (a, c) and Bowtie 0° 1.5 mm (b, d) tissue sections stained with Masson’s trichrome (top images) and picrosirius red (bottom images) demonstrating collagen incorporation between the pores of the mesh. A collagen capsule (arrows) is seen adjacent to the mesh fibers (MF). Although, a capsule is not clearly seen in (b). Collagen fibers in order of decreasing thickness, red>orange>yellow>green.
Figure 86: Collagen is incorporated between the fibers and knots of Gynemesh PS. A collagen capsule (arrows) is seen adjacent to the mesh fiber and knots. Representative tissue sections stained with Masson’s trichrome (a,c) and picrosirius red (b,d). *Single mesh fiber. **Multiple mesh fibers. Collagen fibers in order of decreasing thickness, red>orange>yellow>green.
Importantly, the inflammatory response to the elastomeric fibers in either the 1.0 mm or 1.5 mm size meshes did not overlap with the response to a neighboring fiber indicating an absence of bridging fibrosis and hence, a lack of evidence of mesh encapsulation. In contrast, the cellular response to Gynemesh PS overlapped, particularly when mesh fibers were close together (i.e. mesh knots). A dense collagen shell surrounded the fibers of Gynemesh PS, consistent with bridging fibrosis and encapsulation (Figure 87).

Figure 87: Masson’s trichrome staining of a Gynemesh PS knot. Bridging fibrosis (i.e. the merging of the cellular response around adjacent fibers, arrows) was often observed with the fibers and knots of Gynemesh PS. This response was also associated with a dense collagen shell (blue staining) surrounding the fibers and knots, which is consistent with encapsulation. *Single mesh fiber.
5.3.5 Quantitative Assessment of Fibrosis

Fibrosis was also assessed via quantification of the amount of collagen and collagen fiber thickness using polarized light microscopy after picrosirius red staining. There was a trend toward an increase in the total amount of collagen in the pores of the Bowtie 0° 1.0 mm meshes as compared to the Bowtie 0° 1.5 mm meshes; however, this difference did not reach statistical significance (p-value = .074). Similarly, the percentage of red, orange, yellow, and green collagen fibers as well as the ratio of red to green fibers were not significantly different between the two meshes (Table 15).

Table 15: Total collagen and collagen structure within the pores of the Bowtie 0° 1.0 mm and 1.5 mm meshes. Data represented as mean ± standard deviation or median (interquartile range). aP-value obtained via univariate ANOVAs with a Bonferroni correction (p-value < .008 for significance) or bKruskal-Wallis Test with a Bonferroni correction (p-value < .0125 for significance).

<table>
<thead>
<tr>
<th></th>
<th>Bowtie 0° 1.0 mm</th>
<th>Bowtie 0° 1.5 mm</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Total Collagen (%)</td>
<td>37.75 ± 10.22</td>
<td>28.68 ± 6.20</td>
<td>.074a</td>
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<tr>
<td>Red Fibers (%)</td>
<td>32.75 ± 10.13</td>
<td>29.94 ± 7.92</td>
<td>.585a</td>
</tr>
<tr>
<td>Orange Fibers (%)</td>
<td>56.25 ± 5.43</td>
<td>55.48 ± 2.41</td>
<td>.738a</td>
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<tr>
<td>Yellow Fibers (%)</td>
<td>7.40 ± 3.61</td>
<td>9.81 ± 3.48</td>
<td>.248a</td>
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<tr>
<td>Green Fibers (%)</td>
<td>2.77 (3.60)</td>
<td>3.59 (3.20)</td>
<td>.475b</td>
</tr>
<tr>
<td>Red/Green Ratio</td>
<td>14.73 ± 11.30</td>
<td>14.46 ± 10.33</td>
<td>.964a</td>
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</table>
The fibrotic response to the elastomeric meshes was also compared to Gynemesh PS. Given that the majority of the pores of Gynemesh PS were an order of magnitude less than the Bowtie 0° mesh pores, the total amount of collagen was normalized to the pore area (also referred to as normalized total collagen). Overall, there was a significant difference in the normalized total collagen between the three meshes, p-value = .001 (Figure 88 and Table 16). Specifically, Gynemesh PS had 34% more collagen fibers per area than the Bowtie 0° 1.5 mm, p-value = .004. Similarly, there was 38% more collagen per area within the pores of the Bowtie 0° 1.0 mm meshes relative to the Bowtie 0° 1.5 mm meshes, p-value = .002. Gynemesh PS had the highest percentage of thick collagen fibers and the ratio of red to green fibers for Gynemesh PS was also the largest; however, there was no significant difference between the fiber thicknesses or the ratio of red to green fibers between the three meshes (Table 16).

Figure 88: Gynemesh PS and Bowtie 0° 1.0 mm meshes had significantly more collagen than Bowtie 0° 1.5 mm meshes, p-value = .004 and .002, respectively.
Table 16: Comparison of collagen fiber thickness and the ratio of red to green fibers between Gynemesh PS, Bowtie 0° 1.0 mm, and Bowtie 0° 1.5 mm meshes. Data represented as mean ± standard deviation\textsuperscript{a} or median (interquartile range)\textsuperscript{b}. P-value obtained from univariate ANOVAs\textsuperscript{c} or Kruskal Wallis\textsuperscript{d} tests with a Bonferroni correction (p-value < .017 for significance).

\begin{center}
\begin{tabular}{|l|c|c|c|c|}
\hline
 & Gynemesh PS & Bowtie 0° 1.0 mm & Bowtie 0° 1.5 mm & p-value \\
 & (n=7) & (n=6) & (n=7) & \\
\hline
Red (%)\textsuperscript{a} & 35.61 ± 10.05 & 32.75 ± 10.13 & 29.94 ± 7.92 & .539\textsuperscript{c} \\
Orange (%)\textsuperscript{a} & 56.35 ± 7.37 & 56.25 ± 5.43 & 55.48 ±2.41 & .767\textsuperscript{d} \\
Yellow (%)\textsuperscript{a} & 5.55 ± 2.22 & 7.4 ± 3.61 & 9.81 ± 3.48 & .064\textsuperscript{e} \\
Green (%)\textsuperscript{b} & 2.96 (2.00) & 2.77 (3.60) & 3.59 (3.20) & .370\textsuperscript{d} \\
Red:Green\textsuperscript{b} & 26.98 (84.18) & 10.89 (21.22) & 17.30 (18.90) & .260\textsuperscript{d} \\
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\section{5.4 DISCUSSION}

The primary purpose of this study was to evaluate the host response to elastomeric meshes and to determine if pore size impacts this response. Overall, the host response to elastomeric meshes was consistent with the default host response to a foreign material. Specifically, the cellular response at the surface of Bowtie 0° 1.0 mm and 1.5 mm meshes consisted of primarily rounded mononuclear and spindle-shaped cells with few foreign body giant cells. Additionally, normal host tissue was well integrated within the pores of the elastomeric meshes with evidence of neovascularization. In comparing the Bowtie 0° 1.0 mm and 1.5 mm meshes, there were no differences observed in the cellular response at the surface or in the degree of cellularity and
vascul arity between these two meshes. Additionally, the total amount of collagen and collagen fiber thickness were not significantly different. However, there was less collagen between the pores of the Bowtie 0° 1.5 mm meshes which may suggest that bigger pores may be better, particularly since these pores will expand under tension. Overall, these results suggest that the pore size of 1.0 mm was large enough for an optimal host response and that increasing the pore size further had no apparent benefit. It is possible however that decreasing the pore size will negatively impact the host response, as seen with polypropylene meshes, but that the pore size of the meshes tested in this study had already approached the value needed for an optimal host response. To explore this idea, future studies implanting elastomeric meshes with a wide range of pore sizes should be explored. It is anticipated that there will be a minimum pore size at which a non-favorable host response characterized by increased inflammation and fibrosis will be observed similar to polypropylene meshes.

The fibrotic response is a natural phenomenon that occurs following the implantation of a biomaterial into the body. Overall, the deposition of fibrotic tissue surrounding, and within the pores of porous biomaterials can positively impact the foreign body response by providing support to the implant and aiding integration of the implant with the host tissue. However, fibrotic tissue deposition can also impede the function of a biomaterial if excessive. One common example of this concept is demonstrated with breast argumentation. When a non-degradable biomaterial is implanted into the body, it becomes surrounded/encapsulated by fibrous connective tissue, which functions to separate the biomaterial from the surrounding tissues. In the case of breast implants, this capsule of fibrous tissue helps to anchor the implant in place. However, when an excessive amount of fibrous tissue forms around the implant, capsular contraction can occur. Capsular contraction is a leading complication of breast implants and can ultimately lead to deformed, firm,
and painful breasts [207]. Similar to capsular contraction of breast implants, contraction of prolapse meshes is also problematic for women. Clinically, contraction of prolapse meshes is associated with vaginal pain, dyspareunia (pain with sexual intercourse), and tenderness upon palpation of the contracted portion of the mesh [82,85]. A direct link has not been well established between fibrosis and pain for prolapse meshes. However, recent research investigating the macrophage response to mesh removed from women for pain found a positive correlation between IL-10 (a profibrotic cytokine) and the percentage of M2 polarized macrophages [208]. Additionally, histology of a mesh removed from a woman for a primary compliant of pain shows high mesh density surrounded by a thick fibrous capsule that is consistent with encapsulation and mesh contraction/shrinkage (Figure 89).

Figure 89: Mesh removed from a woman with a primary compliant of pain. A high density of mesh fibers (arrows) are surrounded by a thick fibrous capsule (stars) which is consistent with encapsulation and mesh contraction/shrinkage.
Collectively, these findings provide evidence that fibrosis may be a source of pain for prolapse meshes. Thus, to determine how the fibrotic response to an elastomeric mesh compares to a polypropylene mesh, the fibrotic response to the Bowtie 0° meshes was also compared to that of Gynemesh PS. As anticipated, bridging fibrosis was observed for Gynemesh PS but not for the Bowtie 0° meshes. The lack of bridging fibrosis for the elastomeric meshes is likely a benefit of the design of these meshes. The elastomeric meshes did not contain knots and the pores of these meshes are more open relative to Gynemesh PS, allowing for adequate space to accommodate the foreign body response (Figure 90).

![Figure 90: The pores of the Bowtie 0° mesh are more open relative to Gynemesh PS, and the Bowtie 0° mesh does not contain knots in contrast to Gynemesh PS.](image)

Alternatively, the absence of bridging fibrosis may also be related to the size of the pores analyzed. The smallest distance analyzed between fibers for the Bowtie 0° meshes was 0.14 mm whereas fibers for Gynemesh PS were as close as 0.01 mm. It is possible that the pores of the Bowtie 0°
meshes were not close enough to observe bridging fibrosis. Assessment of the fibrotic response via quantification of the amount of collagen deposited within the pores of the three meshes corroborates this idea. Despite the Bowtie 0° 1.0 mm pores having a similar amount of collagen per area as Gynemesh PS, bridging fibrosis was not observed and this is likely due to the increase in pore width for the 1.0 mm meshes. The average width of the pores analyzed for the Bowtie 0° 1.0 mm meshes was nearly 80% wider than that of Gynemesh PS. Similar to the Bowtie 0° 1.0 mm meshes, the average width of the Bowtie 0° 1.5 mm mesh pores was 85% wider than that of Gynemesh PS, yet the amount of fibrosis for the Bowtie 0° 1.5 mm mesh pores was significantly reduced relative to Gynemesh PS. Interestingly, significantly less fibrous tissue was deposited between the pores of the Bowtie 0° 1.5 mm meshes relative to the Bowtie 0° 1.0 mm meshes, despite the fact that the pore width between the Bowtie 0° meshes were not significantly different. This result suggests that the higher pore size of 1.5 mm is associated with a decreased fibrotic response, which is consistent with the abdominal hernia literature [79,119].

