Mechanical Characterization of Swine Uterosacral and Cardinal Ligaments

Ting Tan

Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Engineering Mechanics

Raffaella De Vita, Chair
Scott W. Case
Saad A. Ragab
Alexander Leonessa

November 12, 2015
Blacksburg, Virginia

Keywords: Uterosacral Ligament, Cardinal Ligament, Scanning Electron Microscopy, Histology, Uniaxial and Biaxial Tests, Nonlinear Elasticity, Viscoelasticity, Isometric Contraction, Isotonic Contraction, Constitutive Modeling
Copyright © 2015 Ting Tan
Mechanical Characterization of Swine Uterosacral and Cardinal Ligaments
Ting Tan

ABSTRACT

The uterosacral ligament (USL) and cardinal ligament (CL) are the two major suspensory tissues of the uterus, cervix, and vagina. These supportive structures can be weakened or damaged, leading to the development of pelvic floor disorders (PFDs) such as urinary incontinence, fecal incontinence, and pelvic organ prolapse. In the surgical treatment for PFDs, the USL and CL are extensively used as anchor structures to restore the normal position of the prolapsed organs. Therefore, the mechanical properties of the USL and CL may be critical for the development of new surgical reconstruction strategies for PFDs.

In chapter 1, we present the first histo-mechanical characterization of the swine USL and CL using histological analysis, scanning electron microscopy and quasi-static uniaxial tensile tests. Our results suggest that the histological and uniaxial tensile properties of the swine CL and USL are very similar to those in humans. The swine is found to be a suitable animal model for studying the mechanical properties of these ligaments.

To capture both the active and passive mechanical responses of biological tissues containing SMCs such as the USL and CL, a new structural constitutive model is proposed in chapter 2. The deformation of the active component in such tissues during isometric and isotonic contractions is described using an evolution law. This model is tested with published active and passive, uniaxial and biaxial, experimental data on pig arteries due to lack of data on the active properties of the USL and CL.

Subjected to constant forces in-vivo, the structure and length of the USL and CL are significantly altered over time. In chapter 3, we present the first rigorous characterization of the fiber microstructure and creep properties of the USL/CL complex by using scanning electron microscopy and planar biaxial testing. Fibers are found to be oriented primarily along the main in-vivo loading direction. In such direction, the creep proceeds significantly faster under lower load.

Overall, our experimental findings advance our knowledge about the passive elastic and viscoelastic properties of the USL/CL complex. The novel structural constitutive model proposed enhances our understanding of the active mechanical behavior of biological tissues containing SMCs. Knowledge about the mechanical behavior of the USL and CL from experimental and theoretical studies such as those presented here will help to improve, in the long term, the medical treatment for PFDs.
To my mother, Xia Yang, who encouraged me to pursue higher education.
To my father, Zhicai Tan, who taught me to overcome difficulties.
I would like to quote from a song by Josh Groban and a poem by Meng Jiao to thank my parents.

I am strong when I am on your shoulders.

You raise me up to more than I can be.

Who dare claim that the green grass might somehow repay the sun for its warm hearth?

I would also like to thank Zhimiao Yan for his support in the last eight years.
First and foremost, I would like to express my most sincere gratitude to my advisor, Dr. Raffaella De Vita, for her guidance to the world of biomechanics, for her mentorship with great patience, for her understanding and support, during my PhD studies.

I am also grateful to my committee members, Drs. Scott Case, Saad A. Ragab, and Alexander Leonessa, for their guidance and suggestions.

I would like to thank Suzanne Nicewonder for the swine pelvic anatomy and dissection, Frances Davis for the uniaxial testing, and Nathan Cholewa for the digital image correlation. I would also like to thank all of the group members of the Mechanics of Soft Biological Systems Laboratory, particularly Matt Webster, Albert Kwansa, Winston Becker, and Adwoa Baah-Dwomoh, for all their help and suggestions.

**Funding Acknowledgments**

This work was supported by NSF PECASE Grant no. 1150397 and the Continent Chinese Government Scholarship.
# Contents

1 Introduction .................................................. 1  
1.1 Motivation .................................................. 1  
1.2 Anatomy and composition of the USL and CL .............. 3  
1.3 Biomechanical passive properties of the USL and CL ..... 4  
  1.3.a In-vivo behavior ......................................... 5  
  1.3.b Ex-vivo behavior ......................................... 5  
1.4 Constitutive modeling for the active response of biological soft tissues containing SMCs .................. 7  
1.5 Outline of the dissertation ................................ 8  

Bibliography .................................................... 10  

2 Histo-mechanical Properties of the Swine Cardinal and Uterosacral Ligaments .................................. 16  
2.1 Introduction .................................................. 16  
2.2 Material and Methods ...................................... 18  
  2.2.a Harvesting Technique .................................. 18  
  2.2.b SEM Examination ........................................ 20  
  2.2.c Histological Examination .............................. 20  
  2.2.d Uniaxial Tensile Testing ............................... 21  
  2.2.e Statistical Analysis ..................................... 22  
2.3 Results ....................................................... 23  
  2.3.a SEM Examination ........................................ 23  
  2.3.b Histological Examination .............................. 24  
  2.3.c Tensile Properties ....................................... 25  
2.4 Discussion ................................................... 27
# Bibliography

## 3 A Structural Constitutive Model for Smooth Muscle Contraction in Biological Tissues

3.1 Introduction ........................................................................ 35
3.2 Model Formulation .......................................................... 37
  3.2.a Constitutive Model for the ACFs and PCFs ................. 37
  3.2.b Evolution Law .......................................................... 38
  3.2.c Isometric Contraction ............................................... 40
  3.2.d Isotonic Contraction ................................................ 41
3.3 Model implementation ...................................................... 41
  3.3.a Reduced 1-D Model .................................................. 41
  3.3.b Reduced 2-D Model ................................................ 42
  3.3.c Parameter Determination ........................................ 43
3.4 Results ........................................................................... 45
3.5 Discussion ....................................................................... 49
3.6 Conclusions ..................................................................... 51

## Bibliography

## 4 Micro-structural and Biaxial Creep Properties of the Swine Uterosacral-cardinal ligament Complex

4.1 Introduction ................................................................... 55
4.2 Material and Methods .................................................... 57
  4.2.a Specimen Preparation .............................................. 57
  4.2.b SEM Examination .................................................. 58
  4.2.c Biaxial Creep Testing ............................................ 61
  4.2.d Statistical Analysis ................................................ 62
4.3 Results ........................................................................... 62
  4.3.a SEM Examination .................................................. 62
  4.3.b Biaxial Creep ........................................................ 66
4.4 Discussion ....................................................................... 74
4.5 Conclusions ..................................................................... 76

## Bibliography

## 5 Conclusions and Future Work

5.1 Conclusions ................................................................... 81
5.2 Future Work ................................................................. 82
  5.2.a Improve three-dimensional structural constitutive model for capturing the active and passive mechanics of the USL and CL ................. 82
  5.2.b Propose three-dimensional nonlinear viscoelastic model for anisotropic ligaments with large deformation .......................... 83
1.1 Uterosacral and cardinal ligaments and pelvic organs (modified from paper by Shahryarinejad and Vardy [17])............................. 2
1.2 Uterosacral and cardinal ligaments attach to the sacrum and pelvic sidewall (modified from paper by Barber [12])............................. 3

2.1 (a) Swine peritoneal cavity showing the bladder, vagina, and tools used for dissection. (b) Left cardinal ligament (LCL), uterosacral ligament (USL), and right cardinal ligament (RCL) and their location relative to the rectum and vagina. (c) LCL, USL, and RCL attached to the cervix. ................. 19

2.2 (a) Swine reproductive organs and in-vivo loading directions of the USL (in blue), RCL (in pink), and LCL (in green). (b) LCL and RCL specimens and their location relative to the vagina/cervix/rectum (frontal plane view). (c) USL specimens and their location relative to the rectum/cervix (transverse plane view). ................................................................. 21

2.3 Scanning electron micrographs of swine CL cross section at (a) 5000× and (b) 20000× magnifications and swine USL cross section at (c) 5000× and (d) 20000× magnifications. ................................................................. 23

2.4 Histological images (40× magnification) of longitudinal sections of swine (a) and (d) LCL, (b) and (e) USL, and (c) and (f) RCL. The Masson’s trichrome stain (blue=collagen, red=muscle and cytoplasm) is used for sections (a), (b), and (c) and the Verhoeff-van Giesson stain (pink=collagen, purple=muscle, black=elastin and nuclei) for sections (d), (e), and (f). ....................... 24
2.5 Histological images of cross sections (a, b, c) and longitudinal sections (d, e, f) of some components of the swine USL and CL: blood vessels (a, d), smooth muscle fibers (b, e), and nerve fibers (c, f). The Masson’s trichrome stain (blue=collagen, red=muscle and cytoplasm) is used for sections (a), (b), (d), and (e) and the Verhoeff-van Giesson stain (pink=collagen, purple=muscle, black=elastin and nuclei) for sections (c) and (f).

2.6 Width (w) and thickness (t) of USL, RCL, and LCL specimens.

2.7 Axial stress-strain curves of the CL/USL complex of one full term sow. Three different symbols are used to report stress-strain data collected from specimens isolated from the three ligaments: RCL, USL and LCL. The different colors denote different specimen locations, within the CL/USL complex, relative to the cervix and rectum as shown in the insert.

2.8 Tangent modulus of (a) toe region and (b) linear region of stress-strain curves obtained by testing $n = 18$ specimens. Different colors denote specimen locations, within the CL/USL complex, relative to the cervix and rectum as shown in the insert.

2.9 (a) Ultimate tensile strength (UTS) and (b) strain at the UTS of $n = 18$ specimens. Different colors denote specimen locations, within the CL/USL complex, relative to the cervix and rectum as shown in the insert.

3.1 (a) Smooth muscle cells (SMCs) and contractile units (CUs). (b) Active collagen fibers (ACFs), passive collagen fibers (PCFs), and SMCs. (c) Model schematic for PCFs, ACFs, and CUs in SMCs showing only a discrete number of elements: $n$ elements for the ACFs (or CUs) and $m$ elements for the PCFs. In the proposed model, the continuous recruitment of these elements under load is described by a probability density function. Note: All the elements are oriented along the unit vector $\mathbf{m}$ in the reference configuration.

3.2 Active and passive uniaxial data [1] and model fits ($R^2 = 0.862$). (a) Active stress-time experimental data (red symbols) at a stretch of 1.5 and model fit (continuous line); (b) Active and passive stress-stretch data (blue and red symbols, respectively) and model fit (continuous lines).

3.3 Active and passive uniaxial data [2] and model fit ($R^2 = 0.982$). (a) Active stress-time experimental data (red symbols) at a stretch of 1.6 and model fit (continuous line); (b) Velocity-force experimental data (green symbols) and model fit (continuous line).
3.4 Active and passive biaxial data [3] \((R^2 = 0.965)\). (a) Active, passive, and total circumferential stress-stretch data at an axial stretch of 1.2 and model fits (continuous lines). (b) Active, passive, and total axial stress-circumferential stretch data at an axial stretch of 1.2 and model fits (continuous lines). (c) Active, passive, total circumferential stress-circumferential stretch data at an axial stretch of 1.3 and and model fits (continuous lines). (d) Active, passive, and total axial stress-circumferential stretch data at an axial stretch of 1.3 and model fits (continuous lines).

4.1 (a) Schematic of the swine USL and its main \textit{in-vivo} loading direction relative to the cervix, vagina, and rectum (transverse plane view). The main \textit{in-vivo} loading direction of the ligaments (parallel direction) is denoted using an orange arrow and the direction that is perpendicular to it is (perpendicular direction) denoted using a green arrow. (b) Locations of the specimen sections used for SEM analysis.

