UNIVERSITY OF CALIFORNIA SAN DIEGO

Development of a Model to Simulate Radiation Induced Vaginal Stenosis

A Thesis submitted in partial satisfaction of the requirements
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by

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The Thesis of Rafaela Mayumi Simoes Torigoe is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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ABSTRACT OF THE THESIS

Development of a Model to Simulate Radiation Induced Vaginal Stenosis

by

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Master of Science in Engineering Sciences (Mechanical Engineering)

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Professor Frank E. Talke, Chair

Radiation induced vaginal stenosis (VS) is characterized by the narrowing or shortening of the vaginal canal following radiotherapy or brachytherapy for the treatment of gynecological malignancies. One of the most prominent gynecological cancers in women is cervical cancer, which has a fairly high survival rate but can have detrimental impacts on the quality of life of those who survive it, mainly due to tissue injury caused by radiation. The current standard of care for the prevention of vaginal stenosis involves the use of vaginal dilators, which usually consist of stiff plastic or silicone rods graded in different sizes to mechanically expand the vaginal canal. Such course of treatment lacks patient adherence and cannot be objectively assessed, as it does not provide monitoring of the progression of the VS syndrome or of dilator use. In order to address this medical need, a vaginal dilation system consisting of an expandable vaginal dilator that can be monitored through pressure measurements was developed. In order to characterize the proposed expandable vaginal dilator prior to its clinical use, a model was developed to simulate different severities of vaginal stenosis, taking into consideration changes in vaginal morphological properties, such as the increase in deposition of stiffer collagen fibers. An established clinical grading criteria for vaginal stenosis was used to modify vaginal dimensions according to a set baseline, which was determined by

average vaginal dimensions reported in literature. A combination of 3D printed thermoplastic polyurethane (TPU) infill and Ecoflex 30 silicone were used to manufacture graded vaginal phantoms and characterize the pressure of the proposed vaginal dilations in a variety of VS scenarios. A variation in diameter, showcasing different severities of VS according to vaginal dimension, as well as a variation in TPU infill density were explored in relation to dilator pressure. Furthermore, the mechanical properties of porcine vaginal tissue were compared to the mechanical properties of the material used to manufacture the phantom model. It was found that while the mechanical properties of vaginal tissue and the composite material used for the phantoms differed, the dilator pressure recorded in both vaginal tissue and the developed vaginal phantoms showed many similarities and was able to highlight different trends (i.e. increasing pressure with decreasing diameter or increasing infill density) depending on the sizing of the vaginal dilator. Thus, the developed model can be used to initially test the vaginal dilators developed. Future considerations should be given to improve both the phantom model and iterate on the dilation system.

Chapter 1: Medical Background

1.1 Anatomy of the Female Reproductive System:

The female reproductive system is a complex organ system that is essential for human reproduction and plays a major role in female development and hormone regulation [1]. It consists of the ovaries, uterus, cervix, and vagina, as shown in **Figure 1**. These organs interact dynamically with the brain, in particular the hypothalamus and the anterior pituitary gland, to regulate hormones involved in pregnancy and female development [2].

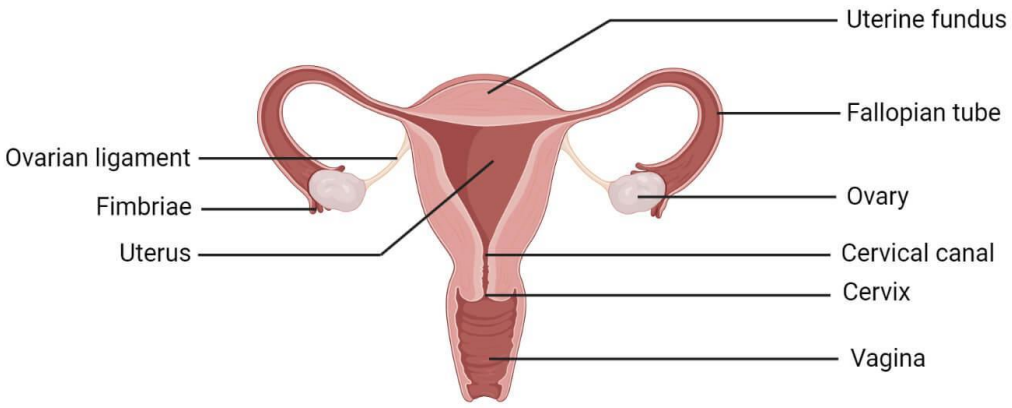

Figure 1: Structure of the Female Reproductive System in Humans [3]

The vagina is the tubular organ that connects the uterus through the cervix and the vestibulum [4]. The anatomy of the vagina consists of a generally tubular shape that is collapsed with its front and posterior wall being in contact. Additionally, the front wall is generally shorter than the posterior wall; however, during sexual stimulation, the posterior wall extends, changing the shape of the vagina slightly [4]. While the female anatomy has a relatively similar structure between different individuals, the dimensions of the vagina are highly variable, as shown in

Table 1. Tan et al. reported the average length of the vagina to be 8-10 cm. **Table 1** shows the variations of vaginal dimensions reported in literature [4], [5].

Table 1: Average vaginal dimensions reported in literature

Source	**Number of Subjects (n)**	**Mid-lower Vagina (cm)**	**Introitus Width (cm)**	**Linear Length of Vaginal Canal (cm)**	**Method**
K.T. Barnhart et al. (2006) [6]	28	2.72	2.61	6.27	MRI analysis
P.B. Pendergrass et al. (1996) [7]	39	-	4.67	11.51	Vaginal Casts
J. Lloyd et al. (2005) [8]	50	-	-	9.6	Vaginal swab
A.M. Weber et al. (1995) [9]	104	-	3.14	10.7	Graded Vaginal Obturators

1.2 Radiation Induced Vaginal Stenosis

Cervical cancer is the fourth most common cancer among women, with approximately 600,000 women developing and 300,000 dying from cervical cancer in 2018 [10]. The incidence and mortality rate of cervical cancer is variable between different countries, due to its relationship with the screening and detection of human papilloma virus (HPV), as HPV serotypes account for approximately 90% of cervical cancers [11]. In the United States, nearly 13 thousand new cases of cervical cancer were estimated in 2016 alone [12]. The curative treatment for cervical cancer is radiation, concurrent chemotherapy and brachytherapy, which can lead to both acute and chronic toxicities affecting the patient's quality of life [13].

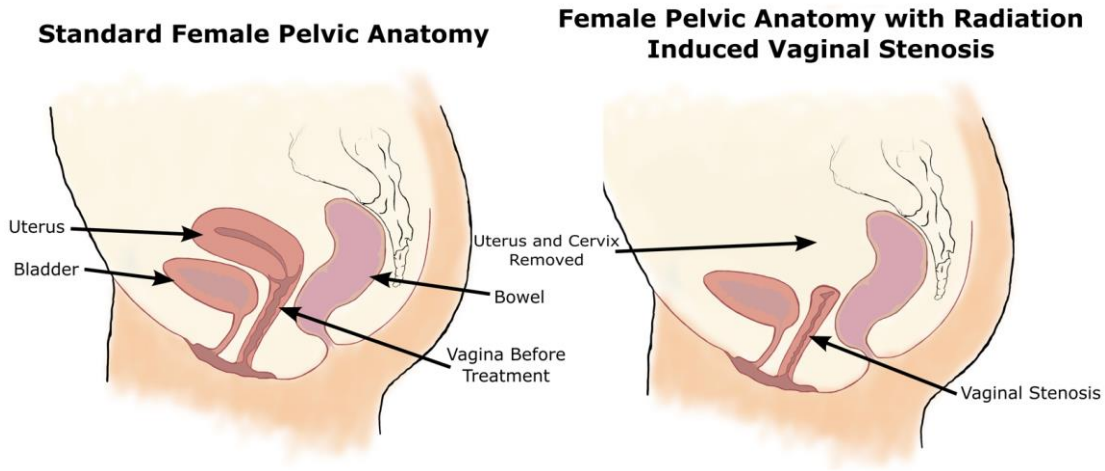

Figure 2: Schematic of radiation induced vaginal stenosis compared to the standard female pelvic anatomy

A common complication of radiotherapy after cervical cancer is the narrowing and shortening of the vaginal canal, termed vaginal stenosis (VS), which is shown in **Figure 2**. The

recorded incidence of radiotherapy induced VS can vary significantly, mainly due to small patient cohorts in the studies reported as well as a lack of consensus in a common grading scale. A summary of the incidence of vaginal stenosis reported in the literature is provided in **Table 2**, which shows the variability in assessment of vaginal stenosis and the incidence reported.

Table 2: Summary of the incidence of radiation induced vaginal stenosis reported in literature

Type of Malignancy	Sample size (n)	VS Scoring Scale	Dilator Use	Incidence of VS	Source
Anal squamous cell carcinoma	96	CTCAE v. 4.0	38%	71%	K.L. Mirabeau-Beale et. al (2013) [14]
Squamous-, adeno-, or adenosquamous carcinoma of the uterine cervix	630	CTCAE v3.0	-	59%	K. Kirchheiner et. al (2016) [15]
Localized endometrial carcinoma	100	CTCAE v4.03	64%	33%	H. Park et. al (2015) [16]
Pathologic Stage I endometrial cancer, Grade 1-2	303	-	Patients given dilators, but use not reported	1.2%	G.H. Eltabbakh et. al (1997) [17]
Invasive cervical malignancies	221	Own scale	-	88%	P. Hartman and A.W. Diddle (1972) [18]
Carcinoma of the cervix	188	Own scale	-	38%	A. H. Brand et. al (2006) [19]
Untreated cervical cancer	57	LENT-SOMA	-	61%	K. Yoshida et. al (2015) [20]

Table 2: continued

Advanced or recurrent cervical cancer and primary vaginal cancer	118	Questionnaire	-	48%	P.T. Jensen et. al (2003) [21]
Cervical cancer and endometrial cancer	130	CTCAE v5	CC: 9.8% EC: 13%	CC: 78.5% EC: 66.4%	T. Morais Siqueira et. al (2021) [22]
Cervical cancer	142	CTCAE v3	Acrylic cylinder once daily	96%	J. Martins et. al (2021) [23]
Endometrial or cervical carcinoma	70	-	Stents	Control: 57% Stent: 11%	S. B. Decruze et. al (1999) [24]
Cervical or endometrial cancer	90	-	Modified vaginal dilator	No % reported	D. W. Bruner et. al (1993) [25]
Carcinoma of the uterine cervix	93	-	-	46%	R. M. Pitkin and L. W. VanVoorhis (1971) [26]
Invasive carcinoma of the cervix	97	Questionnaire	-	60%	M. M. Abitbol and J. H. Davenport (1974) [27]
Carcinoma of the cervix	22	Questionnaire	-	72%	M. M. Seibel et. al (1980) [28]

Table 2: continued

Cervical cancer	34	Questionnaire	Poor compliance with dilators	1 year: reduction in diameter: 13% reduction in length: 24%	L. R. Schover et. al (1989) [29]
Cervical cancer	16	Questionnaire	-	47%	L. D. Flay et. al (1994) [30]
Cervical or endometrial cancer	41	Vaginal cylinder grading	-	Not calculated	A. Katz et. al (2001) [31]
Cervical cancer	5	-	No use of dilators	All patients had VS prior to study	P. A. Poma (1980) [32]
Cervical , vaginal, or endometrial cancer	7	Chart Review	-	All patients had VS prior to study	S. E. Hyde Franzcog (1999) [33]
Cervical, vaginal, uterine cancer	45	-	-	31.60%	M. Jurado (1999) [34]

Currently, there are two prominent existing grading scales that have been commonly used in the literature to study and report the incidence of VS: the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) scale [35] and the Radiation Therapy Oncology Group (EOTG)/European Organization for the Research and Treatment of Cancer (EORTC) late effects of normal tissues, subjective, objective, management (LENT-SOMA) scoring scales [36], which are shown in **Table 3** and **Table 4**. These criteria are used by physicians to establish the severity of complications associated with cancer treatment, in this case VS. The CTCAE scale grades adverse events on a scale of 1-5 relative to the patient's baseline (0), with 5 resulting in death. Because VS rarely, if ever, results in death, the CTCAE

scale only considers subjective measures of narrowing or shortening relative to quality of life on a scale of 1-3 (**Table 3**). The LENT-SOMA scale can be more descriptive, as it combines commonly associated adverse events such as atrophy, dyspareunia, and vaginal stenosis and provides a more quantitative assessment in the objective portion of the scale (**Table 4**).