An in-depth characterization of the host cellular response to the Bowtie 0° meshes and how this response compares to Gynemesh PS was not performed in this study. This is largely because the primary purpose of this dissertation was to develop a prototype mesh that displayed specific mechanical behaviors (i.e. pore expansion and decreased mesh burden with tensile loading and absence of permanent deformation with sudden and repetitive loading) and mechanical properties (i.e. material stiffness that is similar to vaginal tissue). The design of the meshes developed in this dissertation has not been optimized. Additionally, pore size is identified as a critical parameter for determining the host response to synthetic mesh; thus, the primary goal of this in vivo study was to assess the impact of pore size on the host response with the novel mesh developed in this dissertation. However, interesting results were observed when qualitatively comparing the
response of Bowtie 0° meshes to Gynemesh PS. There was a noticeable difference between the cellular response of the Bowtie 0° meshes and Gynemesh PS. The cells aligning the mesh-tissue interface were predominantly rounded mononuclear and spindle shaped cells for the Bowtie 0° meshes whereas the cells within the mesh-tissue interface for Gynemesh PS were mainly rounded mononuclear and multinucleated giant cells (Figure 91). Spindle-shaped cells for Gynemesh PS were observed further away from the mesh surface. Additionally, macrophages lined the mesh-tissue interface for both the Bowtie 0° meshes and Gynemesh PS. Previous studies comparing silicone to polypropylene for glaucoma drainage devices have found that the inflammatory response to polypropylene is more than that to silicone [209,210]. In this study, the inflammatory response was noticeably denser around the knots of Gynemesh PS relative to the fibers of the Bowtie 0° meshes; however, the same qualitative statement could not be made when comparing the inflammatory response around a single fiber of Gynemesh and the Bowtie 0° meshes. Thus, future studies quantifying the inflammatory response around each fiber will aid in determining whether there is a difference in the inflammatory response between the two meshes as found with glaucoma drainage devices.
A noticeable difference was observed in the cellular response between the Bowtie 0° mesh (a) and Gynemesh PS (b). The cells at the mesh-tissue interface are primarily rounded mononuclear and spindle shaped cells whereas the interface for Gynemesh PS contained mainly rounded mononuclear and multinucleated giant cells.

Macrophages are the dominant immune cells that function to clear debris and to initiate the foreign body response following the initial recruitment of neutrophils [211,212]. They are classified as having diverse and plastic phenotypes that range along a spectrum between M1 (classically activated; proinflammatory) and M2 (alternatively activated; remodeling, homeostatic) [213-215]. M1 macrophages secrete reactive oxygen species, proinflammatory cytokines, and chemokines; thus, tissue damage and destruction can occur with long-term persistence of M1 macrophages. M2 macrophages on the other hand secrete anti-inflammatory immune modulators and growth factors, and they are involved in the constructive healing and remodeling phase of the response to a foreign body, which results in tissue deposition and in growth [213,215-217]. Excessive tissue deposition and fibrosis can occur if the M2 response is too strong [218]. Previous studies have found that M1 macrophages are the predominant phenotype at the mesh-tissue interface of polypropylene meshes [151,219]. The predominant macrophage
phenotype to an elastomeric mesh is currently unknown. Given the importance of macrophages in the host response to biomaterials, future studies characterizing the phenotype of macrophages are warranted. It is hypothesized that the Bowtie 0° meshes will have less M1 macrophages and an increased M2/M1 ratio relative to Gynemesh PS. This is based on a study conducted by Blakney et al (2012), which demonstrated that stiffer substrates are associated with increased macrophage activation and have a more pronounced and abnormal host response relative to softer substrates [189].

One of the most interesting findings of this study is that the fibrotic response to the Bowtie 0° meshes was less than that of Gynemesh PS. This finding makes sense if one approaches the analysis of this result focusing only on the differences in pore size. However, when one considers the finding from the abdominal hernia literature which states that less material (i.e. lightweight mesh) is associated with a more favorable host response than heavyweight mesh, the decreased fibrotic result observed with the Bowtie 0° meshes, especially the 1.5 mm mesh, is not consistent with this finding [79,81,134,135]. The thickness and the overall amount of material implanted for the Bowtie 0° meshes was much greater than that of Gynemesh PS. Based on this alone, the fibrotic response to Gynemesh PS should be less; however, this was not the case. The data suggests that there may be other factors contributing to the host response that was not accounted for in this study; for example, differences in material. In previous studies conducted by Ayyala et al (1999 and 2000), polypropylene glaucoma drainage devices were associated with an increased fibrotic response relative to silicone devices [209,210]. This result is consistent with our findings in this study. In addition to differences in material, the shape of the fibers could also be contributing to the differences observed in the fibrotic response between the meshes in this study. Ward et al (2002), found that the foreign body capsule surrounding implants, with a cylindrical shape, was
thinner for smaller implants relative to larger ones [220]. Conversely, an opposite affect was observed with spherical implants. Smaller spheres were associated with an increased foreign body reaction and fibrosis [221]. Relating these findings to the current study, the decreased fibrotic response observed with the Bowtie 0° meshes could be a result of the unique shape of the fibers. Future studies implanting the Bowtie 0° meshes with varying fiber thicknesses and shapes will help to determine how these parameters impact the fibrotic response for the Bowtie 0° mesh. Specifically, the fibers of the meshes implanted should be as small as those of Gynemesh PS and be as large as if not larger than the current Bowtie 0° meshes. Furthermore, in addition to maintaining the current shape of the Bowtie 0° mesh fibers, Bowtie 0° meshes with fibers that are in the shape of rods (i.e. in the shape of the Gynemesh fibers) should also be implanted.

There are a number of limitations with this study. First, an abdominal model was used to assess the host response to an elastomeric mesh, which is intended for urogynecologic applications. Since prolapse meshes are hernia meshes, the abdomen is an acceptable implantation site. However, the host response to a mesh in the abdomen is different from that in the vagina. For example, in this study Gynemesh PS was placed flat against the abdominal wall and was found to have maintained a flat configuration at the time of excision. In contrast, in the vagina, in which meshes are loaded primarily uniaxially, Gynemesh PS contracts due to pore collapse and buckles [75,77]. Additionally, mesh implanted against the vagina is associated with increased inflammation relative to mesh implanted in the abdomen [222]. It is therefore important that in future studies, an elastomeric mesh is implanted into the vagina in order to truly assess the host response to this type of mesh. Another limitation of this study is that the host response was analyzed at only one-time point, 35 days. Limiting the implant time to 35 days provides a cross sectional analysis of the host inflammatory response to mesh, which in reality begins at the time
of implantation and can persist for weeks [211]. Future studies should investigate the host response to mesh at early and late time points in order to provide a more accurate longitudinal assessment. The design of the Bowtie 0° meshes also limited the number of quantitative comparisons that could be made to Gynemesh PS. The thickness of the Bowtie 0° meshes was an order of magnitude greater than that of Gynemesh PS. Additionally, while Gynemesh PS contains knots, the Bowtie 0° meshes do not (they are solid constructs with pores). Normalizing by surface area is often a technique used when trying to compare various parameters (e.g. the number of macrophages or the thickness of the fibrous capsule surrounding a mesh fiber) between different meshes. This technique was not used in this study because the thickness of the Bowtie 0° mesh fibers were so different that there was a concern that any differences found in the host response between the meshes analyzed were more a factor of thickness and not the mesh material. Thus to avoid this, only qualitative comparisons were made when describing the host cellular and fibrotic response surrounding mesh fibers. Another potential way around this concern is to manufacture Bowtie 0° meshes that have the same fiber dimensions and thickness as Gynemesh PS.

5.5 CONCLUSION

Silicone (PDMS) is commonly known for its use as the inner core and outer shell of breast implants. Though popular with breast implants, manufacturing a mesh from silicone is not widely accepted amongst urogynecologists. This is largely due to concerns in the 1990s that there is an association between silicone breast implants and connective tissue diseases [223-225]. However, epidemiologic studies have not found any evidence to substantiate this claim [226-228]. Additionally, there is a misconception that “silicone meshes” are associated with high
complication rates and therefore, they are no longer in use [149,229]. Indeed, silicone was used in the past for synthetic meshes; however, silicone was used to coat polyester meshes, which are inherently associated with complications due to their woven, multifilamentous, and microporous structure. These meshes were not purely silicone. The results of this study revealed that the host response to silicone mesh is not as unfavorable as previously thought. Specifically, the default host response to a foreign material was observed with the silicon meshes developed in this dissertation; however, there was an absence of bridging fibrosis observed with silicone mesh. Additionally, the fibrotic response to the silicone meshes was less than that of Gynemesh PS. The difference between the host response to the silicone mesh in this study relative to previous “silicone meshes” is likely due to structural differences between the two devices. The previous “silicone meshes” were microporous relative to the meshes in this study, which were macroporous. This result further demonstrates the concept that larger pores are associated with an improved host response relative to smaller pores. However, reviewers of these data should keep in mind that the silicone used to manufacture the meshes in this study was not medical grade, the design of the meshes was not optimized, and the host response to a silicone mesh was not fully characterized (e.g. macrophage phenotype was not assessed, among other cell types). Thus, there is still more work to be done and future studies investigating alternative elastomeric polymers may find an alternative material that is superior to silicone.
6.0 CONCLUSION

6.1 SYNOPSIS

Pelvic organ prolapse (prolapse) is a common gynecologic condition; in fact the incidence rate of prolapse repairs is just as prevalent as ACL reconstructions [11,60,230,231]. However, despite the high prevalence of prolapse, many have never heard of this disorder, even women. Prolapse occurs when support to the pelvic organs, provided primarily by the vagina, is lost, resulting in the descent of these organs from their normal anatomical position into the vaginal canal. Depending on the severity of prolapse, women may experience few symptoms while for others the position of the organs may result in the feeling of a bulge or may even cause the vagina to protrude outside of her body. It is commonly assumed that prolapse only impacts a woman’s sexual function and therefore, the severity of this disorder is often disregarded; however, this is not the case. The quality of life of women with prolapse is negatively impacted. Affected women often experience feelings of embarrassment and isolation, and struggle with voiding dysfunction. In addition, women have poor perceptions of their bodies precluding them from being intimate with their partners. As a result, many women suffer in silence withdrawing from their communities and families.

Surgical repair with synthetic mesh is a common treatment for women with prolapse. Though this treatment method has a good success rate, there are many women who experience complications and, as a result, have had multiple revision surgeries. Unfortunately, this has left
women without a surgical treatment option that is not associated with morbidity or failure. The pathogenesis of mesh complications is currently not clear; however, research suggests that multiple factors such as pore collapse, mesh bunching, mesh textile properties, and mesh stiffness are contributing factors. To the scientifically minded, it is not surprising that synthetic meshes are associated with complications in prolapse repair given that these meshes were originally designed for hernia repair and not for prolapse repair. In fact, the phrase “designed for prolapse repair” should be used very loosely as minimal effort was likely placed into the design of these meshes when translating them from hernia to prolapse applications. Hernia meshes appear to have been simply cut into the appropriate shape and then remarketed as urogynecologic meshes. Over the years, companies have modified urogynecologic meshes, for example by increasing pore size and decreasing mesh weight; however, polypropylene is the material of choice. Despite these modifications, a device specifically designed for urogynecologic indications, which was not predicated on another application, has yet to be manufactured.

Recent research suggests that mesh stiffness and pore collapse are critical factors in the pathogenesis of mesh complications. A mesh that is not stiff enough may permanently elongate or fail leading to recurrent prolapse whereas a mesh that is too stiff can result in stress shielding and tissue degeneration [129,131]. *In vivo* prolapse meshes are exposed to predominately tensile loading conditions causing the pores to collapse. Studies in the abdominal hernia literature indicate that large pore, high porosity meshes yield less inflammation, less fibrous tissue, and decreased potential for contraction/pain relative to meshes with small pores and low porosity [79,119-121]. Mesh pore size is inversely related to bridging fibrosis, which is defined as the merging of the foreign body response between neighboring fibers resulting in a continuous fibrotic response or encapsulation of the mesh [79,119]. Indeed, in patients experiencing mesh complications, pore
collapse and encapsulation are often present resulting in pain, contraction, and the perception of shrinkage. Thus, the overall goal of this dissertation was to develop a synthetic mesh prototype that 1) did not experience pore collapse when loaded (i.e. the pores are auxetic), 2) had a material stiffness that is on the same order of magnitude as vaginal tissue, 3) was be able to withstand in vivo loads (both sudden and repetitive) without undergoing permanent deformation, and 4) minimized mesh burden. To accomplish this goal, the use of auxetic geometries for mesh pores and an elastomeric material were explored.