4.2 (a)-(b) SEM of the in-plane specimen cross-section (section 1 in Figure 4.1) at 1,000× and 20,000× magnifications, respectively. (c)-(d) SEM of the out-of-plane specimen cross-section (section 2 in Figure 4.1) at 1,000× and 20,000× magnifications, respectively. (e)-(f) SEM of the out-of-plane specimen cross-section (section 3 in Figure 4.1) at 1,000× and 20,000× magnifications, respectively. (Main \textit{in-vivo} loading direction of the ligaments is denoted using an orange arrow).

4.3 Measured parameters: fiber bundle length \(L_f^{(i)}\), end-to-end fiber bundle distance \(L_0^{(i)}\), and global fiber orientation angle \(\theta^{(i)}\) computed with respect to the parallel direction.

4.4 Area selected on the specimen surface for the measurement of local Lagrangian axial strains via the DIC method. Shown here is the strain map in the parallel direction.

4.5 Fractions of collagen fiber bundles with different straightness parameters and global orientation angles (with respect to the parallel direction). The reported data are obtained by analyzing \(n = 7\) SEM images.

4.6 Fractions of collagen fiber bundles with global angle oriented between \(-10^\circ\) and \(10^\circ\) with straightness parameters that differ by 0.02. The reported data were obtained by analyzing \(n = 7\) SEM images.

4.7 Fractions of collagen fiber bundles with straightness parameters that differ by 0.02. The reported data were obtained by analyzing \(n = 7\) SEM images.

4.8 Fractions of collagen fiber bundles with global orientation angles (with respect to the parallel direction) that differ by \(5^\circ\). The reported data were obtained by analyzing \(n = 7\) SEM images.

4.9 Fractions of collagen fiber bundles with local orientation angles (with respect to the parallel direction) that differ by \(5^\circ\). The reported data were obtained by analyzing \(n = 7\) SEM images.
4.10 Mean and standard deviation of the normalized strain over time in the parallel and perpendicular directions for (a) \( n = 15 \) specimens subjected to constant equi-biaxial loads of 2 N and (b) for \( n = 10 \) specimens subjected to constant equi-biaxial loads of 4 N.

4.11 Mean and standard deviation of normalized strain at \( t = 120 \) min for \( n = 15 \) specimens subjected to constant equibiaxial loads of 2 N and for \( n = 10 \) specimens subjected to constant equibiaxial loads of 4 N in the parallel and perpendicular directions.

4.12 Mean and standard deviation of creep rate for \( n = 15 \) specimens subjected to constant equi-biaxial loads of 2 N and for \( n = 10 \) specimens subjected to constant equi-biaxial loads of 4 N in the parallel and perpendicular directions.

4.13 Normalized strain at \( t = 2.5, t = 12.5, t = 120 \) min versus stress for \( n = 25 \) specimens along (a) the parallel direction and (b) the perpendicular direction.

5.1 Mean isochronal stress-strain data at \( t = 0, t = 2.5, t = 12.5, t = 115 \) min for \( n = 25 \) specimens (a) along to the main in-vivo loading direction and (b) perpendicular to the main in-vivo loading direction.

5.2 (a) Mean normalized strain over time computed over \( n = 5 \) specimens for a stress (mean±S.D.) of 0.105±0.013 MPa, \( n = 8 \) specimens for a stress of 0.132±0.014 MPa, \( n = 8 \) specimens for stress of 0.244±0.068 MPa, and \( n = 4 \) specimens for a stress of 0.356±0.038 MPa along the main in-vivo loading direction. (b) Mean normalized strain over time computed over \( n = 6 \) specimens for a stress of 0.102±0.016 MPa, \( n = 6 \) specimens for a stress of 0.130±0.010 MPa, \( n = 8 \) specimens for a stress of 0.238±0.047 MPa, and \( n = 4 \) specimens for a stress of 0.361±0.046 MPa along the direction that is perpendicular to the main in-vivo loading direction.
List of Tables

3.1 Model Parameters ................................................. 45

4.1 Creep test parameters for \( n = 15 \) specimens (thickness: \( 0.691 \pm 0.226 \) mm) subjected to constant equi-biaxial loads of 2 N. ........................................ 68

4.2 Creep test parameters for \( n = 10 \) specimens (thickness: \( 0.652 \pm 0.152 \) mm) subjected to constant equi-biaxial loads of 4 N. ........................................ 68
1.1 Motivation

Female pelvic floor disorders (PFDs) are characterized by symptoms such as urinary incontinence, fecal incontinence, and pelvic organ prolapse [1]. PFDs are a global health concern affecting millions of women every year. Not only do they decrease the quality of life of women, but they also place an heavy economic burden on the health system.

In United States, women’s lifetime risk of having a single operation for prolapse or incontinence was reported to be 11.1% in 1997, and the risk of re-operation was 29.2% [3]. In the same year, the direct cost for pelvic organ prolapse surgery alone was over $1 billion [4]. A study of PFDs in non-pregnant women over 20 years old conducted between 2005 and 2006 showed that 23.7% women had at least one PFD [2]. Among these women, 15.7%, 9.0%, and 2.9% were affected by urinary incontinence, fecal incontinence, and pelvic organ prolapse, respectively. The number of American women with at least one PFD was predicted to increase from 28.1 million in 2010 to 43.8 million in 2050, with 55%, 59%, and 46% increases in urinary incontinence, fecal incontinence, and pelvic organ prolapse, respectively [5].

Of course, PFDs are not restricted to American women. In 1999, 30.8% Swedish women between 20 to 59 years old were found to have some degree of genital prolapse [7]. In 2005, 8.3% Swedish women had symptomatic pelvic organ prolapse, and 8.9% were affected by urinary incontinence [8]. In the same year, 0.87‰ German women, 1.14‰ French women, and 1.13‰ English women were reported to have pelvic organ prolapse surgeries [9]. The corresponding medical costs were €144 million, €83 million, and €81 million in Germany, France, and England, respectively. In Western Australia, the lifetime risk of a woman having surgery for pelvic organ prolapse was reported to be 19% in 2010 [6]. In the developing regions of Africa, South America and Asia, numbers of women reported having urinary conditions including lower urinary tract symptoms, overactive bladder, urinary incontinence, and bladder outlet obstruction. The number of affected women in Africa, South America,
and Asia were projected to increase from 30.1% to 31.1%, from 20.5% to 24.7% and from 19.7% to 24.4%, respectively during ten years by 2018 [10].

To prevent and treat PFDs, an understanding of the supportive mechanism of pelvic organs is critical. In healthy women, the pelvic supportive ligaments and muscles maintain the anatomical positions of the pelvic organs, such as the uterus, vagina, bladder, and rectum, allowing them to perform their normal physiological function. The pelvic ligaments suspend the pelvic organs to the pelvic sidewalls over the levator plate, while the pelvic floor muscles close the urogenital hiatus and allow the organs to rest on top of them [11, 12]. With the support of the pelvic muscles, the supportive ligaments hold the vagina up over the vaginal introitus [13]. The supported vagina also provides support to other pelvic organs such as the uterus, bladder, urethra and rectum. For example, the vagina and its supportive structures integrated with the vesical neck and urethral sphincter maintain the urethral closure and prevent urinary incontinence [14]. However, pelvic supportive structures can be weakened or damaged by labor, delivery, menopause, aging, and obesity [2, 15, 16], leading to the development of PFDs.

![Figure 1.1: Uterosacral and cardinal ligaments and pelvic organs (modified from paper by Shahryarinejad and Vardy [17]).](image)

The uterosacral ligament (USL) and cardinal ligament (CL) are the two major suspensory tissues of the uterus, cervix, and vagina (Figure 1.1). A theoretical study [18] showed that the impairment of the pubovisceral muscle and uterosacral and cardinal ligaments can lead to anterior vaginal wall prolapse. The USL and CL not only play a pivotal role in supporting the uterus, cervix, and vagina in healthy women but they are also extensively used as anchor structures in surgical procedures for pelvic organ prolapse [19-22]. During these surgeries, the appropriate length of these ligaments should be determined considering their structural and mechanical properties in order to realize a successful outcome. Together with patient’s native tissues, synthetic mesh materials have been also used in surgeries to improve the
support of the pelvic organs to treat PFDs. However, these mesh products may cause serious complications such as pain, infections, bleeding, erosion and organ perforation. For safety and effectiveness, the mesh products should have appropriate biomechanical properties [23, 24] similar to those of healthy and strong suspensory tissues [25]. Therefore, investigating the structural and mechanical properties of the USL/CL complex is critical to the development of new surgical reconstruction strategies and mesh materials for PFDs.

1.2 Anatomy and composition of the USL and CL

As apical supportive structures of the vagina, cervix, and uterus, the USL and CL are connected together distally at the cervix and/or upper part of the vagina without clear boundary between them, attaching the organs proximally to the sacrum and laterally to the pelvic sidewall, respectively (Figure 1.2) [26].

![Figure 1.2: Uterosacral and cardinal ligaments attach to the sacrum and pelvic sidewall (modified from paper by Barber [12]).](image)

The USL is a bilateral band-like structure with ventral-to-dorsal (belly-to-back) arrangement. It is about 12-14 cm long and can be subdivided into distal (distant from the main mass of the body), proximal (close to the main mass of the body), and intermediate sections. The distal section attaches to the posterior (back) of the cervix and/or the upper third of the vaginal wall, where the USL is intermingled with the CL [26]. The distal section is about 2-3 cm long and 5-20 mm thick [27]. The proximal section extends from sacral spine S2-S4 without direct attachment to the sacrum [27, 28]. It is a wide and thick section of 5-6 cm long [27]. The intermediate section is between the distal and proximal sections. It is a thinner section of 5 cm long [27].

The CL is a perivascular sheath with cranial-to-caudal (head-to-tail) orientation. The average total length of the CL is 10 cm. It can be also subdivided into distal, proximal, and
intermediate sections. At the distal section, the CL connects to the cervix and upper vagina, and some of the CL fibers also connect to the bladder [29, 30]. The average length of the distal section is 2.1 cm [29]. The common region shared by the USL and CL at the distal section can be used in both lateral and supero-posterior support for vaginal vault in hysterectomy surgeries [29]. The proximal section attaches to the lateral pelvic sidewall around the internal iliac artery [29, 30]. It is a relatively thick section with an averaging length of 4.6 cm. The intermediate section is between the afore-mentioned two sections and 3.4 cm long on average.

The USL and CL are characterized as visceral ligaments using histological analysis. They are composed of collagen fibers, elastin, smooth muscle cells (SMCs), adipose cells, nerve fibers and blood vessels [28]. Pelvic floor disorders, particularly pelvic organ prolapse, are associate with changes of the ligament components. Loosely arranged thicker collagen fibers, less dense extracellular matrix [31], and impaired smooth muscle cells [32] were observed in the USL and CL of patients with pelvic organ prolapse.

Among the components of the USL and CL, collagen is the main structural element providing the primary passive strength to these ligaments. It has a well-known hierarchical organization of tropocollagen, fibril, and fiber, with sizes from nanometers to micrometers [34]. A collection of tropocollagen molecules forms a collagen fibril, which is cross-striated with an approximate periodic length of 60-70 nm [34]. The tropocollagen molecules are believed to be bent, and the degree of bending is associated with the attachment of water molecules [34]. Bundles of fibrils with diameters on the order of 10 nm form a single collagen fiber. Most collagen fibers are naturally crimped, giving collagenous materials large extension capability.