Table 3: Common Terminology Criteria for Adverse Events (CTCAE) for Vaginal Stricture. Version 5.0 [35], [37]

CTCAE Terminology	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Vaginal Stricture. Definition: a disorder characterized by a narrowing of the vaginal canal	Asymptomatic; mild vaginal shortening or narrowing	Vaginal narrowing and/or shortening not interfering with the physical examination	Vaginal narrowing and/or shortening interfering with the use of tampons, sexual activity or physical examination	-	Death

Table 4: Radiation Therapy Oncology Group (ETOG)/European Organization for the Research and Treatment of Cancer (EORTC) late effects of normal tissues, subjective, objective, management (LENT-SOMA) scoring table for injury to the vagina [36], [37]

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Subjective:				
Dyspareunia	Occasional & minimal	Intermittent & tolerable	Persistent & intense	Refractory & excruciating
Dryness	Occasional	Intermittent	Persistent	Refractory
Bleeding	Occasional	Intermittent	Persistent	Refractory
Pain	Occasional & minimal	Intermittent & tolerable	Persistent & intense	Refractory & excruciating
Objective:				
Stenosis/ length	>2/3 normal length/diameter	1/3-2/3 normal length/diameter	<1/3 normal length/diameter	Obliteration
Dryness	Asymptomatic	Symptomatic	Secondary Dysfunction	-
Ulceration/ necrosis	Superficial, <= 1 cm^2	Superficial, > 1 cm^2	Deep ulcer	Fistulae
Atrophy	Patchy	Confluent	Nonconfluent	Diffuse
Appearance	Telangiectasia without bleeding	Telangiectasia with gross bleeding	-	-
Synechiae	-	Partial	Complete	-

Table 4: Continued

Bleeding	-	On contact	Intermittent	Persistent
Management:				
Dyspareunia/ Pain	Occasional non-narcotic	Regular non-narcotic	Regular narcotic	Surgical intervention
Atrophy	Occasional hormone cream	Intermittent hormone cream	Regular hormone cream	-
Bleeding	Iron therapy	Occasional transfusion	Frequent transfusion	Surgical intervention
Stenosis	Occasional dilation	Intermittent dilation	Persistent dilation	Surgical reconstruction
Dryness	Hormone replacement	Artificial lubrication	-	-
Ulceration	Conservative	Debridement	HBO2	Graft, surgical repair
Analytic:				
MRI	Assessment of wall thickness, sinus and fistula formation			
Ultrasound	Assessment of wall thickness, sinus and fistula formation			
EUA Cytology/ biopsy	Assessment of wall diameter and length and mucosal surface			

Vaginal stenosis as a result of radiotherapy can often lead to intractable long-term complications, including difficulties with sexual intercourse, pain during medical pelvic exams, predisposition to trauma and infections, and even complete vaginal occlusion [19]. VS may prevent adequate internal examinations to monitor disease recurrence after radiotherapy has been completed [19]. Thus, the complication of VS can significantly impact the health and quality of life of cervical cancer survivors.

VS also occurs in women treated with radiation for endometrial and anal cancer, with a somewhat lower frequency than in cervical cancer, but these patients boost the total number of new radiation-induced VS cases per year in the US to approximately 30,000 [10]. This is a large population cohort, which is especially concerning as there are no viable therapeutic options for these patients.

1.3 Current Treatments for the Prevention of Vaginal Stenosis

The common established form of treating stenosis as a result of radiotherapy is vaginal dilation through the use of a vaginal dilator. A dilator is a device which is applied directly to the vagina or cervix, designed to apply pressure to the vaginal cavity and prevent further growth of stenosed tissue. Although dilator designs vary, in general they are plastic or silicone devices meant to be inserted into the vagina for a short period of time, as shown in **Figure 3**. Usage of dilators for the treatment of stenosis has been recommended since at least 1938 [38]. These devices have patient adherence issues due to comfort as well as lack of customizable features that may not be suited for all patients. Recent manufacturing research has been focused on the development of dilator devices that increase patient comfort, contain customizable features while

increasing effectiveness and decreasing cost of devices with the hopes of increasing patient compliance for such devices [39].

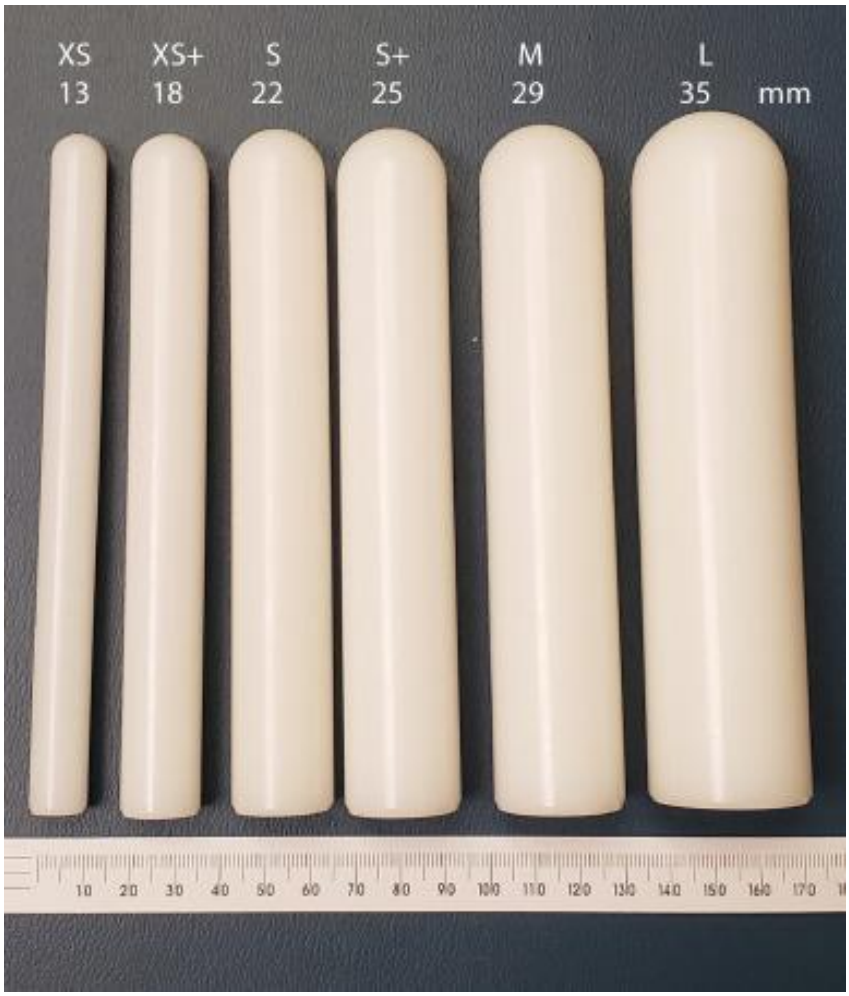

Figure 3: Commercially available vaginal dilators [40]

Velaskar et al. reported that the use of vaginal dilators can be effective at preventing VS by restoring and maintaining vaginal length after radiation therapy [41]. However, the application of this unrefined methodology is often painful, causes bleeding, and is limited by poor patient acceptance [37]. Thus, new designs to improve such treatment are desirable. Decruze et al. created a dilator made out of Perspex that was a better fit for the vaginal anatomy

than existing devices, finding it effective in the treatment of vaginal stenosis [24]. However, the device was not specified to be customizable to patient use and there was not full patient compliance with dilator usage. Besides the aforementioned setbacks, adherence to treatment was increased through improved patient education of dilator usage [24]. Recent dilator designs have used silicone materials to prevent stenosis through increased patient adherence. The design by Patnana et al. claimed that silicone's high elastic recovery, high tear and tensile strength have made the material suitable for dilator fabrication [42]. The researchers have found the fabricated device to be effective in the prevention of stenosis in one patient. Although not mentioned, the elastic behavior of such silicone may potentially increase patient compliance with dilator use as the device may be more comfortable to use. However, this would need to be confirmed through a trial.

One solution to improve patient adherence to the use of vaginal dilators is to improve their design by using inflation and monitoring the pressure of the dilator, correlated to its expansion. The design of the proposed dilation system developed by our research group will be briefly described in Chapter 4. The use of pressurized, inflatable vaginal dilators for the treatment and prevention of radiation-induced VS provides a soft, gradual expansion mechanism to potentially less painful and injurious, load across the vaginal wall that is possible with manual rod-shaped dilators. The design of an improved device would allow for easy insertion due to the initially smaller dilator diameter (before expansion). Additionally, the controlled and compliant expansion could be pressurized to maximize patient comfort. Thus, this proposed solution may be suitable for patients who have undergone irradiation and are facing the long-term vaginal complications of cancer treatment.

1.4 Medical Need: An in vitro test to understand dilator behavior in normal and stenotic conditions

Various forms of vaginal phantoms of several different complexities have been developed for various uses. For instance, Asiedu et al. used a commercially available vaginal phantom model made by Syndaver to test a new speculum design [43]. The Syndaver model is one of the most anatomically accurate vaginal phantom models, as it is anatomically similar to the female reproductive system and somewhat replicates the mechanical properties of vaginal tissue [43]. However, not all vaginal phantom models have the need to be as anatomically accurate as the Syndaver model. For instance, simpler phantom models have been used for clinical applications such as simulating procedures such as the insertion of a needle for use during brachytherapy and for the evaluation of hydrogel packing in the vaginal canal during brachytherapy [24], [44]. While many vaginal phantoms have been designed for several clinical applications and to test new devices designed to be used in the field of women's health, a model designed to simulate vaginal stenosis and test different vaginal dilators has yet to be developed. In this model, it is important to consider the mechanical properties of the vaginal tissue, as well as establish the behaviors of the vaginal dilator in different severities of vaginal stenosis, which can be useful for monitoring the progress of patients during dilation treatment and to objectively define vaginal stenosis grading based on clinically established scales like the CTCAE and LENT-SOMA scales.