6.1.1 Development of Finite Element Computational Mesh Models

Auxetic geometries and materials innately have the ability to expand laterally when stretched longitudinally. This behavior is counterintuitive, as most materials contract when stretched. The ability of auxetics to expand is under-utilized within the biomedical community, and this is unfortunate given the potential for auxetics, particularly in the field of urogynecology. The pores of current synthetic meshes tend to collapse when exposed to uniaxial forces. Given the unique ability of auxetic geometries to expand when stretched, conceptually it makes sense to construct the pores of synthetic meshes using auxetic geometries. To initially explore this concept, finite element analysis was utilized to assess the behavior of computational mesh models containing various auxetic geometries as pores. Thus, the purpose of Specific Aim 1 was two-fold: 1) to establish the design criteria for the creation of finite element prolapse models with standard and auxetic pores and uniform discretization and 2) to develop a protocol for quantifying model and pore deformation, in silico, of computational prolapse mesh models with standard and auxetic pores in response to tensile loading. Initially, eleven computational models were created (referred to as 0º models), three with standard pores (square, diamond, and hexagon) and eight with auxetic
pore geometries (bowtie, spiral, triangle, square chiral_a, chiral hexagon, square chiral_b, hexagon_b, and square grid). CAD models were also created for the auxetic pore models by rotating the pores 45° (referred to as the 45° models) and 90° (referred to as the 90° models) with respect to the longitudinal axis of the models. The 45° models consisted of the bowtie 45°, spiral 45°, triangle 45°, square chiral_a 45°, chiral hexagon 45°, square chiral_b 45°, hexagon_b 45°, and square grid 45°. The 90° models consisted of the bowtie 90°, triangle 90°, chiral hexagon 90°, hexagon_b 90°, and square chiral_b 90°. Overall, the dimensions of the models were based on those of Restorelle, with all models having a minimal pore diameter of 1 mm, the same length, width, and volume of material. An inverse finite element analysis was used to determine the material properties that defined the behavior of the models. Models were then discretized and 3 N of force was applied along the longitudinal axis of the models. The resulting model elongation and pore deformation in terms of the pore dimensions (width and length) were quantified using a custom Mathematica script. Convergence testing was performed to assess the change in model elongation and pore dimensions with increasing elements. Models were considered converged when an increase in the number of elements resulted in a less than 5% change in the model elongation and pore dimensions.

The results of the inverse finite element analysis revealed that a Neo-Hookean material model with a Young’s Modulus of 52.98 MPa and Poisson’s ratio of 0.41 accurately modeled the behavior of Restorelle. Discretized finite element models consisted of between 178,944 and 705,088 elements that were a combination of tetrahedral, pentahedral, and/or hexahedral elements. Additionally, the model elongation, pore width, and pore length, of all discretized finite element models converged within 5%. This result indicates that the finite element models contained enough elements to ensure that the simulated response was based on the geometry, since the material
definitions and boundary conditions were the same for all simulations. Furthermore, this result also suggests that model elongation and pore deformation can be quantified with the custom script utilized.

6.1.2 Preventing Mesh Pore Collapse with Auxetic Geometries: A Computational Analysis

Using the computational models and model/pore deformation analysis techniques developed in Specific Aim 1, the purpose of Specific Aim 2 was two-fold: 1) to examine the overall model and pore deformation of CAD models with auxetic pores compared to CAD models with standard pores and 2) to determine the impact of the loading direction on model behavior of CAD models with auxetic pores. To accomplish these objectives, computational mesh models were subjected to simulated uniaxial tensile tests via 3D quasi-static, large deformation finite element analysis. In total, there were 24 computational models, eleven 0º models, eight 45º models, and five 90º models (See Chapter 3 for specifics regarding these models). To simulate a uniaxial tensile test, 3 N of force was applied along the longitudinal axis of the models. The resulting model behavior was characterized via quantitative measurements of model elongation and pore deformation. Additionally, the porosity and effective porosity as well as the overall expansion of the models were quantified. For the purpose of this synopsis, only a brief description of model/pore deformation will be given. Additionally, the porosity, effective porosity, and model expansion (or contraction) will be reported. For a complete description of the results, the interested reader should refer to Chapter 3.

Overall, the simulated behavior of the 0º standard pore models in response to uniaxial loading was similar to that of the ex vivo uniaxial loading of the respective commercial synthetic
mesh products. Specifically, the pores of the square model contracted but remained relatively stable whereas the pores of the diamond and hexagon_a models contracted and collapsed. Due to the relative stability of the square pore model, the porosity and effective porosity slightly increased; while, contraction of the diamond and hexagon_a model pores was associated with a decrease in the porosity and effective porosity. Assessing model expansion or contraction, all three of the standard pore models contracted, which was indicated by the positive value of the relative lateral contraction for each model. This result was expected given that the pores of these models are non-auxetic.

Unlike the standard pore models, models containing auxetic pores did not experience pore collapse; however, the degree to which the pores opened was dependent on the amount of deformation and on the pore orientation. Specifically, pore and model expansion was visibly apparent for the bowtie 0º, spiral 0º, square grid 0º, bowtie 90º, and triangle 90º models. These qualitative observations were also quantitatively verified. The porosity and effective porosity for all of the previously mentioned models increased with loading while the relative lateral contraction was negative, indicating model expansion. Though not as apparent as the previously mentioned models, pore and model expansion was also observed for the square chiral_a 0º, square chiral_b 0º, hexagon_b 0º, and square chiral_b 90º models. Additionally, both the porosity and effective porosity increased and the relative lateral contraction was negative (i.e. the models expanded) for all of these models.

As previously mentioned, not all pore orientations analyzed yielded pore and model expansion. This was particularly the case when the auxetic pores were rotated 45º. All pores of the 45º auxetic models contracted, which also led to lateral contraction of the models. However, it is worth mentioning that of all of the 45º auxetic models, the chiral hexagon 45º model produced the
most stable pore geometry, one very similar to that of the chiral hexagon 0° and 90° models. Interestingly, pore and model expansion was initially observed for the triangle 0°, chiral hexagon 0°, chiral hexagon 45°, chiral hexagon 90°, and hexagon_b 90° models during the early phase of loading (less than 1.5 N or 2.4 N); however, by 3 N, the pores and models contracted. These results suggest that there is a limit to the amount of deformation or load that can be applied to certain auxetic geometries when used as pores for synthetic meshes. Exceeding this limit will cause the auxetic geometries to lose the unique ability to expand resulting in pore collapse.

Collectively, the finite element analysis suggests that auxetic geometries can be used to prevent pore contraction/collapse; however, the orientation of the auxetically designed pore with respect to the loading direction (or deformation) and the amount of load or deformation applied will impact the ability of the auxetic pore to expand.

6.1.3 Ex vivo Characterization of Auxetic Meshes

In Specific Aim 2, the ability of auxetic geometries to expand when used as the pores of synthetic meshes was demonstrated using computational modeling. Though informative, the predicted behavior of auxetically designed pores in response to uniaxial loading must be experimentally verified. Thus, the objective of Specific Aim 3 was to manufacture synthetic meshes using auxetic geometries as pores and to assess the behavior of these pores, and mesh, in response to mechanical loading. As outlined previously, novel synthetic meshes should be made from a material that has a stiffness that is comparable to vaginal tissue. Thus, in this study, meshes were manufactured from polydimethylsiloxane (PDMS), a soft elastomer made from two parts, a base and curing agent. Prior to manufacturing the meshes, the mechanical properties of PDMS were explored in order to determine the mixing ratio (amount of base to curing agent) and thickness of PDMS that
will yield a material stiffness similar to vaginal tissue. Once these parameters were determined, synthetic meshes were constructed using a mold-fill process. The bowtie and chiral hexagon auxetic geometries were chosen as the pore shapes for the meshes. Additionally, the pores of the synthetic meshes were either aligned along or rotated 45° and 90° with respect to the longitudinal axis of the mesh. In total 30 meshes were created: bowtie 0° (n=5), bowtie 45° (n=5), bowtie 90° (n=5), chiral hexagon 0° (n=5), chiral hexagon 45° (n=5), and chiral hexagon 90° (n=5) meshes.

The mechanical behavior of the meshes was assessed using uniaxial tensile tests. Specifically, 3 N of force was applied along the longitudinal axis of the meshes, and the resulting pore diameter, relative elongation, porosity, effective porosity, and mesh burden were quantified. Additionally, the bowtie 0° and chiral hexagon 0° meshes were cyclically loaded and the amount of permanent deformation was assessed.

Results from the assessment of the PDMS mechanical properties revealed that a mixing ratio of 5:1 and 1.0 mm thickness yielded a material stiffness that is similar to vaginal tissue. Using this mixing ratio and thickness, the bowtie and chiral hexagon 0°, 45°, and 90° meshes were manufactured and mechanically tested. As anticipated, the behavior of these meshes in response to 3 N of force was similar to that predicted by the finite element analysis in Specific Aim 2. Specifically, the pores of the bowtie 0° and 90° meshes remained open and this result was associated with an increase in the porosity and effective porosity of these meshes. Consistent with the previously mentioned results, a decrease in mesh burden was also observed. Unlike the bowtie 0° and 90° meshes, the bowtie 45° mesh pores collapsed, which resulted in mesh contraction and a decrease in the porosity of these meshes. Additionally, there was a complete loss in the effective porosity observed at forces greater than and equal to 1 N. Given these results, it was not surprising that mesh burden was increased for the bowtie 45° meshes.
Overall, the behavior of the chiral hexagon 0°, 45°, and 90° meshes in response to 3 N of force was consistent. The pores of these meshes, which consisted of circles and triangles, elongated and contracted. This was associated with an increase in the porosity of these meshes throughout loading. Unfortunately, the effective porosity could not be calculated for the chiral hexagon meshes and this was a result of the initial diameter of the circles being less than 1 mm. Contraction of the circles would therefore lead to a decrease in the pore diameters and hence, a decrease in the effective porosity. Although not apparent from visualizing the meshes, mesh burden decreased for the chiral hexagon 0°, 45°, and 90° meshes.

Overall, the 0° configurations for both the bowtie and chiral hexagon meshes resulted in the least amount of deformation for these meshes while overall the bowtie 45° and 90° meshes deformed the most. Additionally, there was no amount of permanent deformation observed when the PDMS auxetic meshes were cyclically loaded. This result was observed for both the bowtie 0° and chiral hexagon 0° meshes. Although, cyclic loading was not performed on the bowtie and chiral hexagon 45° and 90° meshes, one can safely assume that these meshes would also not undergo permanent deformation with cyclic loading given that they are made from the same elastomeric material, PDMS.