The active mechanical behavior of the USL and CL is controlled by SMCs in these ligaments. The SMCs are spindle-shaped cells about 2-4 µm wide and 200-1000 µm long [35]. Each SMC has contractile units that function as sarcomeres in skeletal muscle cell. The contractile units are composed of actin filaments, myosin filaments, and dense bodies [35, 36]. The myosin filament has a width of 14-18 nm and a length of the order of µm, and it is aligned between two actin filaments. The actin filaments of diameters about 7-8 nm are anchored to dense bodies [35]. The dense bodies are around 1.2 nm × 0.3 µm, serving to connect the contractile units throughout the SMC and attach to the cell membrane [35]. When the intracellular calcium concentration increases due to stimuli, cross-bridges form between the myosin filaments and the actin filaments leading to SMC contraction [37].

1.3 Biomechanical passive properties of the USL and CL

Biomechanical tests conducted to characterize the USL and CL can be of two types: in-vivo tests and ex-vivo tests. The in-vivo mechanical tests are conducted inside the living
bodies of animals or humans. The ligaments are tested in their physiological environment. The mechanical behavior from the *in-vivo* test is similar to the true mechanical behavior. Moreover, it is subject-specific and more clinically relevant. However, the ligaments and organs are tested as a whole, thus the mechanical response of each component cannot be separated. In addition, the dimensions of the ligaments are not easy to measure inside the body. Since the ligaments are tested in living animals or humans, failure tests cannot be conducted. Therefore, mechanical properties, especially the failure strength, can only be characterized outside the body, using *ex-vivo* tests. To reduce the environment effect on the mechanical properties of biological tissues, specimens are *ex-vivo* tested in a bath that mimics the inside body conditions (e.g. the tissues are submersed in physiological saline solution and tested at body temperature).

1.3.a *In-vivo* behavior

The *in-vivo* mechanical behavior of the USL and CL has been investigated by tension tests [39, 40]. Smith et al. [39] developed a computer-controlled system to measure the *in-vivo* tensile response of the cervix and combined supportive ligaments. The uterine support of women in this study varied from normal to prolapse. The force was applied in the caudal direction through a tenaculum placed on the cervix. The mean stiffness of the cervix and supportive ligaments was reported to be 0.49 N/mm.

Using similar testing technique, Luo et al. [40] studied the *in-vivo* viscoelastic properties of the uterine suspensory tissue including the USL and CL. Tensile and multiple stress-relaxation tests were performed on patients with prolapse (without prior surgeries). The uterine suspensory tissue was found to be visco-hyperelastic. The mean stiffness, mean energy absorbed during the tensile test, and mean normalized force (after 60 s) of the uterine suspensory tissue in the first relaxation tests were 0.49 N/mm, 0.27 J, and 0.56, respectively. A four-cable (two cables for the left and right USLs and two cables for the left and right CLs) model based on magnetic resonance imaging was developed. Using the displacement and force over time data of the uterine suspensory tissue from the first relaxation test in this model, the stiffness of the CL and USL was computed to be 0.2 N/mm and 0.12 N/mm, respectively. Compared with the first relaxation test, the stiffness and normalized force significantly increased while the energy absorbed during the tensile test significantly decreased in the second and third relaxation tests. These significant differences were speculated to be caused by incomplete tissue recovery during the rest time of 60 s between the relaxation tests.

1.3.b *Ex-vivo* behavior

*Ex-vivo* tests including uniaxial tests [41-46] and planar biaxial tests [47] have been conducted to measure the mechanical properties of the USL and CL. These ligaments were found to be
nonlinear elastic and viscoelastic.

Reay Jones et al. [46] studied the effects of the prolapse, vaginal delivery, menopause and aging on the resilience (the area under the force-displacement curve up to the plastic limit) of the USL using female hysterectomy specimens. Significant decrease in the mean resilience was detected between the groups without (0.019 J) and with (0.004 J) symptomatic uterovaginal prolapse, without (0.031 J) and with (0.015 J) vaginal delivery, before (0.021 J) and after (0.013 J) menopause and younger (0.021 J) and older (0.012 J) than 50 years. No significant reduction was detected as the number of deliveries increased. It was speculated that the decreases in the resilience of the pelvic ligaments might facilitate the development of symptomatic pelvic organ prolapse.

Moalli et al. [41] investigated the rat as an animal model for the structural and mechanical properties of the vagina and its supportive tissues. By pulling the rat vagina, they obtained the in-situ force-displacement curve of the vagina-supportive tissue complex. The supportive tissues failed before the vaginal wall. The mean linear stiffness and energy absorbed to failure of the complex were reported to be 2.9 N/mm and 49.4 J, respectively.

Although the ex-vivo force-displacement data from the USL and CL shed light on the mechanical behavior of these ligaments, they were highly dependent on the dimensions of the tested specimens [46, 41]. Mechanical properties of these tissues that were independent of the dimensions of the tested specimens were determined from stress-strain data.

The tensile properties of the USL and round ligament collected from female cadavers were studies by Martins et al. [42]. The USL was found to have significantly higher elastic modulus (14.1 MPa vs. 9.1 MPa) and strength (6.3 MPa vs. 4.3 MPa) than the round ligament. The USL of nulliparous women was found to have significantly lower elastic modulus (10.0 MPa vs. 15.5 MPa) and strength (4.2 MPa vs. 8.2 MPa) compared to the USL of parous women. The stronger USL in parous women was likely due to biomechanical alterations necessary to support higher mechanical loads from increased pelvic floor laxity and the increase in genital hiatus diameter after vaginal delivery.

A comparison study on the mechanical properties of the USL, round ligament, and broad ligament from female cadavers was conducted by Rivaux et al. [43]. The mean tensile strengths of the USL, round ligament, and broad ligament were reported to be around 4 MPa, 4.1 MPa, and 1.5 MPa, respectively. Using the Mooney-Rivlin model, the USL was found to be the stiffest at both low and high strain levels, and the round ligament was stiffer than the broad ligament. This study confirmed that the USL played an important role in supporting pelvic organs.

The aging effect on the tensile properties of the USL, round ligament, and broad ligament from female cadavers was studied by Chantereau et al. [44]. These ligaments were observed to significantly change with aging. Using the Mooney-Rivlin model, the old uterosacral ligament was found to be significantly stiffer than the young USL at both small deformation (elastic modulus 0.83 MPa vs. 0.13 MPa) and large deformation (elastic modulus 5.70 MPa
vs. 0.20 MPa). Similar trend was observed between the old and young round ligaments. No significant differences were detected between the elastic moduli for the young and old broad ligaments. The pelvic floor tissues might naturally become stiffer during aging. However, the old stiffer ligaments might still no longer stabilize the pelvic floor since they were also longer.

Vardy et al. [45] used the monkey as an animal model to study the effect of hormone replacement on the mechanical properties of the USL and round ligament. Incremental stress-relaxation tests were performed at strains that ranged from 5% to 30%. A tensile test to failure was conducted after the stress-relaxation tests on each sample. For ovariectomy monkeys without treatment, a mean failure stress of 0.6 MPa and a mean tensile modulus at 30% strain around 0.75 MPa were reported for the USL, while a mean failure stress of 2.1 MPa and a mean tensile modulus at 30% strain around 14 MPa were reported for the round ligament. Hormone replacement (using conjugated equine estrogens plus medroxyprogesterone acetate or ethinyl estradiol plus norethindrone acetate) was found to increase the elastic modulus of the USL but decrease the elastic modulus of the round ligament. The increase of the elastic modulus of the USL may help in bearing higher weight of the women pregnant uterus including the fetus, amniotic fluid, and placenta. This study supported the hypotheses that hormonal status played a role in pelvic support, and thus menopausal status was a risk factor for prolapse.

The swine was used as an animal model for investigating the biaxial elastic and viscoelastic mechanical properties of the USL/CL complex by Becker and De Vita [47]. The swine USL and CL were observed to undergo large biaxial deformations. An orthotropic nonlinear elastic response of the ligaments was detected, with higher elastic modulus along their main physiological loading direction than along the direction perpendicular to it. However, the ligaments were found to relax to the same amount under equi-biaxial strain along both loading directions. The stress decreased by around 70% over 2000 s. More relaxation was found at lower strain.

1.4 Constitutive modeling for the active response of biological soft tissues containing SMCs

Similar to biological structures such as blood vessels, airways, gastrointestinal tracts and pelvic organs, the USL and CL are mainly composed of an extracellular matrix and SMCs. The extracellular matrix contains primarily elastin and collagen fibers embedded in the so-called ground substance and controls the passive deformation of these structures. SMCs control the active deformation. Histological examination found more than 20% SMCs in 84% of USLs [48]. Since impaired SMCs were reported in patients with pelvic organ prolapse [32], the SMCs in the USL and CL may likely play a role in pelvic support and also in the pathogenesis of pelvic organ prolapse [32]. However, no active mechanical data have been
reported on the USL and CL.

To describe the active mechanical behavior of biological soft tissues containing SMCs, constitutive models have been proposed and assessed using active data on arteries. Rachev and Hayashi [56] first introduced an \textit{ad hoc} parameter which defines the contractile activity of SMCs to capture the active stress, and adopted a parabolic function for the typical isometric length-tension data. Later, Zulliger et al. [57] proposed a structural model for arteries that included the mechanical contribution of SMCs. The active stress was defined by introducing two functions that described the muscle tone level and the isometric length-tension data. To more precisely account for the contraction mechanisms of SMCs, a mechano-chemical model considering Ca$^{2+}$ concentration and temperature was proposed by Stålhand et al. [58]. In this model, the SMC deformation was assumed to be the result of cross-bridge deformation and filament sliding. Recently, Murtada et al. [59, 60] proposed a new theoretical framework in which the active response was defined by considering the dispersion of contractile units, actin-myosin filament overlap and sliding, and chemical activity as done by Hai and Murphy [53]. More specifically, they introduced a parabolic filament overlap function [56, 60], which captured the length-tension data in isometric experiments phenomenologically. Finally, Chen et al. [61] developed a constitutive model which incorporated the experimentally-measured orientation (and its change with the stretch) of vascular SMCs.

1.5 Outline of the dissertation

The mechanical behavior of the USL and CL is critical in understanding the mechanism of PFDs and developing better surgical strategies and materials for PFDs. Since the vagina and its supportive structures including the USL and CL in the swine were reported to be histologically similar to those in humans [62], we selected the swine as the animal model. This dissertation thus aims to characterize the elastic and viscoelastic properties of the swine USL and CL via uniaxial quasi-static tensile tests and planar biaxial creep tests. A novel structural constitutive model is proposed to capture the active and passive mechanical behaviors of soft biological tissues containing SMCs.

The remainder of the dissertation is organized in four chapters. In Chapter 2, we present a study on the structural and mechanical properties of the swine USL and CL. The structural composition of these ligaments is determined by performing histological and SEM analyses. Tensile properties of the USL and CL are investigated by quasi-static uniaxial tensile tests. We then evaluate a possible relation between the composition and structure of these ligaments and their tensile properties.

In Chapter 3, we propose a new structural constitutive model to capture both the active and passive response of biological tissues containing SMCs as the USL and CL. The model is formulated under the assumption that the contractile units in SMCs and the connected collagen fibers are the active tissue component, while the collagen fibers not connected to
the SMCs are the passive tissue component. Due to lack of active experimental data on the USL and CL, the proposed structural constitutive model is tested with published active and passive, uniaxial and biaxial, experimental data on pig arteries.

In Chapter 4, we investigate the micro-structural and viscoelastic properties of the swine USL/CL complex. By using SEM, we reveal the organization of collagen fibers. More specifically, we quantify the straightness and the alignment of collagen fiber bundles. The biaxial creep properties of the swine USL/CL complex are determined by using a planar biaxial testing system in conjunction with the digital image correlation (DIC) method. We then relate the biaxial creep behavior of the USL/CL complex with its microstructure.