Chapter 2: State of the Art

2.1 History of Phantoms and Their Use

Medical phantoms are artificial models that represent an anatomic structure designed to simulate a desired property to be studied without the need of a clinical study [45], [46]. Some of the earliest designs of medical phantoms were for the purposes of studying the effect of radiation on tissue. In order to avoid the harmful side effects that patients were experiencing while X-ray technology was being developed during the early twentieth century, physicists developed phantoms to quantify the doses being delivered to tissue without having to test it on the patients, as they would experience harmful side effects that a synthetic model would not [45]. These early phantoms were made of water or wax and consisted of simple geometries such as blocks [45].

Phantoms have been used for a variety of applications, including imaging applications from as early as the 1940s, as well as more recently, for mimicking the mechanical properties of tissue to test desired procedures [47]. For gynecological applications specifically, some phantoms have been designed; however, they are more focused in dosimetry applications such as a system to practice brachytherapy or imaging applications such as ultrasound imaging [46], [48]. Nattagh et al., for instance, described the design of a training phantom for physicians to practice ultrasound-guided needle insertion [46]. Such a model was manufactured through creating the desired gynecological structures through mold making with gelatin matrices for its imaging properties and coating the model in rubber to simulate the texture of soft tissue [46]. Furthermore, there are commercially available models such as the one manufactured by Syndaver (see section 1.4) that mimic the gynecological anatomy and mechanical properties,

which can be useful for a variety of medical simulations. The described history of phantoms show the benefits of their use and their potential in testing novel therapies.

2.2 Material Considerations in Engineering

The material used to manufacture a phantom can vary significantly, depending on its desired use. One of the most common materials used in the design of phantoms is silicone, as the mechanical properties of the material can be easily tuned by modifying its cross-link density, which would change the ratio of base to cross-linker [47], Furthermore, there is a multitude of commercially available silicone with different mechanical properties, which allow for greater options to improve the phantom by mimicking tissue properties more closely.

With the rise of newer manufacturing technologies such as 3D printing, there is an opportunity to further mimic the structure of tissues in greater detail. 3D printing can be used to establish patterns and use multiple materials to make a composite that is more representative of tissue. Soft materials, such as polymers, can closely mimic tissue at a small strain (<3%); however, for larger deformations such as the ones that the actual tissue experiences, the material properties will differ [49]. Thus, for mimicking vaginal tissue to design a vaginal phantom, it would be advantageous to use 3D printing to make a multi-material model by mixing a stiffer material such as thermoplastic polyurethane (TPU) with a softer material such as silicone.

2.3 Consideration of Mechanical Properties

In order to design a phantom that can mimic the vaginal canal as well as simulate the process of vaginal stenosis, one must consider the mechanical properties of vaginal tissue. It is important to characterize the elasticity of a material, which can be done so using the elastic modulus, as described in equation 1, where E is the elastic modulus, σ is the stress, ε_0 is the

strain, F is the applied force, and A is the area of the applied force, and Δl is the change in length, while l_0 is the sample's original length [47]. It is important to note that most tissue behaves in a viscoelastic manner, which includes more complex mechanical behavior that will not be explored in this project. For the initial design and characterization of the vaginal phantom, linear viscoelastic behavior will be assumed, according to the analysis performed by Griffin et al [50].

$$E = \frac{\sigma}{\varepsilon_0} = \frac{\frac{F}{A}}{\frac{\Delta l}{l_0}} \quad \text{[Equation 1]}$$

Chapter 3: Review of Vaginal Morphological Properties

3.1 Vaginal Morphology: Anatomical Considerations

While radiation induced vaginal stenosis is characterized by the shortening or narrowing of the vaginal canal, several biological events lead to the development of this condition, marked by morphological changes associated with VS. These include the increase in collagen production in the submucosal fibroconnective tissue layer, which can lead to reduction in the blood supply as well as epithelial denudation, causing tissue atrophy, hypoxia, and fibrosis [11]. Such changes lead to the development of telangiectasia (small blood vessels on the surface of the vaginal wall

that can lead to bleeding), the formation of adhesions, loss of elasticity of the vaginal tissue, and occlusion of the vaginal canal [10].

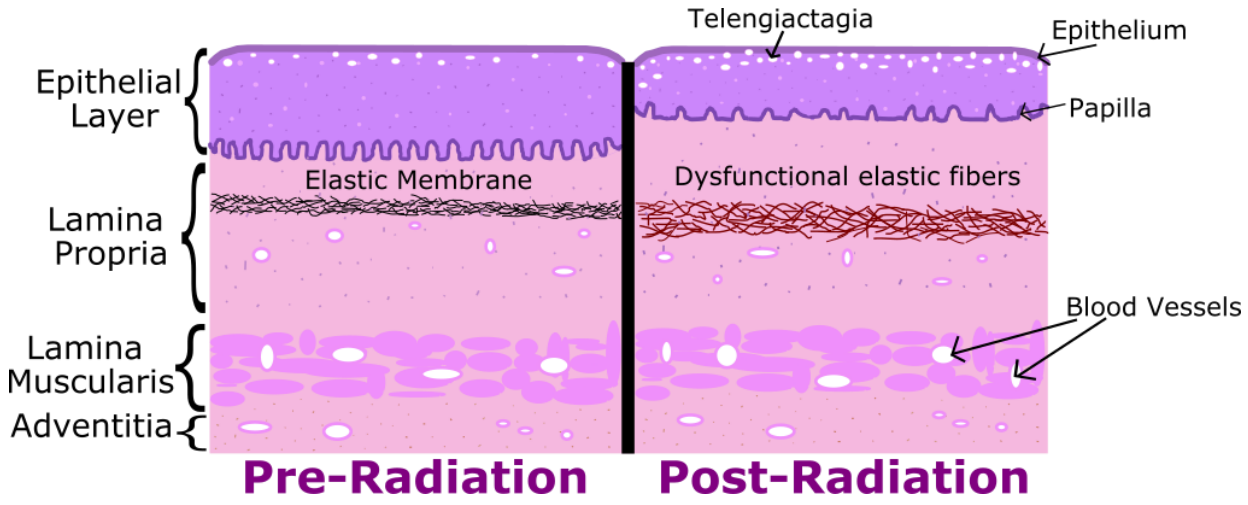

Figure 4: Schematic of Morphological Changes in Vaginal Tissue Associated with Radiation Injury

The use of radiation as a therapy for cervical cancer is extremely common; however, constant exposure to radiation can lead to both acute and chronic injury. With exposure to high energy doses, inflammation and exudates are observed as early side effects to exposure to radiation. In addition, late side effects of radiation are observed as a result of altered biological signaling mechanisms, leading to atrophy and microvasculature changes in the vaginal tissue after treatment has ceased.

Chronic toxicities due to radiotherapy and brachytherapy can include vaginal dryness, dyspareunia, and vaginal stenosis [15], [51]. Vaginal stenosis can affect a large range (1.2-96%, as illustrated in Chapter 1) of gynecologic patients who undergo radiation. In addition to VS, other late effects of irradiation such as vaginal telangiectasia and pallor of the vaginal mucosa

can be observed on follow up clinical examinations [52]. **Figure 4** illustrates some of the morphological changes in vaginal tissue that occur due to radiation injury.

The mechanism of chronic radiation tissue injuries is only partially understood [53]. It is hypothesized that radiation causes progressive endarteritis of the small blood vessels, which results in cellular hypoxia and damages fibroblasts, inhibiting the ability of the irradiated vaginal tissue to repair itself, leading to chronic side effects [4], [53]. Craighead et al. also hypothesized that cells with organ stroma cannot repair DNA damage caused by radiation in people who are sensitive to radiation, which results in a critically low volume of stem cells and is detrimental to tissue healing [53]. The cellular damage can lead to a cascade of related biological events, which might eventually lead to fibrosis, hypoxia, and collagenous scarring, which can contribute to the progression of VS, as shown in **Figure 4**.

Chapter 3.2: Collagen in Irradiated Vaginal Tissue

Several morphological changes in the vagina have been reported as a result of irradiation. Many cervical cancer patients experience vaginal stenosis, vaginal atrophy, and changes in vaginal elasticity following radiation therapy [54]. The morphological changes that are associated with vaginal stenosis include the increase in collagen production in the submucosal fibroconnective tissue layer, which can lead to tissue atrophy and fibrosis [4]. Such changes lead to the development of telangiectasia, the formation of adhesions, loss of elasticity of the vaginal tissue, and occlusion of the vaginal canal [4]. Furthermore, chronic fibrotic changes in pelvic tissue after radiotherapy can worsen or create vaginal atrophy up to two years after treatment, therefore worsening quality of life and sexual function [55].

When exposed to radiation, vaginal tissue can undergo many morphological changes that lead to scarring. While the mechanisms by which this forms are not fully understood, it has been suggested that reactive oxygen species (ROS) as well as their byproducts, are overproduced after exposure to radiation and thus might be responsible for the late stage scarring due to pelvic irradiation [56]. **Figure 5** illustrates the potential biological events that cause the morphological changes observed in irradiated vaginal tissue, which can also be responsible for the progression of VS. Collagen types I, III, and V are the main types of collagen present in the vaginal tissue [4]. While collagen III and V form smaller fibers, collagen type I has larger and stronger fibers. In irradiated tissue, the radio of collagen I to collagen III increases relative to the ratio of collagen types in normal vaginal tissue, which can affect the mechanical behavior of the tissue and lead to scarring [4]. Thus, it is important to consider such morphological changes when designing a phantom to mimic the VS syndrome.

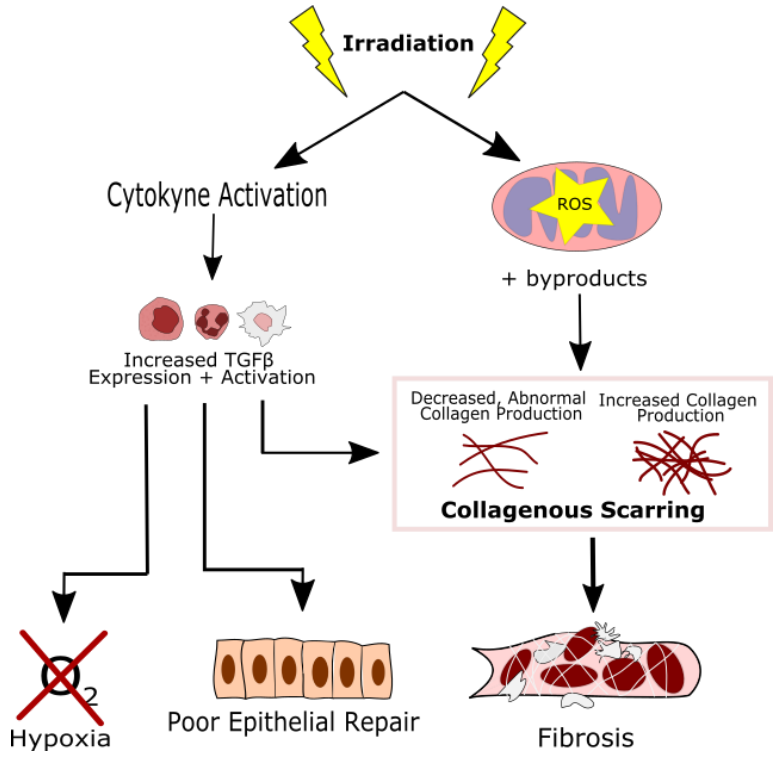

Figure 5: Schematic of biological events following irradiation of the vaginal tissue

Chapter 4: Design and Manufacturing of a Dilation System

4.1 Expandable Dilator Design and Manufacturing

As described in Chapter 1, currently available technologies fail to successfully address the medical need for prevention of VS, as conventional vaginal dilators have low patient adherence. In order to address this medical problem, a vaginal dilator that is able to continuously expand with fluid was designed. The initial design of the expandable dilator is shown in **Figure 6**, consisting of a soft silicone sheath wrapped around a rigid inner rod with fluid filling channels. The dilator can be expanded by connecting a pump or manual syringe to the plastic tube connecting to the insertion rod and pumping air or medically safe liquids such as saline solution into the dilator so that the silicone sheath expands.