Through the manufacture and characterization of synthetic meshes with auxetic pores, we were able to verify the predicted behavior of meshes with auxetic pores in Specific Aim 2. Additionally, the results of Specific Aim 3 demonstrated that: 1) auxetic pores when orientated in the right direction relative to the direction of loading will expand; thus, minimizing mesh burden and 2) manufacturing synthetic meshes from an elastomeric material will give meshes the ability to elongate without permanently elongating in response to repetitive loading.
6.1.4  *In vivo* Evaluation of the Host Response to an Elastomeric Mesh

In Specific Aim 3, the mechanical benefits of pore expansion with an associated decrease in mesh burden were demonstrated through the production and mechanical characterization of mesh with auxetic pore geometries. These meshes were manufactured from an elastomeric material (polydimethylsiloxane, PDMS) which allowed for the creation of a mesh that 1) has a material stiffness that is similar to vaginal tissue, 2) has shape memory, and 3) does not permanently deform with repetitive loading. Thus, from a mechanical perspective, the novel mesh developed in this dissertation shows great promise towards developing a mesh that addresses the current issues with synthetic meshes. Though promising, the *ex vivo* mechanical benefits observed with the novel mesh are meaningless if this mesh is not well tolerated in the body. Thus, the primary objectives of Specific Aim 4 was to 1) evaluate the overall host response to an elastomeric mesh and determine how pore size impacts this response and 2) to compare the fibrotic response of an elastomeric mesh to that of a commercially, available polypropylene mesh. To accomplish these objectives, the following three meshes were implanted into the abdomen of rats for 35 days: Bowtie 0° 1.0 mm (pore size = 1.0 mm, elastomeric mesh), Bowtie 0° 1.5 mm (pore size = 1.5 mm, elastomeric mesh), and Gynemesh PS (polypropylene mesh). At the conclusion of 35 days, the host response to these meshes was evaluated using hematoxylin and eosin (H&E), Masson’s trichrome, and picrosirius red staining. Mesh tissue complexes were imaged by light and polarized light microscopy at 20X. The following parameters were quantified and compared between the Bowtie 0° meshes: pore area, pore width, cellularity, vascularity, number of foreign body giant cells, total amount of collagen, and collagen fiber thickness. In addition, the presence/absence of bridging fibrosis, amount of encapsulation/fibrosis, and the quality of the tissue between the pores of the Bowtie 0° meshes and Gynemesh PS were evaluated.
The default host response to a foreign material was observed for the Bowtie 0° meshes. Specifically, the host cellular response consisted of approximately 1 to 4 cell layers of primarily rounded mononuclear and spindle-shaped cells with few foreign body giant cells present at the mesh-tissue interface. Additionally, avascular, dense connective tissue and macrophages lined the surface of the Bowtie 0° meshes. The tissue within the pores of these meshes consisted of highly vascularized loose connective tissue with very few foreign body giant cells found within the pores. Additionally, the tissue between the pores had a similar appearance to the tissue in which the mesh had been implanted. The cellular response consisted of mainly mononuclear cells with adipose present between some but not all of the mesh pores.

There were no significant differences observed between the pore area and pore width of the Bowtie 0° 1.0 mm and 1.5 mm meshes. Additionally, the degree of cellularity, vascularity, and the total amount of collagen were not different between these two meshes. The presence of a fibrous capsule was observed around the fibers of all three meshes; however, bridging fibrosis was only observed with Gynemesh PS, particularly around the knots of this mesh. Overall, there were no significant differences in the amount of thick and thin collagen fibers present within the pores of the Bowtie 0° meshes and Gynemesh PS. However, there were significant differences observed in the total amount of collagen per area. Specifically, the Bowtie 0° 1.5 mm meshes contained significantly less collagen per area relative to both the Bowtie 0° 1.0 mm meshes and Gynemesh PS.

Overall, the results of this study revealed that the elastomeric meshes developed in this dissertation elicited the default host response to a foreign material with an absence of bridging fibrosis, a phenomenon associated with encapsulation, mesh contraction, and pain. Additionally, the fibrotic response to the elastomeric meshes was less than that of Gynemesh PS. Collectively,
these results suggest that an elastomeric material may serve as an alternative material to polypropylene for urogynecologic mesh.

6.2 CLINICAL IMPLICATIONS

Currently there is no synthetic mesh product available on the market that was specifically designed for prolapse repair. However, through the work of this dissertation, a mesh tailored for prolapse repair, specifically for abdominal sacrocolpopexy, was created. It was created using a mold-fill process, which resulted in a solid construct, i.e. fibers, with pores. This manufacturing process is different from commercially available synthetic mesh products, which are created by knitting fibers together resulting in a mesh with fibers and knots. Knots have the potential to harbor bacteria between the small spaces within the knots (referred to as interstices), which can lead to complications. Thus, by manufacturing meshes without knots, as was the approach in this dissertation, the potential for complications resulting from the consequences of interstices is avoided. Secondly, the pores of the mesh manufactured in this dissertation had an auxetic geometry. Using an auxetic geometry allowed for 1) the pores of the mesh to remain open and expand in response to tension, and 2) the porosity of the mesh to increase while decreasing mesh burden with tensioning. Clinically, this result could translate to the pores maintaining their shape or increasing in size and to the porosity increasing in response to mesh tensioning. This behavior would be particularly beneficial during the early stages of mesh implantation. In vivo, synthetic mesh is arguably most vulnerable to pore collapse at the time of implantation and prior to tissue incorporation within the mesh pores. Supporting this claim, Svabik et al (2011) showed that the greatest amount of deformation (i.e. shrinkage/contraction) in a mesh occurs within 1 week of
implantation [84]. Interestingly, in vivo mesh complications are often located in the areas of the mesh where the pores have collapsed, i.e. mesh burden has increased, and there is no tissue ingrowth between these pores [82,85]. It is well documented in the literature, as well as in this dissertation, that large pores and high porosity meshes yield better biocompatibility and patient outcomes than small pores and low porosity meshes [79,119-121]. Furthermore, the chances of bridging fibrosis decreases with increasing pore size [79,119]. Thus, if pore collapse can be prevented early, then the likelihood of mesh complications due to a decrease in pore size and porosity will also decrease.

In addition to a change in the pore geometry, the mesh material was also changed. The mesh developed in this dissertation was manufactured from PDMS, which is an elastomer, and the stiffness of this material was along the same order of magnitude as vaginal tissue. Manufacturing the mesh from an elastomeric material provided the mesh with shape memory; thus, the mesh did not permanently elongate with cyclic loading. This result would translate clinically to the mesh being able to withstand repetitive loading in vivo without permanently elongating, which is particularly important prior to tissue incorporation between the mesh pores. During this time, the mesh can freely deform with no restrictions by tissue; thus, making it more susceptible to permanent elongation early and likely leading surgeons who utilize this mesh to over tension in order to prevent early recurrences. Over tensioning, likely leads to further pore collapse and mesh deformation. Additionally, it is anticipated that stress shielding and subsequent degeneration of vaginal morphology and smooth muscle function will not be a problem with a mesh manufactured from an elastomer given that the material stiffness of such a mesh will be similar to that of vaginal tissue. Future studies investigating the impact of an elastomeric mesh on vaginal morphology and smooth muscle function in a large animal, vaginal model will be able to address this hypothesis.
Furthermore, the promising results observed with the implantation of silicone mesh into the abdomen of rats may change the clinical perception that silicone is not an appropriate material for synthetic meshes.

Overall, the mesh developed in this dissertation shows promise towards creating a device that is purposely designed for pelvic organ prolapse repair. However, clinicians interpreting the mechanical results of this dissertation should be mindful that these results are reflective of the mesh behavior without tissue incorporated between the pores. The incorporation of tissue and the overall response to the mesh may impact the mechanical behavior; therefore, future studies are needed to investigate this further (future studies are discussed in more detail in Section 6.3). In addition to developing a novel mesh, the techniques used to develop this mesh have the potential to influence the way that future meshes are designed. Instead of modifying a pre-existing hernia mesh, the mesh developed in this dissertation was created from the bottom-up, i.e. from a new material and with a new design. From an economics perspective, this route of manufacturing is more expensive than manufacturing a mesh from an already FDA approved material. A new material would require rigorous scientific data and clinical trials to prove safety and effectiveness, which costs millions. The requirements for an already FDA approved material would more than likely not be as costly. However, with the up-classification of synthetic mesh from Class II to Class III devices, and given the advancements in understanding the pathogenesis of mesh complications (and prolapse in general), it is anticipated that mesh development (and treatment of prolapse) will be moving toward a more tailored/patient specific approach.

In this dissertation, the benefits of using auxetic geometries for prolapse meshes were demonstrated. However, these benefits are not restricted to abdominal sacrocolpopexy meshes (ABS meshes). Indeed, transvaginal meshes would also benefit from these geometries. Similar to
ABS meshes, transvaginal meshes also experience pore collapse, leading to mesh bunching, contraction, and pain [76,82,84,161]. Using an auxetic geometry for the pores of transvaginal meshes may prevent pore collapse and thus obviate the associated complications with pore collapse. Successfully reducing the risk of complications with transvaginal meshes would be especially beneficial clinically given that transvaginal meshes are experiencing more complications relative to abdominal sacrocolpopexy meshes. However, the orientation of the auxetic pores with respect to the loading direction will ultimately determine the true benefit of using auxetic geometries for the pores of prolapse meshes. Recalling from Chapters 3 and 4, auxetic pores in the 45° configuration resulted in pore collapse with uniaxial loading. Similar to current synthetic meshes with square pores, rotating the auxetic pores 45° resulted in alignment of an acute angle with the direction of loading which resulted in pore collapse for the majority of the auxetic geometries with the exception of the chiral hexagon geometry. The chiral hexagon geometry has 6-fold rotational symmetry, which allowed the chiral hexagon pore to maintain a relatively stable pore geometry with pore rotation. Changing the direction of loading applied to a mesh \emph{in vivo} would not be an easy task given that the abdominal sacrocolpopexy and transvaginal procedures are standard for prolapse repair. However, designing a mesh with auxetic pores that have rotational symmetry would aid with the prevention of pore collapse in response to multi-directional loads applied to the mesh. Additionally, designing a mesh with auxetic pores and stressing the importance of implantation direction to surgeons would also aid with the prevention of pore collapse and hence pore expansion.

Outside of urogynecologic mesh, the benefits of auxetic geometries can extend to other applications within the biomedical field, particularly to abdominal hernia meshes. Abdominal hernia meshes, the predecessor of prolapse meshes, are knitted and made of similar materials as
prolapse meshes with polypropylene being the primary material. The material stiffness of hernia meshes is orders of magnitude greater relative to the stiffness of abdominal muscles and tissues. Furthermore, abdominal hernia meshes are also associated with complications similar to prolapse mesh such as pain, erosion, and mesh contraction [185,186]. With the inherent similarities between prolapse and abdominal hernia meshes, future studies investigating a new pore design and mesh material for abdominal hernia meshes, as done for prolapse meshes in this dissertation, may be worth pursuing. Outside of being used as the pores for synthetic meshes, auxetic geometries could also be used in tissue engineering to construct scaffolds that delivery a drug(s) in response to a specific stimuli. For example, Alderson and Simkins in their patent of auxetic materials propose to use auxetic fibers for bandages and pressure pads in wound care which would allow the wound to breathe while concomitantly maintain pressure on the wound to prevent swelling [232]. Similarly, this same concept can be applied to other biomedical applications in which swelling would result in the release of a drug. An example of one such application is a stent made from auxetic geometries with the pores containing an anti-restenosis drug that will aid in preventing restenosis or other drugs that prevent scar tissue buildup and blockage of the stent.

6.3 LIMITATIONS AND FUTURE DIRECTIONS

The overall goal of this dissertation was to develop an initial synthetic mesh prototype that will demonstrate the feasibility of creating auxetic meshes for prolapse repair while concomitantly addressing the following issues with current urogynecologic meshes: 1) mesh pore collapse, 2) stiffness mismatch between the mesh material and vaginal tissue, 3) mesh bunching, and 4) mesh susceptibility to permanently deform. Using computational modeling, the concept that auxetic
geometries used for the pores of synthetic meshes can expand rather than collapse with uniaxial loading was demonstrated. This concept was further confirmed via *ex vivo* mechanical characterization of a synthetic mesh with auxetic pores manufactured from PDMS. Additionally, with the use of mechanical testing and imaging, it was also confirmed that mesh bunching does not occur for meshes in which the pores expand. The decision to manufacture the mesh from PDMS, a soft elastomer, was guided by the principal that 1) the material stiffness of PDMS can be tailored to match that of vaginal tissue and 2) using an elastomer would give the mesh the ability to return to its original shape (hence decrease the susceptibility to permanent deformation). Indeed the prototype mesh manufactured in this dissertation had a material stiffness that was comparable to vaginal tissue and it did not experience permanent deformation with cyclic loading. Thus, the goals of this dissertation were accomplished. However, as with any research, there are several limitations that must be addressed and unanswered questions that warrant further research.