In Chapter 5, conclusions and future work are presented and discussed.


properties of the uterosacral and round ligaments in the monkey model. American journal of obstetrics and gynecology, 192(5), 1741-1751.


2.1 Introduction

Pelvic floor disorders (PFDs) such as urinary incontinence, fecal incontinence, and pelvic organ prolapse affect millions of women every year. These disorders, which are mainly caused by pregnancy, vaginal delivery, and aging [14, 17], have devastating consequences not only on the quality of life of women but also the healthcare system [13]. The annual direct cost of prolapse surgeries alone exceeds 1 billion dollars in the United States [28]. The burden placed by PFDs on women and the healthcare will become even more significant with the projected increase in the aging population. Indeed, it has been estimated that the number of American adult women who suffer for PFDs will rise from 28.1 million in 2010 to 43.8 million in 2050 [31].

PFDs occur due to structural and mechanical alterations of pelvic organs, muscles, ligaments, and fasciae. Recent studies have suggested that “problems of bladder, bowel, prolapse, and some types of pelvic pain, mainly originate from the vaginal ligaments, not from the organs themselves [22].” The vaginal ligaments are mainly composed of collagen fibers interlaced with elastin, smooth muscle cells, nerve fibers, fibroblasts, and vascular structures. During pregnancy and childbirth, these ligaments are likely to lose their strength and increase their laxity due to the release of relaxin, a placental hormone that reduces the production
of collagen and increases collagen breakdown [27]. The mechanical properties of the entire vagina/supportive ligaments complex have been shown to be restored after parturition [15]. However, in many cases, the structure of the ligaments is permanently altered due to childbirth trauma and, consequently, their mechanical function is likely to be compromised. With menopause and aging, elastin and collagen degradation may also lead to laxity of the vaginal ligaments [5, 9, 11]. These morphological changes in the ligaments are, most probably, linked to a reduction in estrogen [21].

The two major suspensory ligaments of the uterus, cervix, and vagina are the uterosacral ligament (USL) and the cardinal ligament (CL) located in a posterior direction over the levator plate of the pelvic diaphragm. The USL provides support to the cervix and the upper vagina and is connected to the sacrum [2, 4, 8]. Pelvic pain in pregnancy, nocturia, urgency, and abnormal bladder emptying are believed to be caused by the laxity of the USL [22]. The CL is linked to the USL at the cervix and extends to the upper fascia of the pelvis. Prolapse of the vagina and uterus has been associated with the laxity of both USL and CL [19, 22].

The importance of investigating the biomechanical properties of the USL and CL for the treatment of PFDs has been recognized only in the past few years [30]. Force and displacement data have been collected on the ligaments by employing different techniques [6, 20, 24], including in-vivo measurement methods [16]. These data are, however, highly dependent on the dimensions of the tested specimens. Consequently, the biomechanical properties computed from them cannot be generalized to specimens of different dimensions. Stress and strain data have been collected to characterize the elasticity and viscoelasticity of USLs via uniaxial tests [18, 25, 29] and of USLs and CLs via biaxial tests [3]. These data describe the mechanical behavior of USL and CL, independently of their size. In the experimental study by Vardy et al. [29], quasi-static tensile tests and incremental stress relaxation tests of USLs from monkeys were performed, demonstrating their nonlinear elasticity and viscoelasticity. The study by Vardy et al. [29] is notable for being the first attempt to determine the mechanical behavior of USLs. Tensile properties such as ultimate tensile strength and stiffness of female cadaveric USLs were computed for the first time by Martins et al. [18]. In the study by Martins et al. [18], stress and strain data were reported, although strain data were computed from the clamp displacement (and not using more accurate video strain measurement methods). Mooney-Rivlin constitutive parameters were employed by Rivaux et al. [25] to quantify the nonlinear elasticity of female cadaveric USLs that were uniaxially tested. Biaxial elastic and viscoelastic material properties were computed very recently by our group for both the swine USL and CL using novel constitutive parameters [3].

In this study, we determine both the histological and mechanical properties of the USL and CL using the swine as an animal model. Toward this end, we perform scanning electron microscopy (SEM) and histological studies on specimens isolated from one entire USL/CL complex. We conduct tensile tests on specimens located in different anatomical regions within another entire USL/CL complex. From accurate stress and strain data measurements, tensile properties such as elastic moduli of the toe and linear regions of the stress-strain curve,
ultimate tensile strength (UTS), and strain at UTS are computed. We then evaluate a possible relation between the composition and structure of these ligaments and their tensile properties.

2.2 Material and Methods

2.2.a Harvesting Technique

Two full term sows (masses=261 kg and 234 kg) were acquired from a different study in accordance with an approved Virginia Tech IACUC protocol. The sows were euthanized immediately after giving birth (each sow delivered 13 piglets) and their lower abdomen and hindquarter were isolated and firmly secured to a dissection table. In order to identify the vaginal canal, the cervix, and uterus, a plastic rod was inserted in the introitus of the vagina (Figure 2.1(a)). By using a scalpel, a midline vertical incision was made until the peritoneal cavity was entered. The pubic symphysis was then separated using a hack saw. A rib spreader was utilized to separate the pubic symphysis for access to the vagina and support structures. Using the plastic rod and a scalpel, the vagina, cervix, uterus, and support structures together with the rectum and bladder were extracted from the abdominal cavity as a single complex. This procedure minimized damage to the CL and USL needed in this study (Figure2.1(b)). The bladder and its connective tissues were carefully removed from the vagina-cervix-uterus complex and discarded.

The USL connected the proximal vagina, from the interdigitating pads (cervix in the swine) to the sacrum. Taking care to retain as much of the USL as possible, a scalpel was used to remove it from its attachments to the vagina and sacrum. The CL fanned out laterally from the lateral vagina, through the broad ligament, including the uterine artery and vein, to the pelvic side wall. The CL was also cautiously dissected to preserve its full course (Figure2.1(b)-(c)).
Figure 2.1: (a) Swine peritoneal cavity showing the bladder, vagina, and tools used for dissection. (b) Left cardinal ligament (LCL), uterosacral ligament (USL), and right cardinal ligament (RCL) and their location relative to the rectum and vagina. (c) LCL, USL, and RCL attached to the cervix.
The vagina and the attached USL and CL were then laid flat on a dissection table, and any excess of adipose or muscular tissue was removed from these ligaments. Finally, the USL and CL were separated from the vaginal wall and kept hydrated with phosphate buffered saline (PBS) solution. After dissection, the ligaments were wrapped in plastic and stored at -20°C. Before each mechanical test, SEM or histological analysis as described hereafter, the ligaments were removed from the freezer and allowed to thaw at room temperature for 30 minutes.

2.2.b SEM Examination

Specimens collected from the USL and CL of one sow (mass=234 kg) were fixed overnight in a 2% glutaraldehyde-0.01 M sodium cacodylate buffer. They were washed with PBS solution, post-fixed in osmium tetraoxide, and dried in a critical point dryer (Model 28000, LADD Research Industries). The specimens were immersed in liquid nitrogen and fractured with a sharp razor blade in order to reveal their cross-sectional area. After being sputter coated with gold, the cross sectional areas were examined using an environmental scanning electron microscope (SEM) (Quanta 600 FEG, FEI).

2.2.c Histological Examination

The USL and CL excised from one sow (mass=234 kg) were fixed in 10% buffered formalin for 24 hours and then stored in 70% ethanol for 48 hours. They were gradually dehydrated in a graded ethanol and xylol series. After dehydration, the specimens were embedded in paraffin wax and cut into 4 µm sections with a microtome. The specimens were stained with Masson’s trichrome (MT) or Verhoeff-Van Giesson (VVG) stain. Smooth muscle and cytoplasm were stained red and collagen fibers were stained blue using the MT method. Elastin and nuclei were stained black, smooth muscle fibers were stained purple, and collagen fibers were stained pink using the VVG method. The histological slides were examined under a light microscope (Olympus IX71/IX51, Olympus) and images were collected using a digital camera (Model D5000, Nikon) at a 40× magnification.
Uniaxial tensile tests were conducted on a total of 7 specimens isolated from the left CL (LCL), 6 specimens from the right CL (RCL), and 5 specimens from the USL of one sow (weight=261 kg). The specimens were strips approximately 8 mm wide and 80 mm long. These strips were aligned along the main \textit{in-vivo} loading direction of the ligaments as indicated in Figure 2.2. Images of each specimen were collected under a stereo-microscope (Stemi 2000C, Zeiss) using a digital camera (Model D5000, Nikon). The width and thickness were measured at six locations using ImageJ (v. 1.44, NIH) and a digital caliper under a 50 g compressive load (Mitutoyo 573-291-20), respectively. The cross-section of each specimen was assumed to be rectangular and its area was calculated using the average width.
and thickness of the specimen. Four black poppy seeds, serving as fiducial markers for strain measurement, were glued to each specimen. The poppy seeds were evenly spaced on the specimens and were all aligned along the longitudinal axis of the specimens (i.e., the main in-vivo loading direction of the ligaments). The ends of each specimen were wrapped in sandpaper and mounted in custom-designed clamps to prevent slippage during mechanical testing.

Uniaxial tensile tests were conducted using an ElectroPuls E1000 (Instron, 50 N load cell) equipped with a bath filled with PBS at room temperature (\(\sim 21^\circ\text{C}\)). Each specimen was pre-loaded to 0.25 N and preconditioned for five cycles from 0.25 N to 1.0 N at 0.75 mm/sec. Five cycles were sufficient to stabilize the response of the specimens. The 0.75 mm/sec displacement rate was selected since it was comparable to the displacement rates used in similar studies by other investigators [18, 25, 29]. Following preconditioning each specimen was allowed to recover for 5 minutes and stretched at 0.75 mm/sec until failure occurred. The load and elongation of the specimen were recorded throughout the tests at 10 Hz. In addition, a video camera (APX-RS, Photron) was used to record images of the specimens during testing at 60 Hz with a 512\(\times\)1024–pixels resolution. The motion of the four poppy seeds was tracked using these images with ProAnalyst (v.1.5.3, Xcitex). The Lagrangian axial strain was then calculated from the motion of the markers. The nominal axial stress was calculated by dividing the load by the initial cross-sectional area.

The axial stress-strain data were analyzed to compute the ultimate tensile strength (UTS) and the strain at the UTS, \(\epsilon_{\text{UTS}}\). Only uniaxial tests in which the specimens failed in their middle region, away from the clamps, were considered successful. The tangent moduli of the toe and linear regions of each stress-strain curve were also computed using simple linear regression. In the toe region, the tangent modulus was calculated by considering only the stress-strain data in the strain interval \([0, 10\%\epsilon_{\text{UTS}}]\) while, in the linear region, was computed by considering only the stress-strain data in the interval \([30\%\epsilon_{\text{UTS}}, \epsilon_{\text{UTS}}]\). Although the choice of these intervals was arbitrary, it provided a consistent method for computing the tangent moduli for the different stress-strain curves.

### 2.2.e Statistical Analysis

Means and standard deviations were calculated for the elastic moduli, UTS, and \(\epsilon_{\text{UTS}}\). One-way analysis of variance was conducted to compare the mean of these mechanical properties for the LCL, RCL, and USL. The Student’s t test was used and the threshold chosen for statistical significance was 0.05. Data were analyzed using the JMP statistical software (JMP, Version 10, SAS Institute Inc.).
2.3 Results

2.3.a SEM Examination

Figure 2.3: Scanning electron micrographs of swine CL cross section at (a) 5000× and (b) 20000× magnifications and swine USL cross section at (c) 5000× and (d) 20000× magnifications.