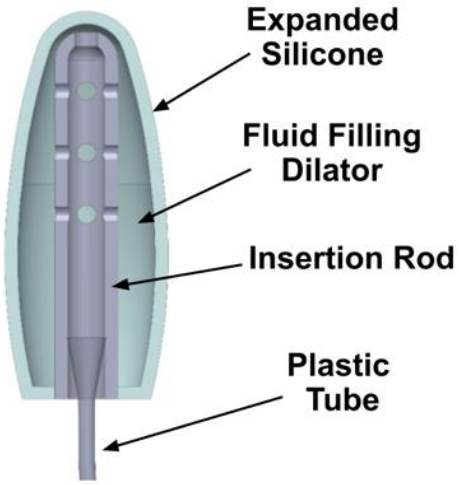

Figure 6: Model of Expandable Dilator Design

Due to the expandable nature of the device, it is envisioned that patient adherence will be greater with this device than with commercially available dilators due to the soft expansion it provides instead of graded steps in commercially available dilators. To accommodate for the variation in vaginal anatomy and severity of VS from the start of dilation therapy, multiple sizes of the dilator prototype were manufactured, as shown in **Figure 7**. These sizes were termed small, medium, and large and are referred to as such in this thesis.

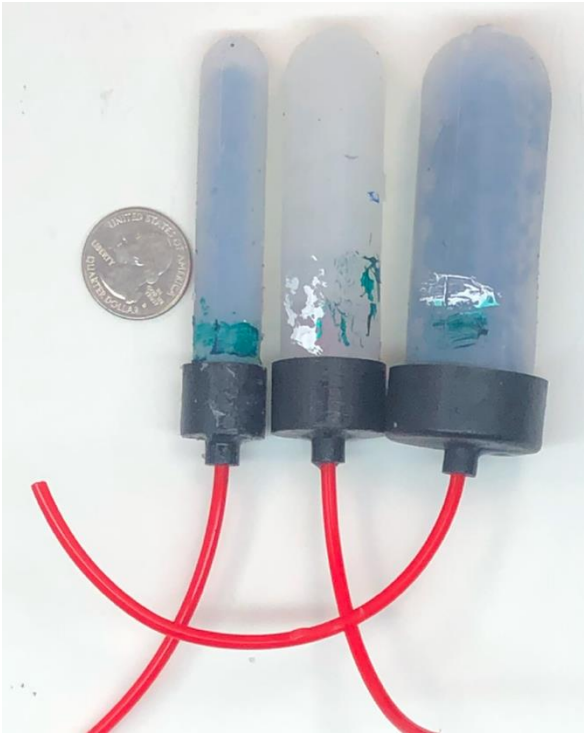

Figure 7: Expandable Vaginal Dilators in Sizes Small, Medium, and Large (from left to right)

4.2 Dilation System Design

In order to monitor the use of the designed vaginal dilator as well as to further characterize the effect of vaginal dilators on the prevention of VS, the expandable dilator was incorporated to a system that allows for automated inflation as well as the monitoring of the

pressure in the dilator (pictured in **Figure 8**). Such a system can be used to ease user experience when using the proposed vaginal dilator as well as to monitor patient adherence and progress through pressure measurements. By recording the pressure response in the dilator whenever a patient uses the system, one would be able to accurately measure how often the patient is using the system and adhering to the proposed treatment course as well as correlate the pressure measurement to the severity of the patient's VS throughout the course of treatment.

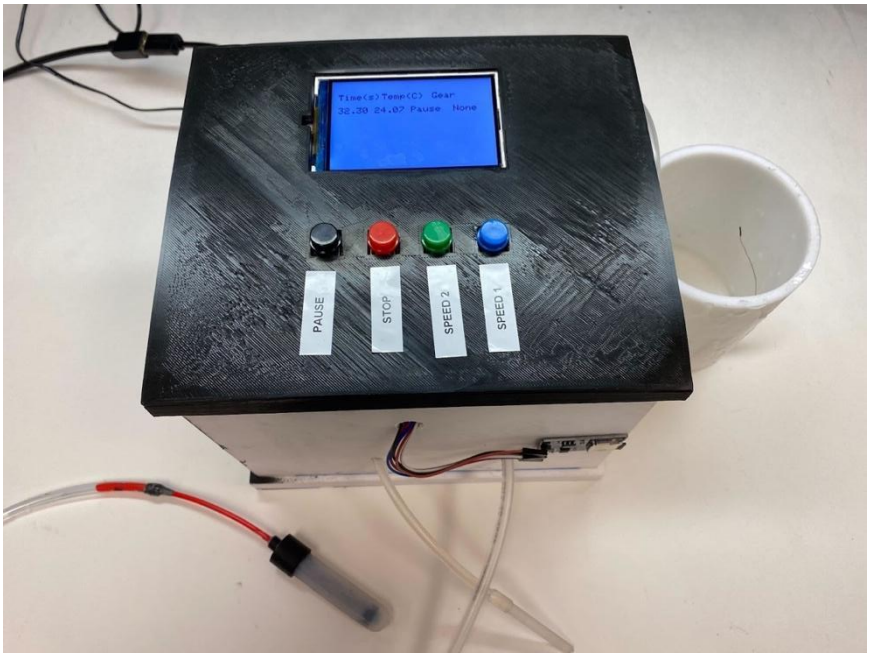

Figure 8: First Prototype of Vaginal Dilation System

Chapter 5: Experimental Methods

5.1 Design of Graded Vaginal Phantoms

In order to understand how the dilation system designed for the prevention of radiation induced vaginal stenosis would behave in a clinical setting, graded vaginal phantoms were designed and manufactured to simulate different stenosis severities. Such a synthetic model can be designed to determine the behavior of the vaginal dilation system according to its pressure in different stenosis scenarios without the need of a clinical trial. The results obtained from the evaluation of the expandable dilators in the vaginal phantoms can then be used to iterate on the dilation system prototype before it is clinically tested.

A literature search was conducted to acquire data on vaginal dimensions, as described in Chapter 1. Average vaginal diameter and length was then calculated and used to design a simplified vaginal phantom, which was 15mm in diameter and 90 mm in length. A simple cylinder design was used to isolate the variability in diameter and stricture of the material utilized to design the phantom. Additionally, the cylindrical design was used to mimic existing phantoms that are used for clinical simulations such as the one seen in **Figure 9**. Such commercially available pelvic models consist of a cylindrical, soft polymer insert into a rigid casing. The manufacturing steps for the designed vaginal phantom are shown in **Figure 10**.

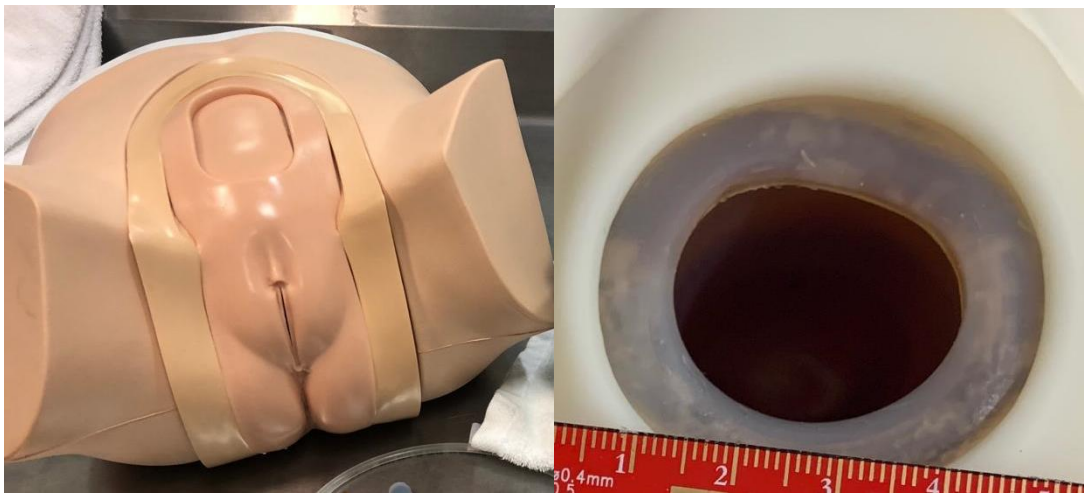

Figure 9: Commercially Available Pelvic Model Available in the Center for Future of Surgery (left) with Normal Vaginal Insert Module (right).

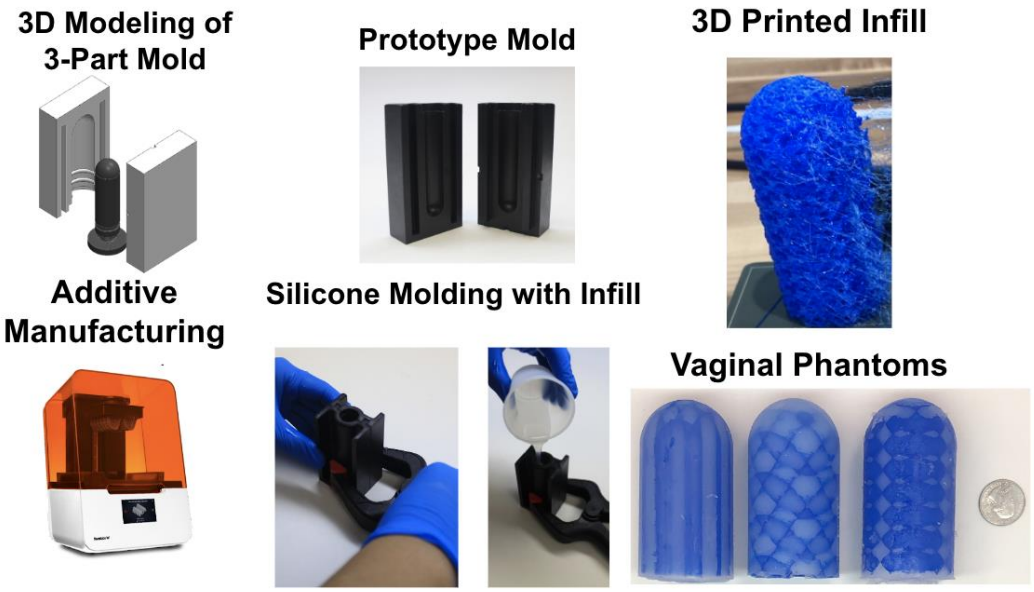

Figure 10: Manufacturing Steps for Proposed VS Vaginal Phantoms

The material chosen to manufacture the proposed vaginal stenosis model was a combination of TPU and silicone (Smooth-On Ecoflex 30). In order to simulate the increase in collagen and elastin fibers in a disorganized pattern, a gyroid infill pattern was designed and 3D

printed with TPU in different infill percentages: 10%,15%, and 20%. Additionally, a cylindrical mold was designed so the 3D printed infill could be cast in silicone to manufacture a vaginal phantom of comparable geometry to commercially available models (**Figure 9**). Several models were manufactured varying TPU infill density, as mentioned, as well as varying diameter (shown in **Figure 10 and 11**). The variations in diameter were scaled according to the LENT-SOMA vaginal stenosis grading scale, with an additional intermediate model that would be categorized in between grade 1 and baseline (Grade 0.5) in the chosen grading scale. The diameters utilized were as follows: 15mm, 12.5m, 10mm, 7.5mm, 5mm.