The primary approach to preventing mesh pore collapse used in this dissertation was to substitute the commonly used pore geometries for synthetic meshes (e.g. squares, diamonds, and hexagons) with auxetic geometries. Although this approach worked, it is limited. First, not all auxetic geometries prevented pore collapse with uniaxial loading (up to 3 N); therefore, these geometries were deemed unsuitable for abdominal sacral colpopexy meshes (*in vivo* loads experienced by an abdominal sacral colpopexy mesh are primarily uniaxial and can exceed 3 N). However, this does not mean that the geometries that contracted or collapsed are not appropriate for transvaginal meshes. *In vivo*, the loads applied to a transvaginal mesh are multi-directional, and perhaps loading the auxetic geometries which contracted, for example the chiral hexagon geometry, in multiple directions simultaneously may lead to pore expansion rather than contraction. With this in mind, FEA using the models developed in Chapter 2 and applying an
additional loading condition will provide a baseline understanding of whether multi-directional loading can reverse pore contraction. Alternatively, creating FE models of transvaginal meshes with auxetic pores and performing FEA with various loading (and boundary) conditions is another way to assess which auxetic geometries may be appropriate for transvaginal meshes. Secondly, the ability to prevent pore collapse is dependent on the orientation of the auxetic geometry with respect to the axis of loading. One way to overcome this limitation is to develop a new auxetic geometry that is isotropic (i.e. it will expand regardless of the loading direction). Designing such an auxetic geometry would be advantageous as it could be used for both abdominal sacrocolpopexy and transvaginal meshes, and from a clinical standpoint, it will also give surgeons more flexibility, in terms of orientation, when implanting the mesh. Thirdly, changing the pore geometry is just one method that was explored to resolve the issue of pore collapse. There are other methods that may work such as coating a synthetic mesh with an agent that can provide pore shape stability. For example, this agent could be a biodegradable covering that is incorporated with the mesh and hence, it would fill the pores of the mesh initially. However, as it degrades over time, the pores of the mesh will become filled with host tissue. The agent could also be a thin coating that increases pore stability by limiting fiber rotation around the knots of the mesh. Additionally, combining these methods is another possible approach that can be used to overcome the obstacle of pore collapse.

The journey from “bench to bedside” is a long process, and although this dissertation may have provided a proof of concept, there is still work to be done before auxetically designed meshes can become a reality. From a design perspective, there are two critical parameters that have yet to be identified: 1) optimal auxetic pore geometry and 2) optimal elastomer. The optimal auxetic pore geometry should allow for maximal pore expansion in response to the loads within the female
pelvis. One of the first steps to identifying an optimal pore geometry is to understand how various textiles features such as pore size, pore shape, and mesh fiber width (and thickness), impact pore expansion. Thus, future studies should aim to determine the impact of the previously mentioned textiles features on pore expansion via a parametric analysis. This analysis can be performed using the FE models developed in Chapter 2 as a starting point. Additionally, despite the promising results that were obtained with auxetic meshes manufactured from PDMS, PDMS is not the most ideal elastomer. One major concern of PDMS is that it is too brittle. When handling the PDMS auxetic meshes, they broke easily, especially if the mesh had an imperfection (such as an air bubble or broken fiber). From a clinical standpoint, failure of the mesh would be a major concern especially when a woman performs an activity that increases intra-abdominal pressure suddenly, for example with coughing and sneezing. Thus, there is a need for a tougher elastomer. Poly(styrene-block-isobutylene-block-styrene) (also referred to as SIBS) may serve as an alternative material. SIBS is a biocompatible, thermoplastic elastomer that is oxidatively, hydrolytically, and enzymatically stable. Additionally, it is relatively inert in the body making it a good candidate for long-term implants. The use of SIBS for biomedical devices was first introduced in 2002 as a coronary stent [233]. Since this time, SIBS has been used in the design of glaucoma drainage tubes, intraocular lenses, and aortic valves. The stiffness of SIBS is reported to be on the same order of magnitude as vaginal tissue. However, further mechanical characterization is needed since SIBS has not been used for prolapse meshes, including analysis of failure properties and structural properties of mesh constructs. Pending that the mechanical properties of SIBS are deemed appropriate for urogynecologic meshes, in vivo studies in the abdomen and vagina can be conducted to determine the host response to meshes manufactured from SIBS and to determine the impact of SIBS on vaginal smooth function and morphology. As a part of these studies, it is also
important to determine the minimal pore size at which bridging fibrosis does not occur. Although the minimal pore size for polypropylene meshes is 1 mm, this may not be the case for other materials. Conze et al (2008) demonstrated that pore sizes greater than 630 μm are not associated with bridging fibrosis with meshes manufactured from co-PVDF (a blend of polyvinylidene fluoride and hexafluoropropylene) [80]. Thus, studies implanting meshes manufactured from SIBS (or other materials deemed appropriate) with varying pore sizes should be performed in conjunction with host response studies.

Finite element analysis played a major role in this dissertation as it allowed us to identify the auxetic geometry (or geometries) that would afford maximal pore expansion with uniaxial loading. Importantly, FEA will continue to be a vital part of the design process of auxetic meshes. It is therefore critical that the models used to simulate ex vivo mesh behavior are accurate. The Neo-Hookean material model was used to define the FE models in this dissertation. Although this model provided a good first approximation, preliminary studies using the Neo-Hookean material model determined that this model could not be used to accurately predict the pore deformation and model elongation of the auxetic meshes manufactured from PDMS in Chapter 4 (see Section 4.3.4 for more details). This result makes sense given that the Neo-Hookean material properties used in Chapter 3 were based on a mesh manufactured from polypropylene not PDMS. Thus, the development of a finite element model that can accurately predict the behavior of a mesh manufactured from PDMS (or another elastomer) is a must. The development of such a model would not only be beneficial for future mesh design optimization studies (e.g. parametric studies proposed in the previous paragraph), but it would also be a valuable model for simulating various scenarios such as multi-axial loading and prolapse repair with mesh. Finite element models using the Arruda-Boyce model as the material model has shown some promising results in terms of
accurately predicting the pore deformation and model elongation of the bowtie $0^\circ$ mesh. However, this work is in the early stages and more research is needed to verify that this model is appropriate for PDMS meshes. See Appendix C for more details regarding the preliminary studies with the Arruda Boyce model.

In order to match the material stiffness of mesh to that of vaginal tissue, the stiffness of vaginal tissue must be known. Due to the ethical dilemmas surrounding the procurement of human vaginal tissue, much of the information collected on the stiffness of vaginal tissue was acquired from animal research. Although studies by Goh et al (2002) and Lei et al (2007) have reported the stiffness of human vaginal tissue ranges between 6.0 MPa and 14.0 MPa, these values are relatively low compared to animal studies which report the stiffness of the vagina to range from 25.0 MPa (rodent nulliparous vagina) to 34.3 MPa (ewe nulliparous vagina) [142,143,197,234]. It is hard to fathom that the rodent vagina is more resistant to deformation than the human vagina. Sample location is one possible factor that may be contributing to the differences observed between the mechanical properties of the animal and human vaginas. For the animal studies, the vaginal samples tested were from the mid-region, along the longitudinal axis of the posterior vagina whereas the vaginal samples for the human studies were taken from the horizontal axis of the anterior vaginal fornix in Lei et al (2007) or an unspecified location along the longitudinal axis of the anterior vagina in Goh et al (2002). The presence of the urethra may influence collagen alignment and composition of the anterior vaginal wall, which can impact the mechanical properties of the vagina. Additionally, differences in the alignment of the collagen along the longitudinal and horizontal axis of the vagina will result in differing mechanical properties, with the longitudinal axis of the vagina being stiffer than the horizontal axis [235]. The differing mechanical properties between the human and animal vaginas may also be related to the different
sources of tissue samples. The human vaginas were obtained from premenopausal and postmenopausal women with and without prolapse in contrast to the animal vaginas which were obtained from animals that were nulliparous or parous. Numerous studies in the literature have shown that age, menopausal status, parity, and pelvic floor disorder status all impact the mechanical properties of the vagina \[31,142,197,198,234,236-238\]. Lastly, the same testing protocol was not utilized for the animal and human studies. Similarly, it is possible that the stiffness of the vaginas in the human studies were calculated differently from the animal studies and/or that the mechanical properties of the human vaginas were determined from samples that failed prematurely which would result in a lower stiffness. In either case, a lack of details and an absence of stress-strain curves for the human studies make it difficult to pinpoint exactly why the stiffness of the human vagina is so low relative to the rodent, primate, and ewe vaginas. Nevertheless, future studies examining the mechanical properties of the human vagina using similar methods as those used for animal studies would aid with identifying which animal model has vaginal mechanical properties that closely match the human vagina. Identifying such a model would not only be advantageous given the ethical dilemmas surrounding the procurement of human vaginal tissue, but this model can also be used to further characterize the mechanical properties of the vagina beyond uniaxial loading. To date, the majority of the studies characterizing the mechanical properties of the vagina focused primarily on only one axis of the vagina. The vagina \textit{in vivo} is loaded in both the longitudinal and circumferential axes, and forces and displacements along one direction impact that of another direction. Uniaxial tensile testing would not be able to capture this coupling; therefore, tests that can load or deform the longitudinal and circumferential axes of the vagina, such as biaxial mechanical testing, are able to provide a better understanding of the mechanics of the vagina. Biaxial mechanical testing has been used to characterize the anisotropic
behavior of various soft tissues such as heart tissues, bladder wall, and the sclera, with limited use of this technique within the gynecology field [239-243]. However, recently researchers within the gynecology field have begun to apply biaxial mechanical testing to characterize the mechanical properties of tissues within the female pelvis. Becker et al (2015) and Tan et al (2016) used biaxial mechanical testing to describe the biaxial elastic and viscoelastic properties of the uterosacral ligaments, cardinal ligaments, and the uterosacral-cardinal ligament complex [244,245]. Applying biaxial mechanical testing to the vagina, Barone 2015 was able to demonstrate, using preliminary studies, the anisotropic behavior of the rat vagina and the impact of smooth muscle function on this behavior [161]. Specifically, the longitudinal axis of the vagina was found to be stiffer than the circumferential axis, and this result was obtained regardless of the contractile state of the smooth muscle (i.e. whether the smooth muscle was relaxed or contracted during loading). Previously in 2010, Tokar et al also demonstrated that the vagina is anisotropic and becomes more distensible in the circumferential direction during mid-pregnancy in the rat [246]. Collectively these studies increase our knowledge of the mechanical behavior of the vagina; however, there is still much work to be done. In addition to smooth muscle, the vagina is also comprised of collagen and elastin, which are two vital components that contribute to the mechanical properties of the vagina. Outside the general knowledge that collagen is the primary load-bearing structure within the vagina and elastin affords the vagina the ability to recoil, little is known regarding how the interaction of these fibrous components collectively impact the mechanical behavior of the vagina. Studies in the arterial wall literature suggest that elastic fibers are under tension and that they directly impact the crimped configuration of collagen fibers, which ultimately impacts the mechanical behavior of the artery [247,248]. In other words, the removal of elastin results in collagen fiber uncrimping and tissue lengthening. Given the link between decreased elastin
content and pelvic organ prolapse, it would be noteworthy to investigate the impact of elastin degradation on the biaxial mechanical properties of the vagina [249-251]. Specifically, coupling biaxial mechanical testing, elastin degradation, and imaging techniques such as multiphoton imaging, would allow for the assessment of changes in collagen and elastin organization, recruitment, and alignment with mechanical loading in real time. Rigorously characterizing the contribution and the interactions of elastin and collagen to the mechanics of the vagina is a crucial next step for the development of constitutive models that can be used to more accurately define the behavior of the vagina in FEA. Additionally, this information would aid in the development of a mesh that is specifically tailored for prolapse repair.