Scanning electron micrographs of swine CL and USL cross-sections were obtained (Figure 2.3). Collagen fibrils in both the CL (Figure 2.3(a)) and USL (Figure 2.3(c)) specimens appeared to be organized into bundles. These bundles were primarily arranged perpendicular to the ligaments’ cross-sections and were more loosely spaced in the CL than in the USL.
The collagen fibrils were found to have a diameter of about 60-70 nm. In both ligaments, a loose network of individual collagen fibrils that intermingled with the collagen bundles was visible at high magnification (Figure 2.3(b) and (d)). These collagen fibrils were oriented along random directions.

### 2.3.b Histological Examination

Presence of loose connective tissue was found in all the specimens with a larger amount of ground substance (in white) in the LCL specimen (Figure 2.4(a) and (d)) and a larger number of smooth muscle fibers and adipose cells (in white) in the RCL specimen (Figure 2.4(c) and (f)). Loose connective tissue was also detected in the USL specimen. However, dense connective tissue, which is characterized by a considerable amount of collagen fibers and elastin fibers, was only identified in the USL specimen (Figure 2.4(b) and (e)). The elastin content was significantly higher in the USL (Figure 2.4(e)) than the CL (Figure 2.4(d) and (f)).

![Figure 2.4: Histological images (40× magnification) of longitudinal sections of swine (a) and (d) LCL, (b) and (e) USL, and (c) and (f) RCL. The Masson’s trichrome stain (blue=collagen, red=muscle and cytoplasm) is used for sections (a), (b), and (c) and the Verhoeff-van Giesson stain (pink=collagen, purple=muscle, black=elastin and nuclei) for sections (d), (e), and (f).](image)

Blood vessels were detected in all these ligaments (Figure 2.5(a) and (d)). Details about
their cross sections, such as the adventitia, media, and intima layers, were observed (Figure 2.5(a)). The blood vessels were noted to be primarily oriented perpendicular to the cross-section of the ligaments (Figure 2.5(a) and (d)). Smooth muscle fiber bundles were also found to be arranged in the plane of the RCL and USL specimens (Figure 2.5(b) and (e)). Nerve fibers had a similar arrangement and were detected in the USL and LCL specimens (Figure 2.5(c) and (f)).

Figure 2.5: Histological images of cross sections (a, b, c) and longitudinal sections (d, e, f) of some components of the swine USL and CL: blood vessels (a, d), smooth muscle fibers (b, e), and nerve fibers (c, f). The Masson’s trichrome stain (blue=collagen, red=muscle and cytoplasm) is used for sections (a), (b), (d), and (e) and the Verhoeff-van Giesson stain (pink=collagen, purple=muscle, black=elastin and nuclei) for sections (c) and (f).

2.3.c Tensile Properties

The dimensions of the CL and USL specimens \( (n = 18) \) obtained from one full term sow and used for mechanical testing are presented in Figure 2.6. This figure presents a map indicating the location of each specimen relative to the cervix and rectum. The width (mean±std) was 7.455±1.536 mm for the RCL, 7.457±1.194 mm for the USL, and 8.489±1.373 mm for the LCL. The thickness (mean±std) was 0.802±0.135 mm for the RCL, 0.490±0.086 mm for the USL and 1.119 ± 0.180 mm for the LCL. Axial stress-strain data obtained by testing these CL and USL specimens are presented in Figure 2.7. The axial stress-strain response of the CL and USL displayed the nonlinear strain stiffening phenomenon which is characteristic
of soft biological tissues. The tensile behavior of specimens collected from different regions within the USL/CL complex varied greatly.

Figure 2.6: Width (w) and thickness (t) of USL, RCL, and LCL specimens.

The tangent moduli of the toe and linear regions of the stress-strain curve are plotted in Figure 2.8 for each specimen. The values (mean±std) of the tangent moduli of the toe region of the stress-strain curve for the RCL, USL, and LCL were found to be 1.154±0.786 MPa, 1.617±1.215 MPa, 0.503±0.396 MPa, respectively. The values (mean±std) of the tangent moduli of the linear region for the RCL, USL, and LCL were found to be 5.385±2.424 MPa, 29.816±7.378 MPa, 3.449±1.449 MPa, respectively. The results of the statistical analysis indicated that there was no significant difference in the tangent modulus of the toe or linear region between the RCL and LCL (p > 0.05). Moreover, the tangent modulus of the toe region of the USL was determined to be significantly different from such modulus in the LCL (p < 0.05) but not significantly different from such modulus in the RCL (p > 0.05). However, the tangent modulus of the linear region for the USL was significantly different than the tangent modulus of such region for the RCL or LCL (p < 0.0001).

The ultimate tensile strength (UTS) for each specimen is reported in Figure 2.9(a). The values (mean±std) of the UTS for the RCL, USL, and LCL were found to be 1.278±0.499 MPa, 2.767±0.444 MPa, and 0.854±0.207 MPa, respectively. The statistical analysis revealed that the UTS of the USL was significantly larger than the UTS of both the RCL and LCL (p < 0.0001). The UTS of the LCL was not found to be significantly different than the UTS of the RCL (p > 0.05). The axial strain measured at the UTS, $\epsilon_{\text{UTS}}$, for each specimen is presented in Figure 2.9(b). The values (mean±std) of $\epsilon_{\text{UTS}}$ for the RCL, USL, and LCL were determined to be 0.337±0.166, 0.216±0.058, and 0.424±0.139, respectively. No significant difference was observed between the $\epsilon_{\text{UTS}}$ in the LCL and RCL (p > 0.05). However, the $\epsilon_{\text{UTS}}$ for the USL was significantly different than $\epsilon_{\text{UTS}}$ for the LCL (p < 0.05)
Figure 2.7: Axial stress-strain curves of the CL/USL complex of one full term sow. Three different symbols are used to report stress-strain data collected from specimens isolated from the three ligaments: RCL, USL and LCL. The different colors denote different specimen locations, within the CL/USL complex, relative to the cervix and rectum as shown in the insert.

but not significantly different than $\epsilon_{UTS}$ for the RCL ($p > 0.05$).

2.4 Discussion

This study focuses on determining the structural and mechanical properties of two major ligaments, the CL and USL, of the uterus-cervix-vagina complex using the swine as animal model. The structural composition of these ligaments was determined by performing histological and SEM analyses. Tensile tests on CL and USL specimens were conducted and the tangent moduli of the toe and linear regions of the stress-strain curves, UTS, and strain at the UTS, $\epsilon_{UTS}$, were computed. The histological, SEM, and mechanical data revealed significant differences between the CL and USL. Due to existing similarities between the histological properties of the swine and human CL and USL [12], this investigation provides crucial information on the material behavior of the supportive structures of the female pelvic floor.

The SEM analysis indicates that the collagen fibers were disorganized but mainly oriented in the \textit{in-vivo} loading direction of the CL and USL. In the swine, the collagen fibers were more densely packed in the USL than in the CL (Figure 2.3(b) and (d)). The presence of collagen
fibers, elastin fibers, smooth muscles, adipose tissues, nerve fibers, and blood vessels in the swine CL and USL was also reported in human CL and USL [23], thus supporting our choice to use the swine as an animal model for mechanical testing. The composition of the CL and USL in the swine was similar but with some significant differences (Figure 2.4). Both these ligaments contained mainly collagen in the swine [10] (as in humans [9]). The amount of elastin in the USL was significantly greater than that found in the CL. Dense connective tissue was only detected in the USL. Loose connective tissues were abundant in both the LCL and RCL. In the LCL, ground substance and nerve fibers were the most abundant whereas, in the RCL, ground substance, adipose cells, and smooth muscle fibers were more prevalent. It must be noted that these results were obtained by analyzing a few representative sections of the whole ligaments in pregnant swine so one must be cautious in generalizing them. Indeed, the compositional difference between the LCL and RCL observed in our study may be caused by a possible selection of neural sections for the LCL and vascular sections for the RCL for our histological analysis [23].

The results of the mechanical tests revealed that the uniaxial stress-strain response of the CL/USL complex strongly depends on specimen location, although the qualitative response of specimens within the LCL, RCL, or USL was similar (Figure 2.7). The USL exhibited a significantly larger tangent modulus in the linear region (Figure 2.8(b)) and larger UTS (Figure 2.9(a)) than the CL while the axial strain at the UTS, $\epsilon_{UTS}$, was significantly lower (Figure 2.9). These differences could be attributed to the different composition and structure of the ligaments. The higher tangent modulus in the linear region and strength of the USL were to be expected given their content of dense connective tissue (Figure 2.5(b)-(e)). Because the collagen fibers were oriented mainly along the loading direction in the USL, its mechanical failure was likely determined by the simultaneous rather than progressive failure of the comprising fibers. Such failure mechanisms could explain the lower axial strain at the UTS for the USL.

Stress-strain curves are presented only up to the UTS in Figure 2.7. Local maxima were observed in the stress-strain curves of some CL specimens. These local maxima were due to partial thickness tears of the loose connective tissues (Figure 2.4(a), (c), (d), and (f)). It must be noted that, despite the tears, the CL specimens continued to carry increasing loads as they were stretched. Interestingly, there were local maxima in the stress-strain curve at strain larger than $\epsilon_{UTS}$ for both of the USL and the CL (data not reported). The ability of the USL and CL complex to sustain tears and continue to support significant loads is a mechanical feature that warrants further investigation in the future.

Different experimental protocols have been used to investigate the elastic properties of human and animal USLs [18, 25, 29]. Vardy et al. [29] conducted incremental stress relaxation tests followed by tensile tests up to failure on USLs in monkeys. They reported a strain-dependent elastic modulus on the order of several hundreds to several thousands of kilopascals and a mean failure stress of 0.6 MPa. Martins et al. [18] found that the mean elastic modulus and UTS of USLs collected from female cadavers via uniaxial tensile tests were 14.1 MPa and 6.3 MPa, respectively. Similarly, Rivaux et al. [25] performed uniaxial tensile tests on USLs
of female cadavers and reported a mean UTS of 4 MPa.

In this study, we identified a mean elastic modulus of 1.6 MPa in the toe region and 29.8 MPa in the linear region and a mean ultimate stress of 2.8 MPa for the swine USL. These values are of the same order of magnitude than those reported in the literature [18, 29]. However, major differences in the results may exist due to differences between our experimental methods and previously used ones. Due to the large size of the swine ligaments, we were able to prepare specimens with an aspect ratio significantly larger (at least 5:1) than those used in the work by Vardy et al. [29] and Martins et al. [18]. Moreover, in the aforementioned studies, engineering strain was reported and its measurement was based on the displacement of the clamps of the uniaxial testing system. In our study, the Lagrangian strain was computed by video-tracking the motion of markers attached to the specimens. *We believe that* the high aspect ratio of the test specimens and optical technique used to determine the Lagrangian strain provide experimental data that more accurately characterize the tensile properties of the USL and CL [1].

Non-human primates have the pelvic anatomy that is most similar to humans [26]. Despite this advantage, the low availability and high expense in maintaining a primate colony has led us to seek a more cost effective animal model to study the mechanical properties of the supportive structures of the pelvic organs. The use of a large animal model such as the swine can help in reducing variation in the measurement of mechanical properties since multiple test specimens can be collected from a single animal. In order to determine the extent to which our mechanical studies can be generalized to humans, additional micro-structural analyses and mechanical experiments are needed on both the swine and human CL and USL. Because humans are bipeds and swine are quadrupeds, their pelvic floors are remarkably different. Nevertheless, our results seem to suggest that the histology and mechanical properties of the swine CL and USL are very similar to those in humans. In light of this evidence, we believe that the swine may be a practical animal model for pelvic floor research, especially since it naturally develops pelvic floor disorder such as prolapse [7, 12].
Figure 2.8: Tangent modulus of (a) toe region and (b) linear region of stress-strain curves obtained by testing \( n = 18 \) specimens. Different colors denote specimen locations, within the CL/USL complex, relative to the cervix and rectum as shown in the insert.
Figure 2.9: (a) Ultimate tensile strength (UTS) and (b) strain at the UTS of $n = 18$ specimens. Different colors denote specimen locations, within the CL/USL complex, relative to the cervix and rectum as shown in the insert.