Figure 11: Vaginal Phantoms Designed According to LENT-SOMA Criteria for Vaginal Stenosis

5.2 Measurement of Mechanical Properties of Porcine Vaginal Tissue

Porcine vaginal tissue was used to measure and understand the mechanical properties of vaginal tissue and compare them to the mechanical properties of the designed phantom. Porcine female reproductive organs (including ovaries, fallopian tubes, cervix, vaginal canal, bladder, and vulva), which are shown in **Figure 12**, were purchased from Sierra Medical. The organs were harvested the day before each experiment was performed. The vaginal tissue was cut and used for both tensile and compression experiments in order to determine its mechanical properties.

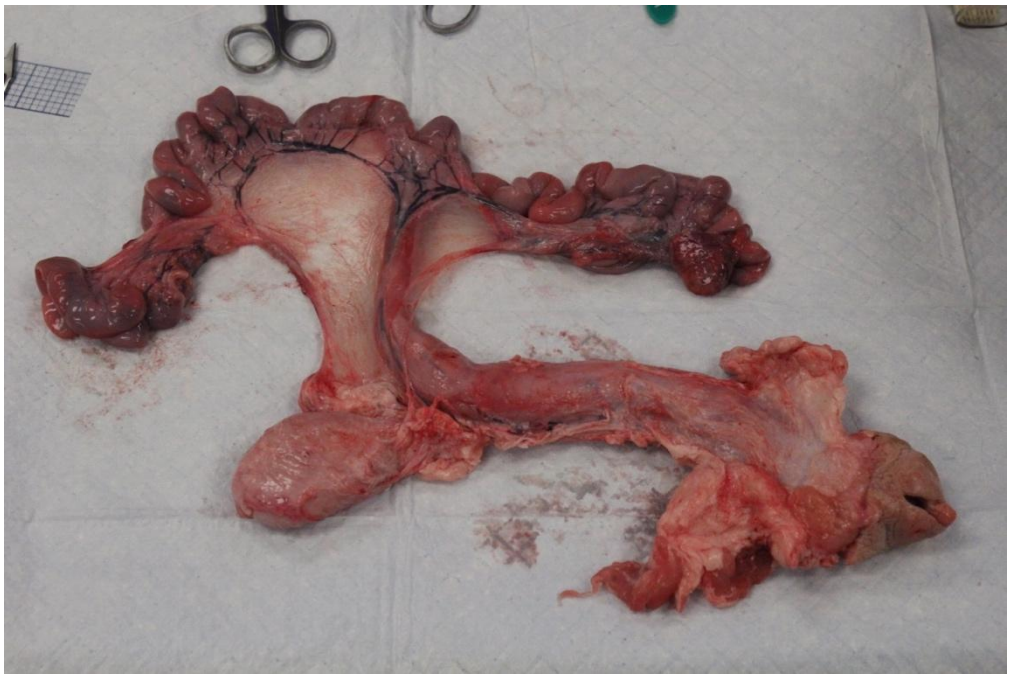

Figure 12: Porcine Reproductive Organs

5.2.1 Tensile Measurements

The tensile properties of porcine vaginal tissue were measured. Rectangular coupons were cut from the tissue with a scalpel, making sure to irrigate the tissue with phosphate buffered saline (PBS) as needed to prevent the tissue from desiccating, which would affect its mechanical properties. Additionally, a thin layer of petroleum jelly was applied to the tissue surface once it was loaded onto the uniaxial tester to prevent tissue desiccation while conducting the tensile tests. When conducting experiments, it was noted that the tissue would easily slip from the grips in the uniaxial tester due to the moisture in the tissue. To resolve this issue, cyanoacrylate adhesive was used on the ends of the tissue samples to fix the tissue onto tape and diminish slipping.

A uniaxial tester (Mark-10) was used to record load as the tissue samples were being pulled. Simultaneously, both the front and side of the tissue samples were recorded in order to keep track of strain throughout each experiment. The pulling speed for all samples was 30mm/min and each test ran until slipping occurred, i.e., until markers placed on the tissue would be significantly displaced relative to their original position. Image processing was conducted with MATLAB and Image J to determine the relationship between stress and strain of porcine vaginal tissue. As previously mentioned, only the linear portion of the results obtained were considered [50]. **Figure 13** shows an example of the tensile tests conducted with porcine vaginal tissue.

Figure 13: Measurement of Porcine Vaginal Tissue Stress and Strain with Uniaxial Tensile Test

5.2.2 Compression Measurements

Porcine tissue samples were prepared by using a metal ring to punch out consistent samples to be tested for compression. The same uniaxial tester as in section 5.2.1 was used by swapping the grips utilized for the tensile measurements for compression plates. The compression speed was 13mm/min, which was the lowest speed the instrument utilized was able to achieve. **Figure 14** shows a porcine tissue compression sample. The dimensions of the sample was recorded using Image J image processing software and used to calculate stress and strain from the load and displacement data obtained from the Mark-10 testing platform.

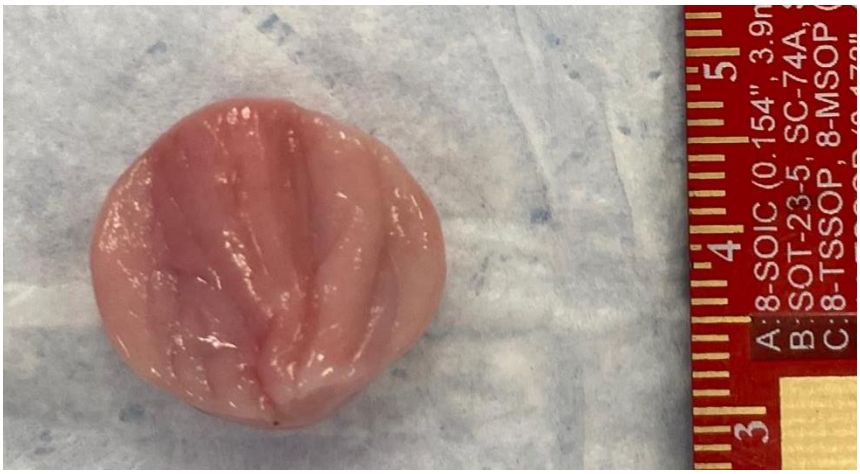

Figure 14: Porcine tissue compression sample

5.3 Measurement of Mechanical Properties of Composite Material for Vaginal Phantom

The same tensile and compression tests were conducted for the material used to manufacture the graded vaginal phantoms described in section 5.1 of this chapter. In order to conduct the following tests with a composite material, a tensile coupon or compression mold was used along with the 3D printed thermoplastic polyurethane (TPU) infill. Coupons of varying infill density, corresponding to the same variation in infill density in the designed phantoms, were tested using a tensile and compression test.

5.3.1 Tensile Measurements

The TPU and Ecoflex 30 composites were uniaxially pulled using the Mark-10 testing platform. The load was recorded as the samples were being pulled at a rate of 100mm/min. Simultaneously, the strain was measured by recording the sample, which was stippled, as shown

in **Figure 15**. This allowed for digital imaging correlation to be performed, which allowed for a more accurate strain measurement than the mechanical tests conducted on tissue samples. Similarly to the tissue tests, only the linear portion of the results were considered in order to compare the results obtained from porcine tissue and the results obtained from the designed composite.

Figure 15: Tensile Testing Coupon made from TPU and Ecoflex 30 Silicone

5.3.2 Compression Measurements

Similarly to the porcine tissue samples, compression tests were performed with the composite material used in the phantoms with the same infill density variation as that of the phantoms. A compression coupon for the phantom material is shown in **Figure 16**. The

compression speed was 13mm/min and the dimensions of the coupon were recorded using Image J processing and used to calculate stress and strain from the load and displacement data recorded on the Mark-10 platform.

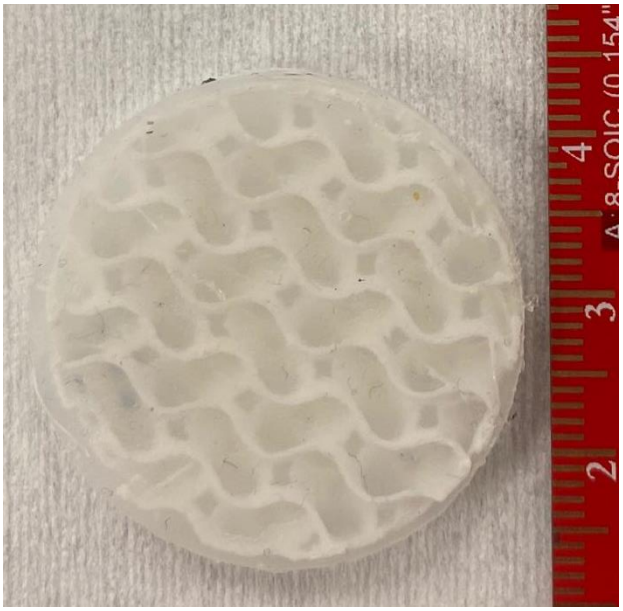

Figure 16: Compression Testing Coupon made from TPU and Ecoflex 30 Silicone

5.4 Comparison of Dilator Pressure on Porcine Tissue and Vaginal Phantoms

Lastly, to further understand the pressure response of the proposed vaginal dilator in different severities of VS, the designed vaginal dilators were used in the graded vaginal phantoms and pressure was recorded. The dilators were tested in both diameter variations in the vaginal phantom as well as infill variation in order to understand how the dilation system would behave in a clinical environment. It is worth noting that due to the variation in length and diameter established by the LENT-SOMA scale, the dilators were not able to be tested on VS

phantom grades 2 and above, as they would not fit in those models. Additionally, vaginal dilators of increasing sizes, such as medium and large dilators, could not be evaluated in the same VS phantom variations as the small dilator, as size was a restricting factor for testing. **Figure 17** shows a small expandable dilator being tested on the developed vaginal phantom.

Small and medium dilators were also tested in porcine vaginal tissue before the tissue samples were prepared for mechanical testing. The pressure in the dilators tested was also recorded and could be compared to the pressure recorded in the synthetic models to determine if the phantom developed accurately mimics vaginal tissue.