Aside from the limitations mentioned previously, designing a mesh for prolapse repair is associated with additional challenges. One of the first challenges is a lack of understanding of prolapse by the public. Many people, including women, are not familiar with pelvic organ prolapse or the profound negative impact of this disorder on the lives of women worldwide. This has resulted in limited attention, in the form of conversation, research, and funding, being allocated to this disorder relative to other fields. It is therefore not surprising, although it may be somewhat shocking at first, that the normal anatomy of the soft tissues in a woman’s pelvis is not known. Additionally, the exact forces and displacements that the pelvic organs, muscles, and connective tissues experience in vivo are not known. Collectively, these unknowns raise several questions regarding the size and geometry of the tissues within the female pelvis, how these tissues are attached and interact, how much force or displacement each experiences, and how disruption of these intricately related structures impacts pelvic organ support. From an engineering perspective, these unknowns make it difficult to develop finite element models that accurately simulate in vivo conditions; therefore, researchers often use simplifications and assumptions in order to simulate in
vivo conditions during the development of FE models. To simplify the complex geometry of the female pelvic anatomy, studies often incorporate the pelvis and only the specific pelvic organs that they deem are the most relevant to address their research question(s) [148,158,252-255]. To approximate these geometries, researchers segment (i.e. trace) them out using MRI scans obtained from patients. Following segmentation, these geometries are smoothed, a mesh is applied to the individual surfaces, and then a 3D FE mesh is generated. Next, material properties are assigned to the model and these properties are based on experimental data, which is rarely available. Thus, researchers often assume that the nonlinear behavior of the pelvic tissues can be modeled with hyperelastic constitutive models such as neo-Hookean and Mooney-Rivlin [256]. Assumptions are also made regarding the boundary conditions that are applied to the FE model, as it is often difficult to visualize boundaries between pelvic muscles and tissues with MR images. Similarly, the loading conditions applied to the model are often estimated with intra-abdominal pressure being a commonly applied loading condition to the surface of the vagina [256]. The estimates for intra-abdominal pressure during various activities, for example with coughing, are obtained from measurements using a catheter placed in the bladder, rectum, or vagina [144-147]. Together, these simplifications and assumptions result in the development of patient-specific FE models; however, it is anticipated that as our knowledge of the female pelvic anatomy and mechano-physiology increases, our ability to create more accurate FE models will also increase. Studies which aim to develop a computational model with the geometry derived from a woman with normal pelvic anatomy and those that aim to characterize the mechanical properties of the pelvic organs and tissues which are unknown or warrant further characterization (e.g. the vagina) are all examples of studies that will aid in the development of more accurate FE models. This in turn will enhance our ability to development a mesh specifically tailored for pelvic organ prolapse.
The work of this dissertation has laid the foundation for the development of an ideal mesh for prolapse repair; however, there is still considerable work to be done. In addition to the future studies mentioned in the previous paragraphs, studies demonstrating the impact of pore size in a vaginal model will substantiate the need for a mesh that maintains (or increases) pore size with loading. Additionally, it is important that future studies investigating a new mesh design and material be evaluated in the vagina of a relevant large animal model as studies have shown that the abdomen is a more forgiving environment to mesh relative to the vagina [222,257].

The primary approach to preventing mesh complications utilized in this dissertation was to re-design the mesh using auxetic geometries for pores and an elastomeric material based on findings from mechanical testing of synthetic meshes. However, one of the first approaches to reducing mesh complications was to mitigate the host response. Polypropylene mesh is shown to induce an intense inflammatory response, which consequently leads to the formation of dense fibrosis tissue in and around the mesh and mesh shrinkage, especially for heavyweight polypropylene meshes [258,259]. Thus, to reduce this response, meshes were manufactured with a reduced density of polypropylene and larger pores. Indeed, lightweight meshes yielded a reduced inflammatory and fibrotic response relative to heavyweight meshes [79,81,134,135]. Despite the improvements in the host response to lightweight meshes, the chronic inflammatory response to polypropylene meshes and mesh complications still exist. Thus, researchers continue to search for ways to improve these devices. Currently, the majority of approaches to improve the host response to mesh utilize a mesh coating technique whereby the mesh is coated with an agent, such as collagen and ECM. The results from studies analyzing the impact of collagen coating on the host response are conflicting. Pierce et al (2011), Huffaker et al (2008), and de Tayrac et al (2007) all found that there was no significant differences in the long-term host response observed between
collagen coated and uncoated polypropylene meshes [150,260,261]. These results are in contrast to studies by Dias et al (2015) and Siniscalchi et al (2013) which demonstrate that polypropylene mesh coated with highly purified collagen yielded better tissue adherence and integration, increased angiogenesis, and impacted the amount and duration of MMP-2 and MMP-3 in tissue relative to non-coated polypropylene meshes [262,263]. Unlike collagen, the impact of an ECM coated mesh on the host response is more consistent. In 2013, Wolf et al demonstrated that coating a heavyweight mesh with a hydrogel form of porcine dermal extracellular matrix (ECM) mitigates the host response to the mesh [63]. Specifically, the pores within the ECM coated mesh consisted of loose connective tissue relative to the dense connective tissue present within the pores of the uncoated polypropylene meshes. Additionally, the inflammatory response to the ECM coated mesh was reduced relative to the uncoated meshes. The improved host response to an ECM coated mesh is believed to be a result of macrophage polarization; the M1 macrophage response, and subsequently the number of foreign body giant cells, is reduced while the M2/M1 ratio is increased [264]. The benefits of coating a mesh with ECM were further confirmed in a long-term study conducted by Faulk et al (2014). The chronic inflammatory response and deposition of scar tissue that is characteristic of polypropylene mesh was attenuated with an ECM coated mesh [265]. Aside from collagen and elastin, S-nitrosoglutathione (GSNO) and IL-4 have also shown promise as a mesh coating [266,267]. Compared to a pure polypropylene mesh, the inflammatory response to a GSNO coated mesh was reduced while angiogenesis was increased [266]. Additionally, the presence of apoptotic cells was decreased in the presence GSNO. In a mouse implant study, Hachim et al (2017) found that the percentage of M1 macrophages at the mesh-tissue interface was decreased while the percentage of M2 macrophages increased for an IL-4 coated mesh relative to meshes with no IL-4 [267]. The capsule area and thickness was also reduced with an IL-4 coated
mesh, and this capsule consisted of an increased amount of thin collagen fibers and less thick collagen fibers compared to mesh without IL-4. Aside from using a mesh coating technique, the majority of the previously mentioned studies that showed positive results in terms of mitigating the host response with a coated mesh were performed in the abdomen. It would be interesting to evaluate the impact of these coatings on the host response in a vaginal implantation model given the differences between the host response to mesh implanted in the abdomen and vagina.

One of the biggest challenges to resolving the complications associated with urogynecologic mesh is that the pathogenesis of mesh complications \textit{in vivo} is currently not clear. Although evidence in the literature suggests that factors such as pore collapse and stiffness mismatch with vaginal tissue resulting in stress shielding may be contributing to complications, the inability to purposely recreate mesh complications in an animal model limits our ability to develop solutions that address mesh complications. However, in a recent study conducted by Nolfi et al (2016), a link between the macrophage response and patients experiencing complications due to mesh exposure or pain may shed light on a potential mechanism for mesh complications [208]. The results of this study found that pain and mesh exposure are associated with a proinflammatory response and this response persists years after implantation. Additionally, the macrophage response surrounding mesh fibers was predominantly M1 for meshes removed for both pain and exposure. In the mesh excised for exposure, MMP-9 was elevated, which is suggestive of tissue degradation, whereas with the meshes removed for pain, there was a positive correlation between M2 macrophages and interleukin-10, which is consistent with fibrosis and encapsulation. In a separate study, a dominating proinflammatory M1 macrophage response was also observed with mesh implanted on the vagina of nonhuman primates [219]. Outside of the macrophage response, characterization of the impact of polypropylene mesh on vaginal collagen and elastin metabolism
also may shed light on mechanisms for mesh complications. Increased degradation of collagen and elastin as well as increases in active matrix metalloproteinase (MMP)-1, -8, -13, and total MMP-2 and -9 all were observed following implantation of Gynemesh PS (one of the stiffest polypropylene, prolapse meshes available on the market) on the nonhuman primate vagina, and resulted in structurally compromised tissue [268]. Based on these findings, perhaps coating urogynecologic meshes with ECM or IL-4 may be advantageous for reducing the inflammatory response to mesh and increasing the pro-remodeling response. Liang et al (2017) recently explored the option of using a noncross-linked degradable acellular procine urinary bladder matrix, MatriStem (Matrix RS, MatriStem RS, ACell, Inc, Columbia, MD) to alleviate the negative impact of polypropylene mesh on the vagina in a nonhuman primate model [269]. The results revealed that Gynemesh PS combined with MatriStem as a composite mesh yielded a reduced inflammatory response compared to Gynemesh PS alone. Additionally, the thickness of the smooth muscle layer as while as the contractile function of the smooth muscle for the composite was comparable to that of sham tissue. This result is in contrast to Gynemesh PS, in which a decrease in smooth muscle thickness and contractile function was observed relative to sham tissue. Furthermore, the increase in apoptotic cells surrounding mesh fibers with Gynemesh PS was also obviated with the composite mesh. Liang et al (2017) also compared the host response to MatriStem alone and found that the biomechanical, histomorphologic, and biochemical endpoints assessed in this study were similar to sham. Overall, the results of this study demonstrated the potential of using extracellular matrix to mitigate the host response to urogynecologic mesh. Given the positive impact of MatriStem alone on the vagina and the current popularity of using tissue engineering for medical applications, it would not be surprising if future treatment of prolapse is approached from a more regenerative medicine perspective. For example, instead of implanting synthetic mesh to restore
apical support to the vagina, a biodegradable graft that aims to reinforce or regenerate the uterosacral and cardinal ligaments may be implanted. Similarly, modifying the surface of polypropylene mesh with ECM or an anti-inflammatory/regulatory drug eluting coating may be the approach taken. These techniques could also be combined with the concept of using auxetic geometries as pores. Ultimately, the direction of prolapse treatment will be dictated by the information that we learn about the pathogenesis of prolapse and mesh complications.

Current synthetic meshes used in the repair of pelvic organ prolapse are simply hernia meshes re-marketed for prolapse repair. This was allowed given that hernia meshes were in use prior to the 1976 Medical Device Amendment Act; hernia meshes were shown to be safe and effective therefore it was assumed that synthetic meshes for prolapse repair would also be safe and effective. Ultimately this resulted in the implantation of synthetic meshes that had not undergone costly clinical trials and experiments to demonstrate safety and effectiveness, as far as public reports indicate. Additionally, these meshes were also not specifically designed for the loading conditions and tissues within the female pelvis. Thus the current design of knitted, lightweight, and large pore (high porosity) meshes were determined by implanting mesh into women on a trial and error basis. Not surprisingly, through this trial and error process many women experienced complications with some women undergoing multiple revision surgeries. In result of mesh complications, the FDA released public health notifications in 2008 and 2011. Since the release of these warnings, many manufacturers have discontinued their prolapse products, numerous lawsuits have been filed and millions of dollars have been awarded to patients. Additionally, the FDA recently up-classified prolapse meshes from Class II to Class III devices. Given all that has transpired with synthetic meshes over the years, there is no better time to design a mesh for prolapse that is not predicated on another device than now. This dissertation provides a first
example of how taking a fresh look at the design of prolapse meshes and combining the knowledge gained from exploring the mechanical behavior of mesh, mesh complications, and the host response to mesh, we were able to design a mesh in a logical and scientific manner that is specifically tailored for pelvic organ prolapse repair via an abdominal sacral colpopexy. The advantages of the novel mesh developed in this dissertation include: 1) that the pores expand in response to tensile loading, 2) mesh burden is minimized with loading, 3) the material stiffness of the mesh is similar to vaginal tissue, and 4) this mesh does not permanently elongate with repetitive loading. All of these advantages we feel will significantly improve the current state of the art of synthetic meshes for prolapse repair as well as reduce the risk of mesh related complications.
POSTFACE

Looking back on the past seven years, I can honestly say that this journey has been long, hard, frustrating, interesting, and rewarding. I have learned a lot and have grown as a researcher and as a person. I am truly humbled and I thank God for blessing me with the talent, ability, and opportunity to pursue and obtain a PhD in bioengineering. I also thank my parents for providing me with a solid foundation and I thank all of the villagers who helped me along the way. Although, I would not want to go through this experience again, I would not trade it in for the world. To my fellow African Americans, I did it and so can you!
APPENDIX A

PORE DIAMETER AND POROSITY ANALYSIS

Pore Diameter and Porosity Analysis of Computational Models and Meshes
February 5, 2016

Copy and paste the folder location where the data will be exported.

path = "/Users/Katrina/Desktop/Dissertation\ Work";
Copy and paste the file location of the image to be analyzed. Adjust the threshold value if greater contrast between the background and model or mesh is needed.