Ting Tan

[22] Petros, P. E. The female pelvic floor: function, dysfunction and management according to the integral theory. Springer Verlag, 2010.


A Structural Constitutive Model for Smooth Muscle Contraction in Biological Tissues

This chapter was originally published as a journal article in the International Journal of Non-Linear Mechanics. It is available at http://dx.doi.org/10.1016/j.ijnonlinmec.2015.02.009. ©<2015>. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/

3.1 Introduction

Biological hollow structures such as blood vessels, airways, gastrointestinal tracts and pelvic organs are mainly composed of an extracellular matrix and smooth muscle cells (SMCs). The extracellular matrix contains mainly elastin and collagen fibers embedded in the so-called *ground substance* and controls the passive deformation of these structures. The SMCs govern and maintain the active deformation. They have contractile units that function as sarcomeres in skeletal muscles and are composed of actin filaments, myosin filaments, and dense bodies [4, 5] (Fig. 3.1(a)). The actin filaments are anchored to dense bodies. The dense bodies serve to connect the contractile units throughout the cell and are attached to the cell membrane [6]. Each myosin filament is aligned between two actin filaments, with the myosin heads uniformly spaced between these filaments [6]. When the intracellular calcium concentration increases due to electric, chemical, and mechanical stimuli, cross-bridges form between the myosin heads and the actin filaments, leading to SMC contraction [7]. SMCs generate a contraction force that is comparable to the force generated by skeletal muscle cells. However, unlike skeletal muscle cells, SMCs maintain this contraction force over a longer time, and they have a much lower contraction speed so as to accomplish their physiological functions (e.g., maintain proper pressure in blood vessels, propelling food in
the gastrointestinal tracts) [8].

The contraction mechanism in skeletal muscle has been explained by H. E. Huxley, A. F. Huxley and co-authors [10-13], who proposed the so-called *sliding-filament theory*. According to this theory, the contraction force in skeletal muscle is generated by the attachment of myosin heads to actin filaments (i.e., the formation of cross-bridges) during actin-myosin filament sliding. Based on the sliding-filament theory, Hai and Murphy presented a new model that includes the *latch state* introduced by Dillon et al. [2, 9] to capture the characteristic cross-bridge kinetics of the SMCs [10]. In this state, a high contraction force is maintained at a very low or even zero contraction speed.

Constitutive models that describe the active mechanical contribution of SMCs in biological hollow structures have been proposed over the years. The passive response has been usually assumed to be due to the collagen and elastin fibers, while the active response has been assumed to be determined by the contractile units in SMCs. For vascular tissue, Rachev and Hayashi [11] introduced an *ad hoc* parameter that defined the contractile activity of SMCs to model the active stress, and adopted a parabolic function for the typical isometric length-tension data. Later, Zulliger et al. [12] proposed a structural model for arteries that included the mechanical contribution of SMCs. The active stress was defined by introducing two functions that described the muscle tone level and the isometric length-tension data. To more precisely account for the contraction mechanisms of SMCs, a mechano-chemical model considering Ca$^{2+}$ concentration and temperature was proposed by Stålhand et al. [13]. In this model, the SMC deformation was assumed to be the result of cross-bridge deformation and filament sliding. Recently, Murtada et al. [14, 1] proposed a new theoretical framework in which the active response was defined by considering the dispersion of contractile units, actin-myosin filament overlap and sliding, and chemical activity as done by Hai and Murphy [10]. More specifically, they introduced a phenomenological parabolic filament overlap function [1, 11], which captured the length-tension data in isometric experiments. Finally, Chen et al. [3] developed a constitutive model that also incorporated the experimentally-measured orientation of vascular SMCs.

In this study, a new structural constitutive model for the active and passive mechanical behavior of biological tissues containing SMCs is proposed. The SMC contraction force is assumed to be equal to the force acting on the surrounding collagen fibers. This assumption is justified by the fact that the contraction force generated by SMCs can be transmitted, via their connection to the dense bodies, to the extracellular matrix [4]. Thus, the active stress can be computed from the stress of the collagen fibers that are connected to the SMCs, without introducing a chemical kinetics model as done by other investigators [1, 14]. Within the framework of Hill’s three-element model [15], we develop an evolution law for the deformation of SMCs and connected collagen fibers. Following the sliding filament theory, in this evolution law the contraction force is the sum of a motor force that initiates contraction, a viscous force that describes the actin-myosin filament sliding, and an elastic force that accounts for the cross-bridge deformation. The passive response of the collagen fibers is captured by the nonlinear elastic model proposed by De Vita et al. [16]. The proposed
The structural constitutive model is then tested using uniaxial isometric length-tension [1] and isotonic quick-release experimental data [2] on pig carotid arteries and biaxial isometric inflation-extension experimental data on pig coronary arteries [3].

### 3.2 Model Formulation

In the proposed model, the mechanical behavior of biological tissues with SMCs is assumed to be determined by the collagen fibers. We assume that there are two different types of collagen fibers based on their interaction with SMCs (Fig. 3.1(b)). Collagen fibers of the first type are directly connected to SMCs. These collagen fibers determine the active mechanical response of the tissues. The activation of SMCs is assumed to be transmitted to the neighboring collagen fibers. For this reason, the collagen fibers connected to SMCs are called the *active collagen fibers* (ACFs). Collagen fibers of the second type are not connected to SMCs. These determine the passive mechanical response of the tissues and are called the *passive collagen fibers* (PCFs). The mechanical contributions of other components (e.g., ground substance or elastin) are neglected. In summary, the active and passive mechanical behaviors of biological tissues with SMCs are determined by the ACFs and PCFs, respectively.

#### 3.2.a Constitutive Model for the ACFs and PCFs

Within the framework of nonlinear elasticity, the active or passive first and second Piola-Kirchhoff stress tensors, \( P \) and \( S \), respectively, are expressed as [17]:

\[
P = -pF^{-T} + 2F \cdot \frac{\partial W}{\partial C}, \quad S = -pC^{-1} + 2 \frac{\partial W}{\partial C}, \tag{3.1}
\]

where \( p \) is the Lagrange multiplier that accounts for incompressibility, \( F \) is the deformation gradient, \( C = F^T \cdot F \) is the right Cauchy-Green strain tensor, and \( W \) is the strain energy of ACFs or PCFs, which can be defined as:

\[
W = \int_{\Sigma} R(m)w(\lambda(C, m)) \, d\Sigma, \tag{3.2}
\]

where \( \Sigma \) is the set of all material directions, \( R(m) \) is the probability density function for ACFs or PCFs with mean axis parallel to the unit vector \( m \) in the reference configuration, \( w \) is the strain energy of ACFs or PCFs along \( m \), and \( \lambda(C, m) = \sqrt{m \cdot C \cdot m} \) is the ACF or PCF axial stretch. After defining the ACF or PCF axial stress as \( \sigma = \frac{dw}{d\lambda} \), the first and second Piola-Kirchhoff stress tensors in Eq. (3.1) can be rewritten as:

\[
P = -pF^{-T} + F \cdot \int_{\Sigma} R(m)\frac{\sigma}{\lambda} m \otimes m \, d\Sigma, \quad S = -pC^{-1} + \int_{\Sigma} R(m)\frac{\sigma}{\lambda} m \otimes m \, d\Sigma. \tag{3.3}
\]
Both ACFs and PCFs are assumed to support load only after becoming taut and are linear elastic with elastic modulus $K$, as done in our previous work [16]. A Weibull probability density function with shape parameter $\kappa$, scale parameter $\gamma$, and location parameter of 1 is then introduced to model the gradual straightening and recruitment of collagen fibers. Then, the axial stress $\sigma$ of ACFs or PCFs is expressed as [16]:

$$\sigma = \int_{\lambda_t}^{\lambda_2} \frac{\kappa}{\gamma^\kappa} (\lambda_t - 1)^{\kappa-1} e^{-\left(\frac{\lambda_t - 1}{\gamma}\right)^\kappa} K \left(\frac{\lambda}{\lambda_t} - 1\right) d\lambda_t,$$

where $\lambda_t$ is the stretch at which the ACFs or PCFs become taut, and $\lambda_1$ and $\lambda_2$ are the lower and upper bounds for $\lambda_t$, respectively. It must be noted that the above equations describe both the active and passive mechanical response of the tissue. In the sections below, two subscripts, “a” and “p”, will be introduced to distinguish active and passive axial stretches, which lead to active and passive stresses for axisymmetric deformations. Thus, $\lambda_a$ will denote the axial stretch for ACFs and $\lambda_p$ will denote the axial stretch for PCFs.

### 3.2.b Evolution Law

The axial stretch of ACFs, $\lambda_a$, along the direction $m$ is a function of the SMC contraction time $t$. In order to determine this function, which is also known as the evolution law, we assume that, along $m$, SMCs and ACFs are subjected to the same force due to their connections and, therefore, are arranged in series (Fig. 3.1(c)). Within the contractile units in SMCs, the actin-myosin filament sliding is assumed to generate a viscous force and the cross-bridge deformation is assumed to generate an elastic force. The viscous force is modeled using a dashpot element and the elastic force using a spring element. Moreover, the existence of a motor force that initiates SMC contraction is postulated as done by other investigators [14]. SMCs that are connected to ACFs along the direction $m$ are thus modeled as a combination of three parallel elements (Fig. 3.1(c)). As mentioned in Sec. 3.2.a, ACFs are assumed to be linear elastic and are modeled as a spring element. Because along $m$ the ACFs are arranged in series with the contractile units of SMCs, one has:
Figure 3.1: (a) Smooth muscle cells (SMCs) and contractile units (CUs). (b) Active collagen fibers (ACFs), passive collagen fibers (PCFs), and SMCs. (c) Model schematic for PCFs, ACFs, and CUs in SMCs showing only a discrete number of elements: \( n \) elements for the ACFs (or CUs) and \( m \) elements for the PCFs. In the proposed model, the continuous recruitment of these elements under load is described by a probability density function. Note: All the elements are oriented along the unit vector \( \mathbf{m} \) in the reference configuration.

\[
k_a u_a(t) = \eta_c \dot{u}_c(t) + k_c u_c(t) + f_c ,
\]

where \( k_a \) and \( u_a(t) \) are the spring stiffness and axial displacement of the ACFs, respectively, \( \eta_c, k_c, u_c(t) \), and \( f_c \) are the viscous coefficient, spring stiffness, axial displacement, and motor force of the contractile units, respectively, and \( t \) is the contraction time. We emphasize that \( u_a \) in Eq. (3.5) is the axial displacement of the ACFs (and not the axial displacement of the taut ACFs). We also note that the ACFs contribute to the total active stress only when
they are taut and, in that case, they behave as a linear elastic material. However, during SMC contraction, the ACFs generate a force, which is equal to the force in the SMCs, even when they are not taut. This force will not contribute to the total active stress of the tissue unless the ACFs are taut.