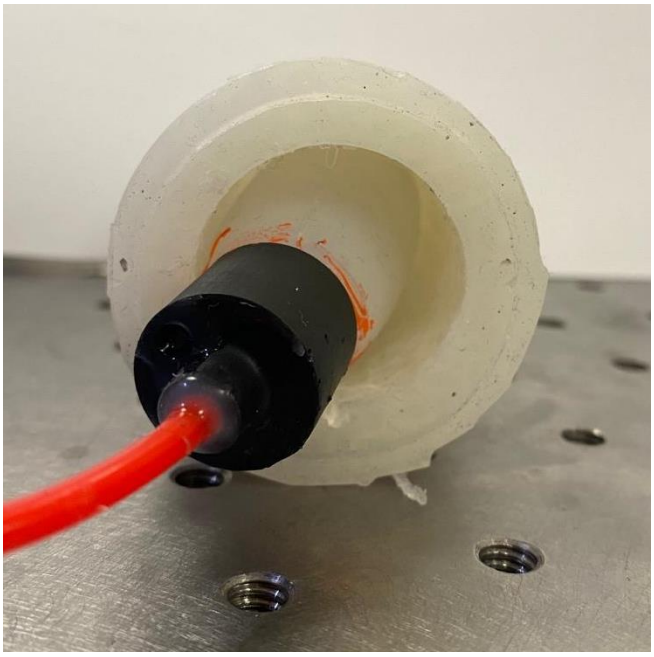

Figure 17: Small Expandable Vaginal Dilator being Tested on the Designed Vaginal Phantom

Chapter 6: Experimental Results and Discussion

6.1 Uniaxial Tests: Composite Material used for Phantoms

The uniaxial tests conducted in composite coupons showed a slight increase in stiffness across the different infill densities explored. As shown in **Figure 18**, at strains comparable to what was obtained in the tensile tests with animal tissue (0-40% strain), there is nearly no difference between the behavior of the composited with 10% TPU infill or 15% TPU infill. This difference, however, visibly increases when the infill density is increased by another 5%, as the 20% TPU composite showed to be significantly stiffer than the 10% and 15% TPU composites. **Table 5** shows the elastic modulus, calculated for the different infill densities explored (10%,15%,20%, and 100%), which was calculated by isolating the linear region of the plots in **Figure 18** and finding the slope of the region. From these results, it is clear that infill density does not behave in a linear manner and appears to exhibit greater stiffness differences at higher infill densities rather than lower.

Furthermore, **Figure 19** showed the compressive behavior of the composite material at different infill densities. The results showed the same behavior during compression regardless of infill density. There are several possibilities for this result; however, it is likely that the compression test results are less reliable than the tensile test results due to the limited speed range of the uniaxial tester used for these experiments, as the minimum speed used for compression was 13mm/min. This compression speed could not obtain more than 85 readings

before the sample was completely compressed and the apparatus reached capacity. Therefore, the tensile test results should be given prior consideration to the compression test results.

One possible physiological significance to this study is that small variations in the morphology of the vaginal tissue might not be detectable or significant to the development of vaginal stenosis, as the variation in stiffness relative to the increase in stiffer fibers in the composite is not significant at a lower infill density. On the other hand, if there were to be a great increase in collagen, similar to the increase in stiff fibers in the composite, the stiffness difference to baseline tissue characteristics would be detectable, as there was a 74% increase in the elastic modulus of the material for an infill density of 20% relative to 10% infill density. Therefore, the deposition of collagen in irradiated tissue should be further studies to determine if it plays a major role in the mechanical changes of the vaginal tissue and be compared to the results obtained for the designed vaginal phantom.

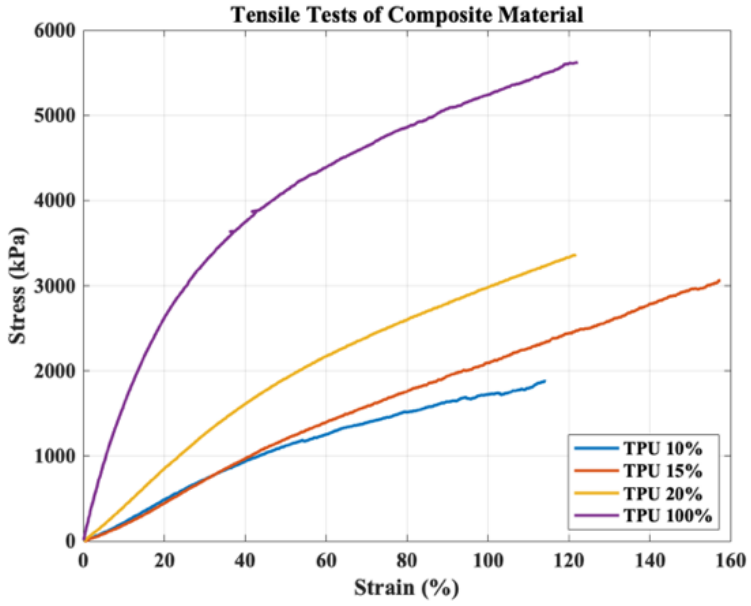

Figure 18: Mechanical behavior of composites made of a varying percentage of TPU infill and Ecoflex 30 silicone

Table 5: Elastic modulus for varying infill densities calculated from the linear region of the stress vs. strain curves in **Figure 18**

TPU Infill %	Calculated Elastic Modulus from Figure 17
10% TPU	**24.87 kPa**
15% TPU	**22.76 kPa**
20% TPU	**43.49 kPa**
100% TPU	**159.4 kPa**

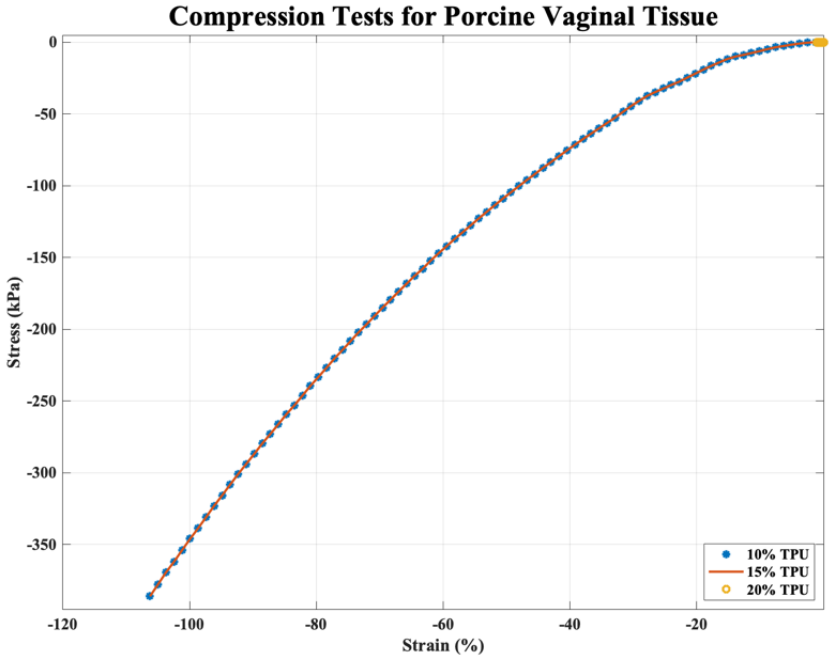

Figure 19: Mechanical behavior of composites of varying TPU densities obtained from compression tests

6.2 Uniaxial Tests: Porcine Vaginal Tissue

The results for the uniaxial tests conducted are shown in **Figures 20-22**, which show both the tensile and compression test results. **Figure 20** shows the tensile test results for vaginal tissue located in the middle to lower (distal) vagina, as the porcine anatomy is different from human anatomy especially in vaginal length, as described previously in Chapter 5. All measurements obtained from the middle and distal vagina were taken with the tissue fibers being aligned, or parallel, to the direction it was being pulled. **Figure 21** shows the tensile test results for vaginal tissue located in the apex of the vaginal canal, which was closer to the cervix and was qualitatively different than the other anatomical locations analyzed, as it contained several bumps across the surface. Additionally, the measurements obtained for tissue near the cervix were taken both with fibers parallel and perpendicular to the extension of the tissue. The elastic modulus of each experiment was calculated from the linear region of the stress versus strain graph as in section 6.1 and as described by Griffin et al. are summarized in **Table 6** [50].

When comparing the mechanical properties of the porcine vaginal tissue tested with the composite material used for the design of the vaginal phantom, the composite material shows a greater elastic modulus by an order of magnitude from the tissue. It is worth noting that the vaginal tissue evaluated also showed some anisotropy, as the perpendicular fiber alignment yielded in an elastic modulus higher than that of parallel fiber alignment, as shown in **Table 6**.

While the results for the compression testing for porcine tissue differed slightly, the same error source should be considered as in section 6.1, as the uniaxial tester could not gather enough data points to make significant conclusions from the results obtained. While the behavior obtained from both the compression test in section 6.1 and the compression test shown in **Figure**

22 were as expected since the compression curve showed increasing compressive stress over greater strains, the rate at which these results were obtained were too fast to draw significant conclusions.

Table 6: Elastic modulus of porcine vaginal tissue depending on tissue location and fiber alignment

Tissue Region:	Fiber Alignment:	Elastic Modulus:
Apex	Parallel (trial 1)	8.827 kPa
Apex	Parallel (trial 2)	5.907 kPa
Apex	Perpendicular	15.64 kPa
Middle	Parallel	8.817 kPa
Distal	Parallel	5.856 kPa

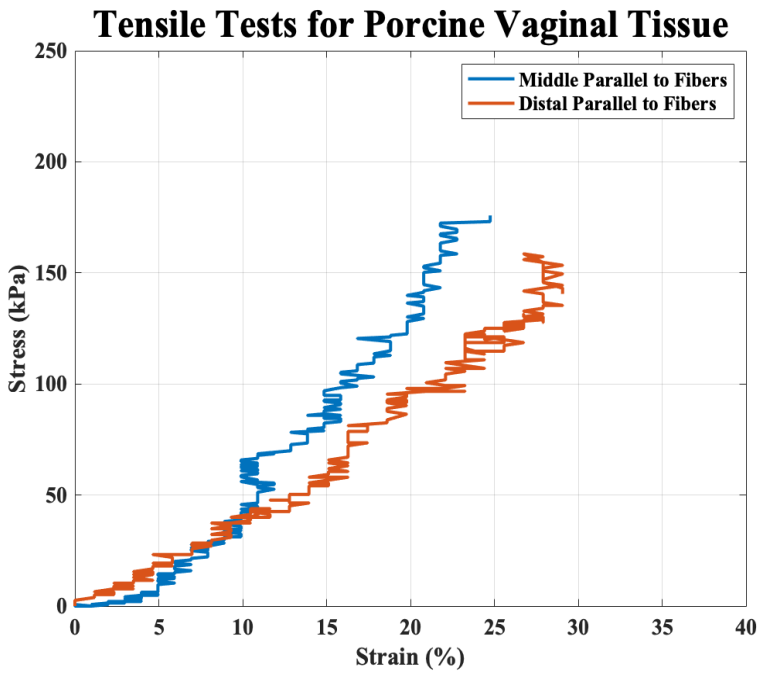

Figure 20: Tensile test of middle and distal portion of porcine vaginal tissue

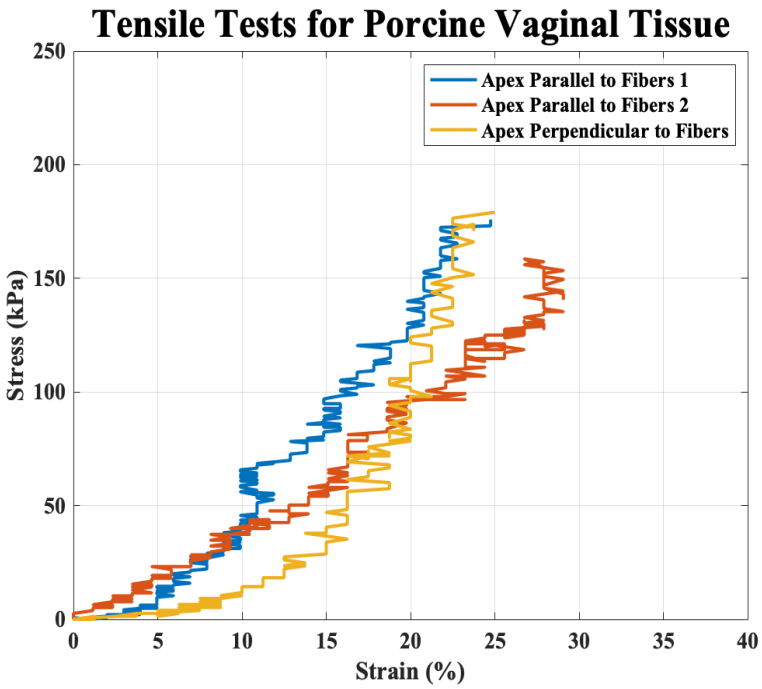

Figure 21: Tensile test of top portion of porcine vaginal tissue (apex)