ActualImage = Import[
   "/Users/Katrina/Desktop/Dissertation\ Work/Updated\ Meshes/RotatedSquares/FEA\ Pore\ Diameter/DeformedR52860ele_38x4.6mm.jpg", "JPG"];
Grid[{{Print["Threshold"], Slider[Dynamic[threshold], {0, 1, .01}],
   Dynamic[threshold]]},
Grid[{{Print["Erosion"], Slider[Dynamic[radius], {0, 10, .1}], Dynamic[radius]]},
Grid[{{Print["Fill"], Slider[Dynamic[radius2], {0, 5, .1}], Dynamic[radius2]]},
Grid[{{Print["Gamma"], Slider[Dynamic[gamma], {0, 10, .01}], Dynamic[gamma]]}]
gamma = 1;
contrast = 0;
brightness = 0;
radius = .5;
radius2 = .5;
threshold = .2;
Grid[{{Magnify[ActualImage, 3],
   Dynamic[Magnify[GeodesicOpening[GeodesicClosing[ContourDetect[
      ImageAdjust[ActualImage, {contrast, brightness, gamma}], threshold],
      radius], radius2], 3]]], Frame -> All}]
(* from ContourDetect, 1=void space, 0=mesh*)
(*GraphicsRow[{ActualImage, ProcessedImage}, ImageSize=Large]*)
ProcessedImage = ColorNegate;
    GeodesicOpening[GeodesicClosing[ContourDetect[ImageAdjust[ActualImage, 
        {contrast, brightness, gamma}], threshold], radius], radius2]];
BinaryPixelData = ImageData[ProcessedImage];
Dimensions[BinaryPixelData]
Dimensions[BinaryPixelData][[1]] * Dimensions[BinaryPixelData][[2]];
BorderPoresPic = MorphologicalComponents[ImageSubtract[ColorNegate[ProcessedImage],
    DeleteBorderComponents[ColorNegate[ProcessedImage]], .3] // Colorize
NoBorderPoresPic = MorphologicalComponents[
    DeleteBorderComponents[ColorNegate[ProcessedImage]]] // Colorize
EdgePoreArea = ComponentMeasurements[BorderPoresPic, "Area"];
EdgePoreArea = EdgePoreArea[[All, 2]];
AreaToRemove = Total[EdgePoreArea];
Centroids = ComponentMeasurements[NoBorderPoresPic, "Centroid"];
Centroids = Centroids[[All, 2]];
MaxDiameter = ComponentMeasurements[NoBorderPoresPic, "Length"];
MaxDiameter = MaxDiameter[[All, 2]];
MinDiameter = ComponentMeasurements[NoBorderPoresPic, "Width"];
MinDiameter = MinDiameter[[All, 2]];
PoreArea = ComponentMeasurements[NoBorderPoresPic, "Area"];
PoreArea = PoreArea[[All, 2]];

To convert the dimensions of the image from pixels to millimeters, enter the height of the image in 
physical space. For example, the height of the image was 4.6 mm (see below).
ScalingFactor = 4.6 / Dimensions[BinaryPixelData][[1]];
ActualMinDiameter = MinDiameter * ScalingFactor;
ActualMaxDiameter = MaxDiameter * ScalingFactor;
NoBorderPoresPicNoColor = DeleteBorderComponents[ColorNegate[ProcessedImage]];
a = ListPlot[Centroids, PlotStyle -> {Red, PointSize[.Large]}];
Show[(NoBorderPoresPicNoColor, a)]
CirclePic = Show[a,
    Graphics[{Red, Thick, Rotate[Circle[p[[1]], {p[[2]]/2, p[[3]]/2}], p[[4]]] & @
        ComponentMeasurements[NoBorderPoresPic, {"Centroid", "Length", 
            "Width", "Orientation"}][[All, 2]]]}, AspectRatio -> .8];
Show[(NoBorderPoresPic, CirclePic)]
Histogram[{ActualMinDiameter}, {8, 5, .1},
ChartLegends -> {"Min Diameter", "Max Diameter"},
AxesLabel -> {"Diameter (mm)", "Frequency"}, ChartStyle -> {Red, Blue}]
(*Number of pores which meet the criteria. Here it is set to include all shapes with a minimum diameter greater than 1 mm*)
GoodLengths = Select[ActualMinDiameter, # > 1 &];
(*Lengths which are greater than 1 mm*)
GoodLengthElementNumbers =
  Table[Flatten[Position[ActualMinDiameter, GoodLengths[[i]]]],
    {i, 1, Length[GoodLengths]}];
(*Elements that are pulled out for calculations*)
GoodLengthElementNumbers = GoodLengthElementNumbers[[All, 1]];  (*OUTPUTS:*)
Print["Porosity (new)"]
Porosity = Total[PoreArea] / Dimensions[BinaryPixelData][[1]] * Dimensions[BinaryPixelData][[2]] - AreaToRemove
GoodPoreAreas = Flatten[Table[Take[PoreArea, (GoodLengthElementNumbers[[i]])],
    {i, 1, Length[GoodLengthElementNumbers]}]];  (*Effective porosity")
EffectivePorosity = Total[GoodPoreAreas] / Dimensions[BinaryPixelData][[1]] * Dimensions[BinaryPixelData][[2]]
Print["Corrected effective porosity"]
CorrectedEffectivePorosity =
  Total[GoodPoreAreas] / {Dimensions[BinaryPixelData][[1]] * Dimensions[BinaryPixelData][[2]] - AreaToRemove}
Print["Skewness"]
Skewness[ActualMinDiameter]
Print["Number of pores"]
Length[ActualMinDiameter] - Length[GoodLengthElementNumbers]
(*Number of pores that don't touch the border of the image. This is used to not skew the effective histograms")
Print["Effective pores"]
Length[GoodLengthElementNumbers]
Print["Total Pore Area (mm^2)"]
TotalPoreArea = Total[PoreArea];
TotalPoreArea *= (ScalingFactor)^2 (*Converts to mm^2*)
Print["% Pore Area that is effective"]
Total[GoodPoreAreas] / TotalPoreArea

Enter the file names for the data that will be exported.
(*Export area, porosity, and other variables*)
PoreDimensions = Transpose[{ActualMinDiameter, ActualMaxDiameter}];

FileName = 
FileNameJoin[{path, "/OutputMeasure_DeformedRSQ228480ele_30x4.6mm.xls"}];

Export[FileName, {{threshold, gamma, Porosity, EffectivePorosity, 
CorrectedEffectivePorosity, Skewness[ActualMinDiameter], 
Length[ActualMinDiameter] - Length[GoodLengthElementNumbers], 
Length[GoodLengthElementNumbers], TotalPoreArea * {ScalingFactor}^2, 
Total[GoodPoreAreas]/TotalPoreArea}]]; 

(*Export pore diameter and pore area*)
PoreDimensions2 = Transpose[{ActualMinDiameter, ActualMaxDiameter, 
PoreArea*(pix^2), PoreArea*(mm^2)}];

FileName = FileNameJoin[{path, 
"/ActualDiameters_DeformedRSQ228480ele_30x4.6mm.xls"}];

Export[FileName, PoreDimensions2];
APPENDIX B

RELATIVE LATERAL CONTRACTION ANALYSIS

Relative Lateral Contraction Analysis of Computational Models
February 8, 2016

Enter the folder where the data will be exported.
path = "/Users/Katrina/Desktop/Dissertation\ Work";
Copy and paste the file location for the undeformed image to be analyzed.
ActualImage =
  Import["/Users/Katrina/Desktop/Dissertation\ Work/Updated\ Meshes/98\ Square\ Chiral\ 2\ FEA\ Images/3N96SQChiral2ele432512_Undeformed.png", "PNG"];
ImageAdjust[ActualImage,
  {1,
   1,
   1}]
ProcessedImage = ActualImage;
BinaryPixelData = ImageData[ProcessedImage];
Dimensions[BinaryPixelData] = Dimensions[BinaryPixelData][[2]]
(*Identify the pores of the image*)
b = MorphologicalComponents[ColorNegate[ProcessedImage], .3] // Colorize
FixedPic = MorphologicalComponents[ColorNegate[ProcessedImage], .3];
Centroids = ComponentMeasurements[
  MorphologicalComponents[ColorNegate[ProcessedImage], .3], "Centroid"
]
Centroids = Centroids[[All, 2]]
(*Display the centroids and the pores. Note there may be extra centroids that will need to be removed*)
a = ListPlot[Centroids, PlotStyle -> {Red, PointSize[Large]}]
Show[{ProcessedImage, a}]
Remove any extra centroids using the Drop function. Depending on the number of extra centroids, this function may be used more than once.

UndeformedCentroids = Drop[Centroids, 1];
ListPlot[UndeformedCentroids, PlotStyle -> {Red, PointSize[Large]}]

Repeat the steps above for the deformed image.

ActualImage2 = Import["/Users/Katrina/Desktop/0.6N9SQChiral432512ele.png", "PNG"]; ImageAdjust[ActualImage2, (1, 1, 1)]

ProcessedImage2 = ActualImage2;
BinaryPixelData2 = ImageData[ProcessedImage2];
Dimensions[BinaryPixelData2]
Dimensions[BinaryPixelData2][[1]] == Dimensions[BinaryPixelData2][[2]]

b2 = MorphologicalComponents[ColorNegate[ProcessedImage2], .3] // Colorize
FixedPic2 = MorphologicalComponents[ColorNegate[ProcessedImage2], .3];
Centroids2 = ComponentMeasurements[
    MorphologicalComponents[ColorNegate[ProcessedImage2], .3], "Centroid"
]
Centroids2 = Centroids2[[All, 2]]

c = ListPlot[Centroids2, PlotStyle -> {Red, PointSize[Large]}]
Show[{ProcessedImage2, c}]

DeformedCentroids = Drop[Centroids2, 1];
ListPlot[DeformedCentroids, PlotStyle -> {Red, PointSize[Large]}]

Enter a name for the Excel file that will be exported containing the centroid positions for the undeformed and deformed images. This file will be exported to the folder defined at the beginning of the code.