Along the direction $m$, PCFs are modeled as springs that are parallel to the series of ACFs and contractile units (Fig. 3.1(c)). Thus, $u_a(t)$ and $u_c(t)$ are related to the axial stretch of PCFs, $\lambda_p(t)$, by

$$\lambda_p(t) = \frac{L_0 + u_a(t) + u_c(t)}{L_0}, \quad (3.6)$$

where $L_0$ is the original length of PCFs. We assume that the axial stretch of the PCFs is equal to the axial stretch of the tissue along $m$ and can be expressed as

$$\lambda_p(t) = \begin{cases} 
\lambda_m & \text{for isometric contraction}, \\
\lambda_m + \frac{V(e^{-\mu t} - 1)}{\mu} & \text{for isotonic contraction},
\end{cases} \quad (3.7)$$

where $\lambda_m$ is the constant tissue stretch along $m$ in isometric experiments, $V$ is the normalized initial velocity of the tissue with respect to $L_0$ in isotonic experiments, and $\mu$ is a constant parameter that controls the rate and amount of change of the constant stretch. Note that, for the isotonic experiments, $\lambda_p(t)$ can be obtained by integrating the axial velocity of the PCFs, i.e. $\lambda_p(t) = -Ve^{-\mu t}$. This exponential decaying function for the axial stretch of PCFs is assumed based on experimental data and previous models [18, 19].

### 3.2.c Isometric Contraction

For isometric contraction, $\lambda_p(t) = \lambda_m$. After replacing $\lambda_p(t)$ with $\lambda_m$ in Eq. (3.6), one can express $u_a$ in terms of $u_c$, $\lambda_m$, and $L_0$ then substitute it into Eq. (3.5). The solution of the resulting differential equation, $u_c(t)$, has the following form:

$$u_c(t) = \beta(f - \lambda_m + 1)(e^{-\alpha t} - 1)L_0 + u_c(0)e^{-\alpha t}, \quad (3.8)$$

where $\alpha = \frac{k_a + k_c}{L_0}$, $\beta = \frac{k_a}{k_c + k_a}$, and $f = \frac{f_c}{k_c L_0}$. The initial axial displacement of the SMC contractile units is unknown from the experiments, so we assume that

$$u_c(0) = a(\lambda_m - 1)L_0, \quad (3.9)$$

where $0 \leq a \leq 1$ is a fractional length parameter. This assumption implies that the initial axial displacement of the SMC contractile units is a fraction of the axial tissue displacement. After introducing the parameter $a$, the axial stretch of ACFs can then by written as

$$\lambda_a(t) = 1 + \frac{u_a(t)}{(1 - a)L_0}. \quad (3.10)$$
By substituting Eq. (3.8) into Eq. (3.6), one obtains $u_a(t)$. This can then be substituted into Eq. (3.10) so that, for an isometric contraction,

$$
\lambda_a(t) = 1 + \frac{(\lambda_m - 1)(1 - ae^{-\alpha t}) + \beta[f - (\lambda_m - 1)](1 - e^{-\alpha t})}{1 - a}.
$$

(3.11)

### 3.2.d Isotonic Contraction

For isotonic contraction, $\lambda_p(t) = \lambda_m + \frac{V(e^{-\mu \tilde{t}} - 1)}{\mu}$, where $\lambda_m$ is the constant tissue stretch in isometric experiments. This expression for $\lambda_p(t)$ can be substituted in Eq. (3.6) and $u_a$ can be written in terms of $u_c$, $\lambda_m$, and $L_0$ and then substitute it into Eq. (3.5). The solution of the resulting differential equation, $u_c(t)$, has the following form:

$$
u_c(t) = \beta(f - \lambda_m + 1)(e^{-\alpha \tilde{t}} - 1)L_0 + a(\lambda_m - 1)L_0e^{-\alpha \tilde{t}} + VL_0\beta \left[ \frac{e^{-\mu t} - 1}{\mu} - \frac{e^{-\mu t} - e^{-\alpha t}}{\mu - \alpha} \right],
$$

(3.12)

where $\alpha$, $\beta$, $f$, $L_0$, $a$ are defined as above, and $\tilde{t}$ is a constant that represents the duration of the isometric contraction that precedes the isotonic contraction. The first two terms of the right hand side of Eq. (3.12) represent the axial displacement of the contractile units at the end of the isometric contraction (i.e., Eq. (3.8) evaluated at $t = \tilde{t}$).

By substituting Eq. (3.12) into Eq. (3.6), one obtains $u_a(t)$ for an isotonic contraction. This can then be substituted into Eq. (3.10), so that $\lambda_a(t)$ for an isotonic contraction becomes

$$
\lambda_a(t) = 1 + \frac{(\lambda_m - 1)(1 - ae^{-\alpha t}) + \beta[f - (\lambda_m - 1)](1 - e^{-\alpha t})}{1 - a} + \frac{V}{1 - a} \left[ (1 - \beta)(e^{-\mu t} - 1) + \frac{\beta(e^{-\mu t} - e^{-\alpha t})}{\mu - \alpha} \right].
$$

(3.13)

Again, we note that the first two terms of the right hand side of Eq. (3.13) represent the axial stretch of the ACFs at the end of the isometric contraction (i.e., Eq. (3.11) evaluated at $t = \tilde{t}$).

### 3.3 Model implementation

#### 3.3.a Reduced 1-D Model

In order to test the constitutive model with published uniaxial data collected from isometric and isotonic experiments on carotid arteries [1, 2], we assume that the tested specimens undergo a homogeneous isochoric axisymmetric deformation. Thus, the deformation gradient
of the ACFs or PCFs has the following form:

\[ F = \lambda^{-\frac{1}{2}}e_1 \otimes E_1 + \lambda^{-\frac{1}{2}}e_2 \otimes E_2 + \lambda e_3 \otimes E_3 , \]  

(3.14)

where \( \lambda \) is the axial stretch of the ACFs or PCFs. The orthonormal bases \( \{ E_1, E_2, E_3 \} \) and \( \{ e_1, e_2, e_3 \} \) are defined so that the \( E_3 \) and \( e_3 \) are the loading directions in the reference and current configurations, respectively.

In the reference configuration, the ACFs or PCFs are assumed to be all aligned along the loading direction \( E_3 \). Thus, in Eq. (3.3), \( R(m) = \delta(m - E_3) \), where \( \delta \) is the Dirac delta function. Substituting Eqs. (3.4) and (3.14) into Eq. (3.3), the non-zero components of the active or passive first Piola-Kirchhoff stress tensor are the following:

\[ P_{11} = P_{22} = -p\lambda^{-\frac{1}{2}} , \quad P_{33} = -p\lambda^{-\frac{1}{2}} + \sigma(\lambda) . \]  

(3.15)

After applying the traction-free boundary condition \( P_{11} = P_{22} = 0 \) on the lateral surface of the specimens, we obtain \( p = 0 \). Thus, the constitutive equation that defines the response of the ACFs or PCFs is

\[ P_{33}(\lambda) = \sigma(\lambda) = \int_{\lambda_1}^{\lambda_2} \frac{\kappa}{\gamma^\kappa} (\lambda_t - 1)^{\kappa-1} e^{-\left(\frac{\lambda_t - 1}{\gamma}\right)^\kappa} K \left( \frac{\lambda}{\lambda_t - 1} \right) d\lambda_t . \]  

(3.16)

The above equation defines the response of the ACFs for \( \lambda = \lambda_a(t) \), \( \lambda_1 = \lambda_m \), and \( \lambda_2 = \lambda_a(t) \). More specifically, for an isometric contraction \( \lambda_a(t) \) is given by Eq. (3.11) and for an isotonic contraction \( \lambda_a(t) \) is given by Eq. (3.13). Equation (3.16) also defines the response of the PCFs for \( \lambda = \lambda_p(t) \), \( \lambda_1 = 1 \), and \( \lambda_2 = \lambda_p(t) \) where \( \lambda_p(t) \) is given by Eq. (3.7). The total stress of the tissue is obtained as the sum of the stress of ACFs, \( P_{33}(\lambda_a) \), and the stress of PCFs, \( P_{33}(\lambda_p) \). We note that the model parameters \( \kappa, \gamma, \) and \( K \) in Eq. (3.16) are assumed to have the same values for both ACFs and PCFs.

### 3.3.b Reduced 2-D Model

The constitutive model proposed is also tested with biaxial data that are obtained from isometric inflation-extension tests on coronary arteries. Thus, we assume that the specimens undergo a homogeneous isochoric axisymmetric deformation defined by

\[ F = (\lambda_\theta \lambda_z)^{-1} e_t \otimes E_t + \lambda_\theta e_\theta \otimes E_\theta + \lambda_z e_z \otimes E_z , \]  

(3.17)

where \( \lambda_\theta \) and \( \lambda_z \) are the circumferential stretch and axial stretch of the ACFs or PCFs. The orthonormal bases \( \{ E_t, E_\theta, E_z \} \) and \( \{ e_t, e_\theta, e_z \} \) are defined so that the \( E_t, E_z \) and \( e_t, e_z \) are the biaxial loading directions in the reference and current configurations, respectively. In the reference configuration, the collagen fibers are assumed to be aligned in two preferred directions \( \mathbf{m}_1 = \cos(\psi)E_\theta + \sin(\psi)E_z \) and \( \mathbf{m}_2 = \cos(\psi)E_\theta - \sin(\psi)E_z \), where \( \psi \) and \( -\psi \) are
the angles off the circumferential axis $E$. Then, $R(m) = \frac{\delta(m-m_1) + \delta(m-m_2)}{2}$ and the strain energy in Eq. 3.2 can be re-written as

$$W = \frac{w(\lambda(C, m_1)) + w(\lambda(C, m_2))}{2},$$

where $\lambda(C, m_1)$ and $\lambda(C, m_2)$ are the axial stretches of the fibers. It then follows from Eqs. (3.7), (3.11), and (3.13) that, since $\lambda_m(C, m_1) = \lambda_m(C, m_2) = \sqrt{\lambda_\theta^2 \cos^2 \psi + \lambda_z^2 \sin^2 \psi}$, then $\lambda(C, m_1) = \lambda(C, m_2)$. Then the axial stresses along $m_1$ and $m_2$ defined by Eq. (3.4) are equal: $\sigma(\lambda(C, m_1)) = \sigma(\lambda(C, m_2))$.

Substituting Eqs. (3.4) and (3.18) into Eq. (3.3), one gets the following non-zero components of the second Piola-Kirchhoff stress tensor:

$$S_{rr} = -p (\lambda_\theta \lambda_z)^2, \quad S_{\theta\theta} = \frac{\sigma(\lambda)}{\lambda} \cos^2(\psi) - \frac{p}{\lambda_\theta^2}, \quad S_{zz} = \frac{\sigma(\lambda)}{\lambda} \sin^2(\psi) - \frac{p}{\lambda_z^2}. \quad (3.19)$$

By following Fung et al. [20], we assume that $S_{rr} = 0$ and, hence, $p = 0$. Then, it follows that

$$S_{\theta\theta} = \frac{\sigma(\lambda)}{\lambda} \cos^2(\psi), \quad S_{zz} = \frac{\sigma(\lambda)}{\lambda} \sin^2(\psi), \quad (3.20)$$

where

$$\sigma(\lambda) = \int_{\lambda_1}^{\lambda_2} \frac{\kappa}{\gamma^\kappa} (\lambda_t - 1)^{\kappa-1} e^{-\left(\frac{\lambda_t-1}{\gamma}\right)^\kappa} K\left(\frac{\lambda}{\lambda_t} - 1\right) \, d\lambda_t. \quad (3.21)$$

Equations (3.20) and (3.21) define the non-zero components of the second Piola-Kirchhoff stress tensor of the ACFs for $\lambda = \lambda_a(t)$, $\lambda_1 = \lambda_m$, and $\lambda_2 = \lambda_a(t)$, where $\lambda_a(t)$ is given by Eq. (3.11). Equations (3.20) and (3.21) are the non-zero components of the second Piola-Kirchhoff stress tensor of the PCFs for $\lambda = \lambda_p(t)$, $\lambda_1 = 1$, and $\lambda_2 = \lambda_p(t)$, where $\lambda_p(t)$ is given by Eq. (3.7). Again, the model parameters, $\kappa$, $\gamma$, and $K$, in Eq. (3.21) are assumed to have the same values for both ACFs and PCFs.