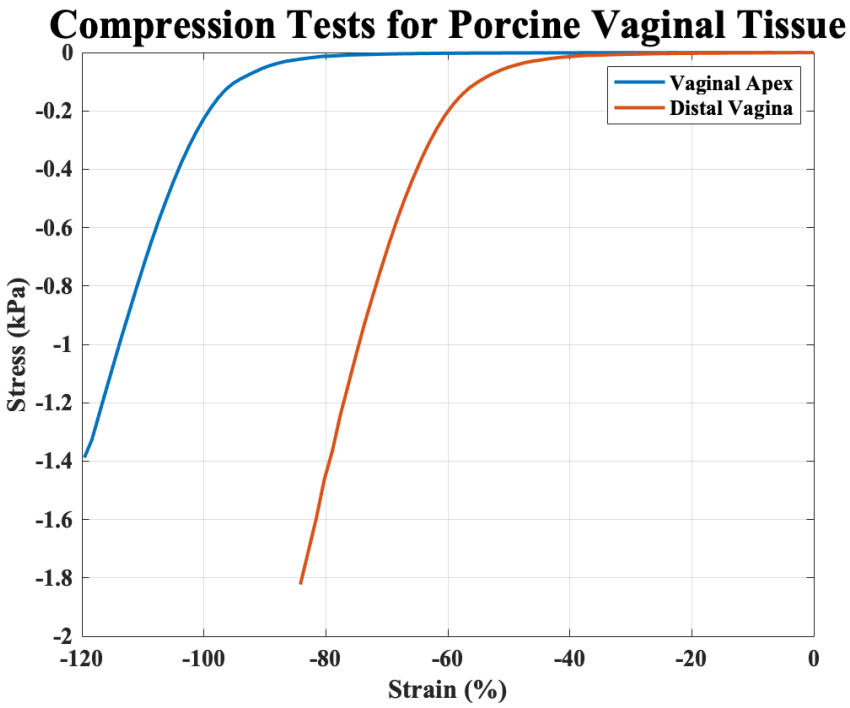

Figure 22: Compression tests of both the apex portion and distal portion of porcine vaginal canal

6.3 Dilator Pressure: Variation in Diameter in Phantoms

While section 6.1 and 6.2 showcased the comparison of mechanical properties of the designed vaginal phantom and vaginal tissue, in order to evaluate the proposed expandable dilator model the pressure response of the dilation system must be measured in both environments. When varying the diameter of the dilator according to the LENT-SOMA criteria, the pressure response observed in the dilators varied depending on the size of the dilator being used as well as the condition, or VS grade, it was being used in. **Figures 23-25** show the pressure response of the proposed vaginal dilators in different VS grades, according to each dilator size. It is important to note that due to the graded sizes in the VS phantom models, more severe grades of VS (grade 2 and above) could not be used to measure the dilator pressure response, as even the smallest dilator size would not fit that particular model. Additionally, increasing dilator sizes, such as medium and large, were not able to be evaluated in smaller VS phantom grades (e.g. grade 1 for medium and 0.5 for large); however, all dilator sizes were evaluated in the control phantom.

Apart from the large dilator size, which could only be used in the control (Grade 0) VS phantom model, there was an increasing pressure response as the diameter of the phantom decreased, simulating the progression of vaginal stenosis. Furthermore, the small dilator experienced a large increase in its pressure response from the phantoms of grade 0.5 to grade 1, showing a peak near 600 mmHg for a grade 1 phantom versus a peak at approximately 310 mmHg for the intermediate grade 0.5. The medium dilator exhibited a similar increase in pressure; however, due to the difference in sizing, it was able to detect a bigger pressure

difference between grades 0 and 0.5. These results can be useful in monitoring the progression of patients from either prevention or recovery from stenosis. Additionally, the results show that a small expandable dilator may be beneficial in more severe cases of VS, while the medium dilator might be useful to determine less severe or preventative cases of VS. Thus, without considering other factors such as patient specific anatomy and preference, a smaller sized dilator may be useful to treat already occurring VS, while a medium or larger-sized dilator may be more useful to monitor prevention of VS. Further mechanical analysis should be conducted to evaluate this problem with the evaluation of both hoop and longitudinal stress.

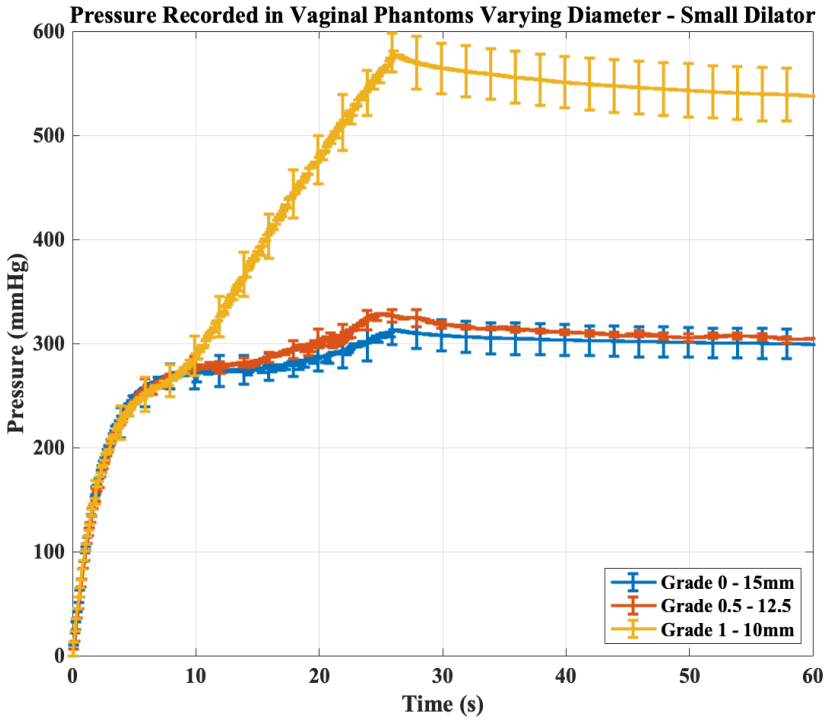

Figure 23: Pressure response of small vaginal dilator in VS phantoms graded 0, 0.5, and 1 following the LENT-SOMA criteria

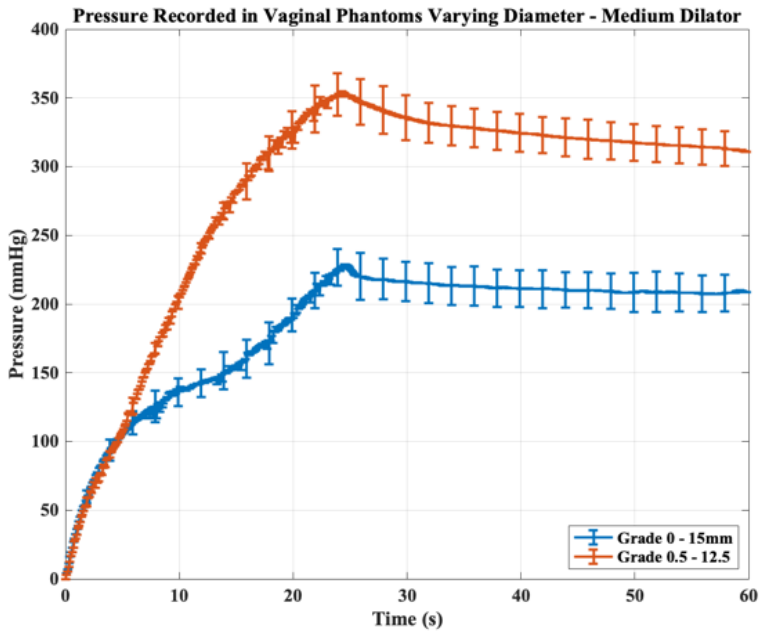

Figure 24: Pressure response of medium vaginal dilator in VS phantoms graded 0 and 0.5 following the LENT-SOMA criteria

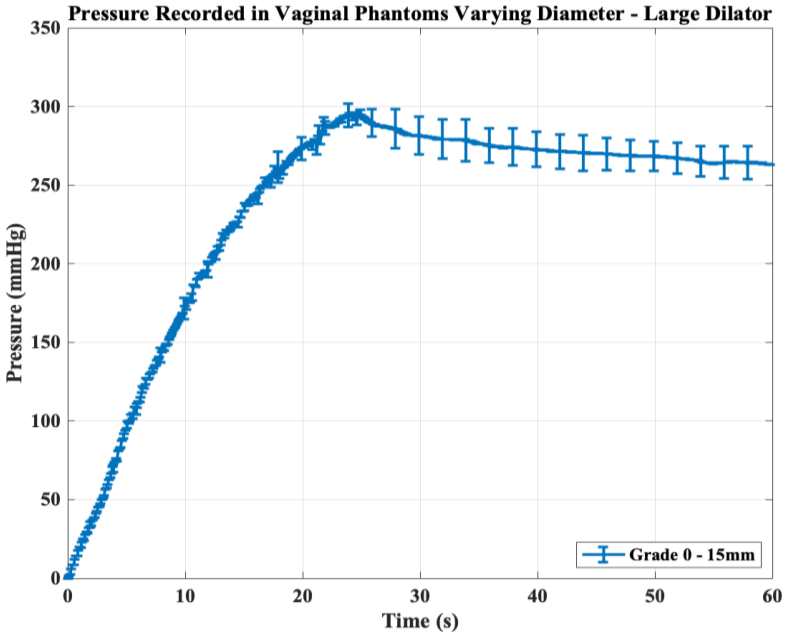

Figure 25: Pressure response of large vaginal dilator to VS phantom grade 0 (baseline average vaginal dimensions)

6.4 Dilator Pressure: Variation in Infill Density in Phantoms

Similarly to section 6.1, which showed that the mechanical properties of the material being used for the phantoms did not show a drastic change for lower infill densities such as 10% and 15%, but showed a stiffness increase at 20% TPU, the pressure response showed a similar trend when the dilator being used was a large enough size, as seen in **Figure 26**. While **Figures 27-28** show a slight increase in pressure with increasing infill density, the recorded trend does not show a sufficient pressure difference between different infill densities to be considered significant. The pressure response recorded with the large vaginal dilator, however, showed a larger increase at 20% TPU, which could be considered significant.