FileName = FileNameJoin[{path, "/0.6NCentroids_9SQChiral2ele432512.xls"}];
Export[FileName, {UndeformedCentroids, DeformedCentroids}];
APPENDIX C

FINITE ELEMENT MODEL DEVELOPMENT OF BOWTIE 0° MESH: A PRELIMINARY STUDY

Previously in our lab, the Neo-Hookean model was shown to accurately predict the non-linear, load-elongation behavior of Restorelle (Coloplast, Minneapolis, MN), a commercially available synthetic mesh [161]. However, the results from preliminary computational studies revealed that the Neo-Hookean model was not able to accurately predict the pore deformation of the bowtie 0° meshes as observed via mechanical testing in Section 4.3.3.1. Thus, the Arruda-Boyce model was considered as an alternative to the Neo-Hookean. In the remaining sections of this Appendix, the Arruda Boyce model will be introduced and the methodology as well as the results from our preliminary analysis with using the Arruda Boyce model to develop a finite element model of the bowtie 0° mesh will be presented.
C.1 ARRUDA BOYCE

The Arruda-Boyce model is used to predict the non-linear stress-strain relationship of a hyperelastic material that undergoes large deformation. Specifically, the Arruda-Boyce model was developed to describe the deformation of rubber materials. This model uses a statistical mechanics approach in which the underlying macromolecular network structure of rubber is modeled as an eight-chain network. Each chain is allowed to stretch to its respective maximum length and this behavior is incorporated within the Arruda-Boyce model. Only two parameters are required to describe an Arruda-Boyce material: the initial modulus and the limiting chain extensibility (or chain locking stretch), which is defined as the final length of the chain divided by the initial chain length. For a hyperelastic material, the stress and strain relationship is defined by a strain energy function, $\Psi$, (also referred to as a stored-energy function). The general form of the strain energy function for the Arruda-Boyce model is derived from a series expansion of the inverse Langevin function with the first five terms as follows:

$$
\Psi = nk\Theta \left[ \frac{1}{2} (I_1 - 3) + \frac{1}{20N} (I_1^2 - 9) + \frac{11}{1050N^2} (I_1^3 - 27) + \frac{19}{7000N^3} (I_1^4 - 81) \right. \\
\left. + \frac{519}{673750N^4} (I_1^5 - 243) \right] + \ldots
$$

where $n$ represents the chain density, $k$ is Boltzmann’s constant, $\Theta$ is temperature, $I_1$ is the first invariant of the right Cauchy-Green tensor, and $N$ is the number of links in a chain (this parameter is also related to the stretch at which the chains reach full extension). For an incompressible Arruda-Boyce material, the uncoupled strain energy function is defined as:

$$
\Psi = \mu \sum_{i=1}^{5} \frac{C_i}{N^{i-1}} (I_1^i - 3^i) + U(f)
$$
where \( \mu \) is the initial modulus, \( C_1 = 1/2, C_2 = 1/20, C_3 = 11/1050, C_4 = 19/7000, C_5 = 519/673750, \) and the function \( U(J) \) defines the volumetric strain as:

\[
U(J) = \frac{1}{2} k (\ln J)^2
\]

where \( k \) is the bulk modulus and \( J \) is the Jacobian of the deformation gradient tensor [270].

### C.2 DETERMINATION OF MATERIAL PROPERTIES

In order to determine the material properties of the Arruda-Boyce model for PDMS, an inverse finite element analysis was performed. For this analysis, experimental data obtained from uniaxial tensile tests (load to failure) of PDMS 5:1 ratio dog-bone samples (described in Section 4.2.3) was fit to a computational simulation of the central portion (also referred to as the gauge length region) of the PDMS dog-bone. In other words, instead of the CAD model being dog-bone shaped, the model was in the shape of a rectangle, which corresponds to the shape of the central portion of the dog-bone. This was done in order to diminish the effects of clamping. During uniaxial testing of PDMS dog-bone samples, a large degree of elongation in the PDMS occurs at the clamps. This elongation is a direct result of the clamps and is not captured during the collection of data (elongation is quantified using markers placed within the gauge length region of the dog-bone). Thus, by modeling only the central portion, we are able to focus on the precise location where the data was collected.

The CAD model of the central dog-bone region was created in SolidWorks 2013 x64 Edition (Dassault Systèmes SOLIDWORKS Corporation, Waltham, Massachusetts). The dimensions of this model matched those of the dog-bones manufactured in Section 4.2.2.1: length
- 33 mm, width - 3 mm, and thickness -1 mm. This model was discretized using methods described previously (Sections 2.4) and consisted of 43,008 linear hexahedral elements. In order to simulate a uniaxial tensile test that is reflective of the central region, the following boundary conditions were prescribed. Translation in the z-direction was fixed for the bottom face of the discretized model while one node (located in the central region) on the top face was fixed to a rigid body. The remaining nodes on the top face as well as the rigid body were allowed to displace in the z-direction. Additionally, translation in the x-direction was fixed for one of the two faces aligned along the thickness of the discretized model while translation in the y-direction was fixed for one of the two faces aligned along the width of the model. A prescribed displacement of 30.79 mm was applied to the rigid body. This displacement corresponds to the maximum amount of elongation that the PDMS dog-bone samples experienced prior to failure. The inverse FE method was performed in FEBio using a Levenberg-Marquardt optimization method. For the optimization, the sum of the squared difference between the experimentally obtained forces for the PDMS dog-bones, uni-axially loaded, and the resulting reaction force of the computational simulation of the PDMS dog-bones, uni-axially loaded, (with the “unknown” parameters) was minimized with a convergence tolerance of 0.001. From this analysis, the optimized material properties were as follows: \( \mu = 0.146 \) MPa, \( N = 1.305 \), and \( K = 100 \) MPa. In order to check the accuracy of these parameters, a uniaxial test was simulated using the optimized parameters and the same model and boundary conditions as outlined above. The results of this study were then post-processed in PostView (University of Utah, MRL). A load-elongation curve for this simulation was created by plotting the observed elongation of the model versus the reaction force of the rigid body (which is similar to the reading of the load cell experimentally). For comparison, the load-elongation data from the experimental test of PDMS dog-bone samples was plotted with the simulation data.
The Arruda Boyce model with the optimized parameters listed above was able to accurately predict the non-linear, load-elongation behavior of the PDMS dog-bone samples (Figure 92). Although there were some slight deviations of the model from the experimental data between 10 mm and 20 mm, overall there was good agreement between the two. Thus, the optimized parameters determined in this study were used as the initial inputs to define the material properties for the bowtie 0° mesh models.

![Figure 92: Resulting load-elongation curves from experimental data of PDMS 5:1 dog-bone samples (black) and the Arruda Boyce, FE model of the PDMS dog-bones (red) uniaxially loaded to failure. The Arruda Boyce model, with the following parameters: \( \mu = 0.146 \text{ MPa}, N = 1.305, \text{ and } K = 100 \text{ MPa}, \) determined via inverse FEA, closely captured the load-elongation behavior of the PDMS dog-bones as indicated by the fit of the finite element data to the experimental data.]
C.3  BOWTIE 0° MESH CAD MODEL AND DISCRETIZATION

A computational bowtie 0° mesh model was generated using SolidWorks 2013 x64 Edition (Dassault Systèmes SOLIDWORKS Corporation, Waltham, Massachusetts). This model is simply the CAD model of the bowtie 0° meshes created in Section 4.3.2.1; therefore, the same design criteria that was used to create the bowtie 0° meshes was also used to create the CAD model in this study. To recall, the design criteria was as follows:

1) The minimal pore dimension was at least 1 mm. Although, it should be noted that models containing angles less than 90° had small regions of the pore where the fibers were closer than 1 mm.

2) The smallest allowable angle within a pore was 45°.

3) The width of the fibers equaled 0.75 mm.

4) The thickness of the model was 1 mm.

The bowtie 0° CAD model was 103.3 mm x 17.15 mm (Figure 93). Following the creation of this CAD model, the model was discretized using a similar method as outlined in Section 2.3. Briefly, the bowtie 0° model was imported into Autodesk Simulation Mechanical 2015 (Autodesk, Inc., San Rafael, CA). The default settings of this software were used to discretize the model. Additionally, the refinement of the discretized model was adjusted until a homogenous discretization (relatively coarse) was achieved (i.e. the number and type of elements were evenly distributed across the model as much as possible). The discretized model was further refined to create a denser model (i.e. the number of elements were increased) and all refinements consisted of 1st order elements. The final discretized finite element model consisted of 413,474 elements (tetrahedral elements: 28,520, pentahedral elements: 27,653, hexahedral elements: 357,301).
C.4 FINITE ELEMENT ANALYSIS

C.4.1 Methods

Following discretization, the discretized bowtie 0° finite element model was imported into PreView (University of Utah, MRL) and a simulated uniaxial tensile test via 3D quasi-static, large deformation FEA was performed using FEBio (University of Utah, MRL) as described in Section 2.5. Briefly, the bottom edge of the discretized model was fixed in translation and rotation while the top edge was fixed to a rigid body. Both the top edge of the discretized model and the rigid body were allowed to displace in the y-direction, along the length of the model. Additionally, all deformation was constrained to be in the plane of the model. A load of 3 N was applied to the rigid body. The material for all models was defined to be Arruda Boyce with the optimized parameters determined in Section C.2. The solution was then obtained using FEBio (University of Utah, MRL)
and was post-processed to examine pore deformation (i.e. changes in pore size, porosity, and effective porosity) in response to 3 N.

C.4.2 Results

Theoretically, determining the Arruda Boyce material properties for PDMS and then applying this same material model and properties to the bowtie 0° FE model should produce the same pore deformation as observed experimental for the bowtie 0° meshes in response to 3 N. Overall, the pores of the bowtie 0° model expanded and elongated as predicted by the bowtie 0° FE model (Figure 94). Quantifying pore deformation of the FE model and comparing this data to the experimental results, the FEA was able to predict the increase in the width of the pores that was observed experimentally (Figure 95). However, the model was not able to predict the thinning of the vertical fibers in the bowtie 0° meshes that occurred with loading (Figure 94, yellow arrows). Additionally, the FEA was not able to predict the increase in the length, porosity, and effective porosity (Figure 94). In an attempt to better predict the previously mentioned parameters, the bulk modulus K was reduced. Reducing K yielded a closer prediction to the previously mentioned parameters; however, the predictions were still not as close as the width, which suggested that adjusting the other parameters μ and N may produce results more similar to the experimental (Figure 94 and 95). Thus, a small parametric analysis was performed adjusting μ and N while keeping K at 100 MPa. Indeed, adjusting μ and N resulted in changes in pore size, porosity, and effective porosity that were similar to the experimental results (Figure 96 and 97). Additionally, the thinning of the vertical fibers that was observed with the bowtie 0° meshes was captured with the new set of parameters, μ = 0.3 MPa, N = 6, and K = 100 MPa (Figure 96).
Figure 94: Bowtie 0° mesh and model (Arruda Boyce $\mu = 0.146$ MPa, $N = 1.305$, and $K = 100$ MPa, 1 MPa, and 0.5 MPa) results in response to 0.1 N and 3 N of force applied along the longitudinal axis of the mesh and model. Similar to the bowtie 0° meshes, the pores of the bowtie 0° models elongated; however, thinning of the vertical fibers did not occur with the models as it did experimentally with the bowtie 0° meshes.
Figure 95: The bowtie 0° models (Arruda Boyce $\mu = 0.146$ MPa, $N = 1.305$, and $K = 100$ MPa, 1 MPa, and 0.5 MPa) were able to predict the increase in the width of the pores relatively well compared to the prediction of the changes in length, porosity, and effective porosity.
The Arruda Boyce model with $\mu = 0.3$ MPa, $N = 6$, and $K = 100$ MPa was able to predict the pore elongation and vertical fiber thinning that was observed with the bowtie $0^\circ$ meshes.

Figure 96: The Arruda Boyce model with $\mu = 0.3$ MPa, $N = 6$, and $K = 100$ MPa was able to predict the pore elongation and vertical fiber thinning that was observed with the bowtie $0^\circ$ meshes.
Figure 97: Quantifying the changes in pore size, porosity, and effective porosity for the Arruda Boyce model with \(\mu = 0.3\) MPa, \(N = 6\), and \(K = 100\) MPa confirmed that this model was able to predict the pore deformation of the bowtie 0° meshes.

C.5 DISCUSSION AND CONCLUSION

The results of this preliminary study demonstrated that the Arruda Boyce model was able to provide a good approximation of the pore deformation for the bowtie 0° meshes. However, the material properties determined for the Arruda Boyce model were only optimized for the bowtie 0° meshes.
meshes. Studies investigating whether these properties will work for the other bowtie pore configurations or the chiral hexagon meshes have yet to be performed. Additionally, convergence testing was not done with the current FE model, which is a necessary next step if this model is going to be used for future optimization studies. Initially, it was believed that the Arruda Boyce material properties optimized for PDMS would provide a good approximation of the bowtie 0° meshes; however, this was not the case. It is possible that the tiny air bubbles created when pouring the PDMS into the bowtie mesh molds may have impacted deformation more for the meshes than the PDMS dog-bone samples, hence the same properties could not be used for both. Thus, future studies (using the same manufacturing methods) may have to use inverse finite element analysis to determine the material properties for a PDMS mesh with the load-elongation data for the mesh itself rather than the PDMS dog-bone sample data.
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