This result indicates that the larger vaginal dilator may be most useful in the cases where there is little to no change in vaginal dimensions but already occurring morphological changes such as increase in collagen fiber deposition as outlined in Chapter 3. Thus, this dilator size might be indicated for the very early stages of VS and can be monitored by examining its pressure response and comparing it to the results outlined in **Figure 26**.

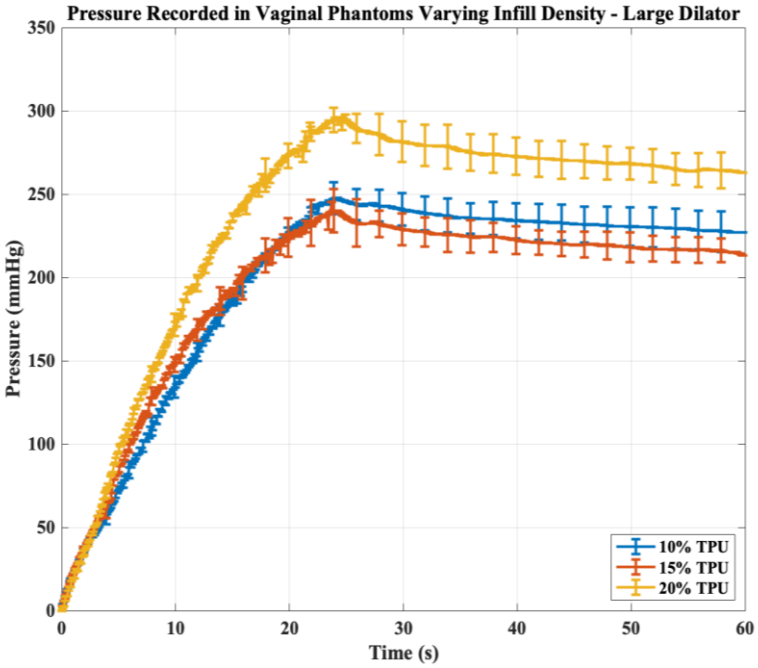

Figure 26: Pressure response of large vaginal dilator with varying infill density (10%, 15%, 20%) on baseline vaginal phantom (Grade 0)

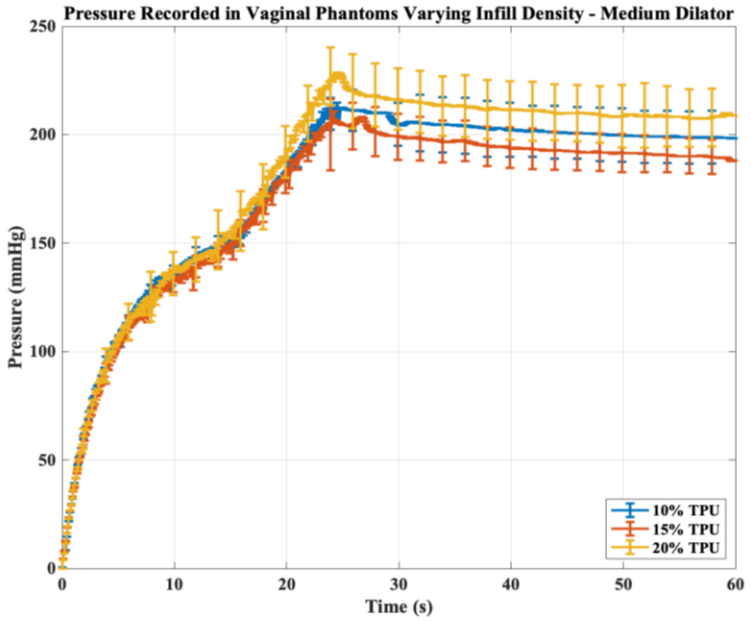

Figure 27: Pressure response of medium vaginal dilator with varying infill density (10%, 15%, 20%) on baseline vaginal phantom (Grade 0)

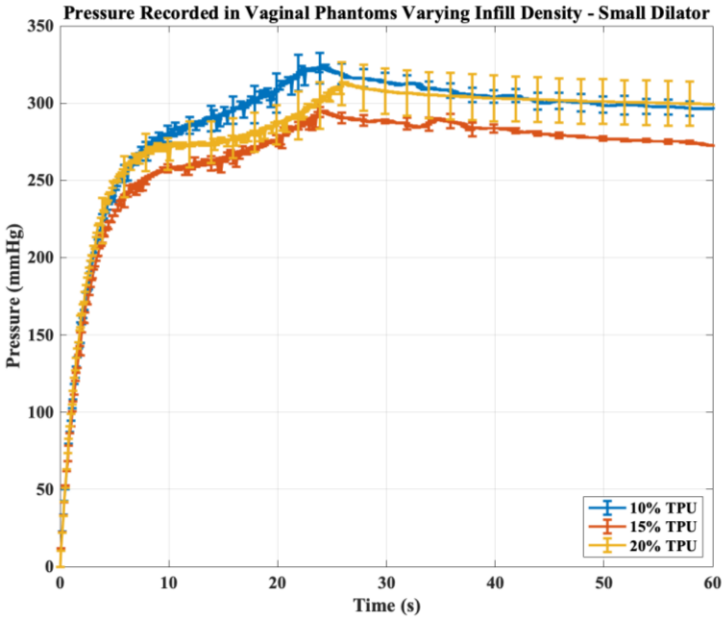

Figure 28: Pressure response of small vaginal dilator with varying infill density (10%, 15%, 20%) on baseline vaginal phantom (Grade 0)

6.5 Dilator Pressure in Porcine Vaginal Tissue

Lastly, the dilator pressure rerecorded in porcine vaginal tissue was evaluated, which is shown on **Figure 29** and can be compared to the pressure response in the designed vaginal phantom to determine if the synthetic model can be used to accurately characterize the pressure response of the proposed vaginal dilators. Due to sizing restrictions, the pressure response of the large vaginal dilator could not be recorded; however, the pressure in both the small and medium dilator was recorded. **Figure 29** shows a peak in pressure at approximately 310mmHg for the small dilator, which is consistent with the pressure observed in the vaginal phantom shown in section 6.3. Furthermore, the pressure in the medium dilator peaks at approximately 225mmHg, which is also consistent with the results in section 6.3. This is a promising result, which can indicate that the model developed sufficiently mimics vaginal tissue for the purposes of

characterizing the pressure response in the proposed vaginal dilators and thus can be used to simulate vaginal stenosis for this purpose. However, it is important to consider external factors such as the resolution of the pressure sensor utilized.

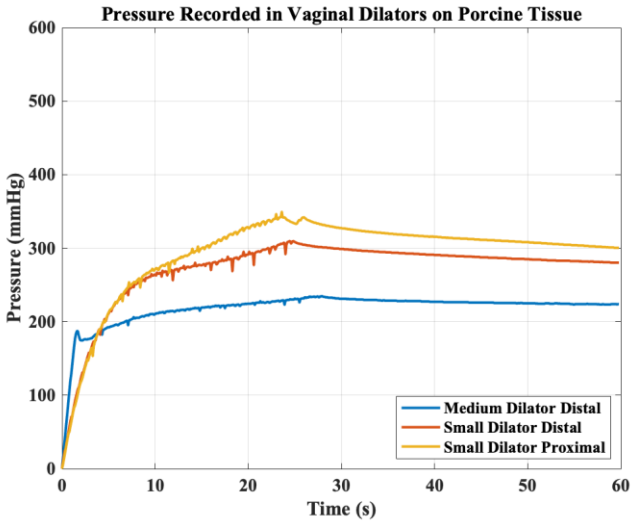

Figure 29: Pressure response of proposed vaginal dilators in porcine vaginal canal

Chapter 7: Conclusions and Future Considerations

7.1 Conclusions

This project described the design of a vaginal phantom for the purposes of characterizing a proposed vaginal dilator model for use in the prevention and treatment of radiation induced vaginal stenosis. By using a composite material consisting of 3D printed TPU infill that was cast in a soft polymer (Ecoflex 30), the model could be varied not only in size but also in infill density, which would consider the effects of underlying morphological changes leading to the VS syndrome, such as the increase in deposition of stiff collagen fibers due to radiation injury. Furthermore, the mechanical properties of both the composite material used to manufacture the vaginal phantom models as well as of porcine vaginal tissue were measured using uniaxial tests and the elastic modulus for such materials was extracted from the uniaxial tests results.

While the mechanical properties of the composite material and that of the vaginal tissue were fairly different, the pressure response recorded in both porcine vaginal tissue and the designed VS phantoms were similar and exhibited similar trends. The pressure response of different vaginal dilator sizes with respect to varying vaginal dimensions (e.g. diameter) and infill density provided a multitude of information that can be used when applying the VS dilation system in the clinic. For severe VS, a small vaginal dilator can be useful, as it is able to detect variations in smaller vaginal dimensions through a change in the pressure being measured in the dilator while it is being used. A medium dilator, on the other hand, can be most useful to detect

changes in vaginal dimensions for earlier stage VS, as it exhibits a larger pressure difference within larger vaginal dimensions than that of the small dilator. Lastly, while the large dilator was only characterized in the baseline phantom model, it was able to detect changes in pressure according to increasing infill density, which suggests this particular dilator size may be useful in early stages of VS prevention, as it may detect pressure difference relating to not only vaginal dimensions, but also its morphological changes.

7.2 Future Considerations – Short Term

Future considerations should be given to this project, as it opens opportunities to further explore the mechanical properties of vaginal tissue, especially the mechanics of irradiated tissue, which was not explored in this project. In order to more accurately simulate vaginal stenosis in a synthetic model, the mechanical properties of radiation injured vaginal tissue that shows properties of vaginal stenosis should be explored. Additionally, further consideration of in-depth tissue mechanics should be given to future experiments, such as the hyperelastic behavior of tissue and the isotropy of both the composite material as well as tissue.

Additional considerations should be given to iterating on the VS dilation system, as the phantoms designed to showcase severe cases of VS were not able to be utilized due to the limited sizes of expandable dilators being used. Furthermore, the sizing criteria should not only be weighed against the designed vaginal phantoms, but also the trends in vaginal dimension seen in the clinic. Another consideration that can be given to the system is to test a pressure sensor of higher resolution in order to validate the results showcased in this research project.

7.3 Future Considerations – Long Term

A long term consideration that could be of value to this project includes the research into active ingredients such as medication of hormones that can be applied either through the VS dilation system or in conjunction with dilator treatment in order to address the underlying biological events that lead to the VS syndrome. Such a study would involve a comprehensive review of biochemistry and pharmacokinetics; however, it would address an area that is overlooked in the field of radiation injury and could have the potential for application in various condition associated with radiation treatment.

References

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