### 3.3.c Parameter Determination

The model parameters were identified by using three sets of published experimental data on pig arteries that were obtained by performing uniaxial isometric length-tension tests [1], uniaxial isometric and isotonic quick-release tests [2], and biaxial isometric inflation-extension tests [3]. These parameters were calculated by minimizing three different error functions, as described in detail below, using the fmincon function with the interior-point method in MATLAB (MATLAB R2013b, MathWorks). All the model parameters were constrained to be non-negative. Furthermore, the parameters $a$ satisfied the inequality $0 \leq a \leq 1$.

When using the isometric length-tension data [1], the seven parameters $\{\alpha, \beta, a, f, \kappa, \gamma, \}$. 

---

**3. Structural Constitutive Model**

Ting Tan
were obtained by minimizing the error function, $E_{r_1}$, defined as

$$E_{r_1} = \sum_{\lambda_m} (P_{33}^{\text{exp}}(\lambda_a(\lambda_m)))^2 + \sum_{\lambda_m} (P_{33}^{\text{theor}}(\lambda_a(\lambda_m)))^2 + \sum_{t} (P_{33}^{\text{exp}}(\lambda_a(t)))^2,$$

where $P_{33}^{\text{exp}}(\lambda_a(\lambda_m))$ and $P_{33}^{\text{theor}}(\lambda_a(\lambda_m))$ are the experimental and theoretical non-zero components of the first Piola-Kirchhoff stress tensor for the ACFs, respectively, and $P_{33}^{\text{exp}}(\lambda_p(\lambda_m))$ and $P_{33}^{\text{theor}}(\lambda_p(\lambda_m))$ are the experimental and theoretical non-zero components of the first Piola-Kirchhoff stress tensor for the PCFs, respectively. We note that the active and passive stretches, $\lambda_a$ and $\lambda_p$, are functions of the tissue stretch $\lambda_m$ via Eq. (3.11) and Eq. (3.7), respectively. Experimentally, the stresses were always measured at a contraction time $t = 300$ s. $P_{33}^{\text{exp}}(\lambda_a(t))$ and $P_{33}^{\text{theor}}(\lambda_a(t))$ are the experimental and theoretical non-zero components of the first Piola-Kirchhoff stress tensor for the ACFs. These vary with the contraction time $t$ for a constant tissue stretch $\lambda_m = 1.5$.

Uniaxial isometric and isotonic experimental data [9] were also used to compute the model parameters. Toward this end, the eight model parameters $\{\alpha, \beta, a, f, \kappa, \gamma, K, \mu\}$ were identified by minimizing the error function, $E_{r_2}$, defined as

$$E_{r_2} = \sum_{t} (P_{33}^{\text{exp}}(\lambda_a(t)))^2 + \sum_{V} ((F/F_0)^{\text{exp}}(V) - (F/F_0)^{\text{theor}}(V))^2,$$

where $P_{33}^{\text{exp}}(\lambda_a(t))$ and $P_{33}^{\text{theor}}(\lambda_a(t))$ are the isometric experimental and theoretical non-zero components of the first Piola-Kirchhoff stress tensor for the ACFs at a constant $\lambda_m = 1.6$. These components change with the contraction time $t$. $(F/F_0)^{\text{exp}}(V)$ and $(F/F_0)^{\text{theor}}(V)$ define the experimental and theoretical normalized forces applied to the tissue during isotonic experiments as functions of the isotonic initial velocities $V$ computed at a constant $\lambda_m = 1.6$ and constant contraction time $t = 3000$ s. $F/F_0$ is the normalized force with respect to the maximum force $F_0$ obtained in isometric experiments. It is equal to the ratio of the total stress, $P_{33}(\lambda_a(V) + P_{33}(\lambda_p(V)))$, in isotonic experiments and the total stress obtained in isometric experiments, which was reported to be 290 kPa. We note that $\lambda_a$ and $\lambda_p$ depend on $V$ via Eq. (3.13) and Eq. (3.7), respectively.

Biaxial isometric data from inflation-extension tests [3] were used to evaluate the eight model
parameters \{\alpha, \beta, a, f, \kappa, \gamma, K, \psi\}. The following error function, \(\text{Er}_3\), was minimized:

\[
\text{Er}_3 = \sum_{\lambda_\theta} \left\{ [(S_{\theta\theta}^{\exp}(\lambda_\theta)) - S_{\theta\theta}^{\text{theor}}(\lambda_\theta)]^2 + [(S_{zz}^{\exp}(\lambda_\theta)) - S_{zz}^{\text{theor}}(\lambda_\theta)]^2 + [(S_{\theta\theta}^{\exp}(\lambda_\theta)) - S_{\theta\theta}^{\text{theor}}(\lambda_\theta)]^2 \right\} \bigg{|}_{\lambda_\theta = 1.2}
\]

where \(S_{\theta\theta}^{\exp}(\lambda_\theta)\) and \(S_{\theta\theta}^{\text{theor}}(\lambda_\theta)\) are the experimental and theoretical circumferential components of the second Piola-Kirchhoff stress tensor for the ACFs, respectively, and \(S_{zz}^{\exp}(\lambda_\theta)\) and \(S_{zz}^{\text{theor}}(\lambda_\theta)\) are the experimental and theoretical axial components of the second Piola-Kirchhoff stress tensor for the ACFs, respectively. \(S_{\theta\theta}^{\exp}(\lambda_\theta)\) and \(S_{\theta\theta}^{\text{theor}}(\lambda_\theta)\) are the experimental and theoretical circumferential components of the second Piola-Kirchhoff stress tensor for the PCFs, and \(S_{zz}^{\exp}(\lambda_\theta)\) and \(S_{zz}^{\text{theor}}(\lambda_\theta)\) are the experimental and theoretical axial components of the second Piola-Kirchhoff stress tensor for the PCFs. The active and passive stretches, \(\lambda_a\) and \(\lambda_p\), are functions of the circumferential tissue stretch \(\lambda_\theta\) at a constant axial tissue stretch \(\lambda_\alpha\) of 1.2 or 1.3. The stresses were always measured experimentally at a constant contraction time \(t = 900\) s.

<table>
<thead>
<tr>
<th>Experimental Data</th>
<th>(\alpha) (s(^{-1}))</th>
<th>(\beta)</th>
<th>(a)</th>
<th>(f)</th>
<th>(\kappa)</th>
<th>(\gamma)</th>
<th>(K) (Mpa)</th>
<th>(\mu) (s(^{-1}))/(\psi) (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniaxial data [1]</td>
<td>0.023</td>
<td>0.328</td>
<td>0.777</td>
<td>0.641</td>
<td>1.103</td>
<td>1.048</td>
<td>0.188</td>
<td></td>
</tr>
<tr>
<td>Uniaxial data [2]</td>
<td>0.0875</td>
<td>0.987</td>
<td>0.810</td>
<td>0.124</td>
<td>21.1</td>
<td>0.733</td>
<td>74.0</td>
<td>0.0261</td>
</tr>
<tr>
<td>Biaxial data [3]</td>
<td>0.020</td>
<td>0.138</td>
<td>0.190</td>
<td>0.652</td>
<td>4.235</td>
<td>0.398</td>
<td>2.614</td>
<td>37.00</td>
</tr>
</tbody>
</table>

### 3.4 Results

In the study by Murtada et al. [1], active and passive stress-stretch data were collected from strips of pig carotid media arteries in the circumferential direction. Stretch data were computed by normalizing the tissue length with respect to the tissue length in the slack configuration. It must be noted that, although isometric length-tension data have been published by several investigators [7, 21, 22], the stretch of the tissue is often measured as the tissue length normalized with respect to the optimal length, i.e. the length at the maximal active tension. The data reported by Murtada et al. [1] captured the tissue deformation independently of the tested specimen’s dimensions and optimal length. For this reason, these data were selected to compute the model parameters in Eqs. (3.11) and (3.16).
The digitized experimental data and our model fit are shown in Fig. 3.2. The values of the model parameters are reported in Table 3.1. Active stress-time data recorded at an optimal stretch of 1.5 were presented by Murtada et al. [1]. The constitutive model fits well these data: it captures the increase in the active stress with the contraction time of SMCs (Fig. 3.2(a)). The active stress was found to be almost constant after reaching its maximum value at 200 seconds. In Fig. 3.2(b), the digitized active and passive stress-stretch data and model fits are shown. The constitutive model describes well the typical passive nonlinear elastic response of soft biological tissues. It also reproduces the increase in active stress with tissue stretch and the decrease in active stress after reaching the maximum value at the optimal stretch. The coefficient of determination $R^2$ was found to be 0.862. Considering the variation in the active and passive stress-stretch experimental data, the constitutive model appears to be capable of fitting the uniaxial isometric experimental data by Murtada et al. [1].

Uniaxial isometric data obtained from length-tension experiments and uniaxial isotonic data obtained from quick-release experiments on swine carotid media specimens were reported by Dillon et al. [2]. We note that many experimental studies have focused on determining either isometric length-tension relationships or isotonic force-velocity relationships [7, 9, 18, 10]. However, since both isometric and isotonic data were reported by Dillon et al. [2], these data were selected and used simultaneously to determine the model parameters in Eqs. (3.7), (3.13), and (3.16). The digitized active stress-time experimental data at an optimal stretch of 1.6 [2] and the model fit are shown in Fig. 3.3(a). The constitutive law with the values of the model parameters reported in Table 3.1 reproduces the results of the experiments: there
is a quick increase in the active stress followed by a plateau region that starts at around 100 seconds. The model also successfully illustrates the nonlinear force-velocity relationship, which is characterized by a decrease in velocity with increasing force while the tissue is shortening (Fig. 3.3(b)). Overall, the proposed model can simulate the uniaxial isometric and isotonic behavior of pig carotid arteries ($R^2 = 0.982$).

![Figure 3.3: Active and passive uniaxial data [2] and model fit ($R^2 = 0.982$). (a) Active stress-time experimental data (red symbols) at a stretch of 1.6 and model fit (continuous line); (b) Velocity-force experimental data (green symbols) and model fit (continuous line).](image)

Recently, biaxial mechanical data for pig coronary arteries obtained from inflation-extension experiments were presented by Chen et al. [3]. To our knowledge, among published data, this is the most complete set of experimental data that characterizes the biaxial active and passive stress-stretch relationships [23, 24, 25]. For this reason, we utilized these experimental data to determine the model parameters in Eqs. (3.20) and (3.21). In Fig. 3.4, both the
Figure 3.4: Active and passive biaxial data [3] \((R^2 = 0.965)\). (a) Active, passive, and total circumferential stress-stretch data at an axial stretch of 1.2 and model fits (continuous lines). (b) Active, passive, and total axial stress-circumferential stretch data at an axial stretch of 1.2 and model fits (continuous lines). (c) Active, passive, total circumferential stress-circumferential stretch data at an axial stretch of 1.3 and model fits (continuous lines). (d) Active, passive, and total axial stress-circumferential stretch data at an axial stretch of 1.3 and model fits (continuous lines).

Digitized active and passive experimental stress data collected in circumferential and axial directions under a constant axial stretch of 1.2 or 1.3 are plotted versus the circumferential stretch with the model fits. The biaxial passive and active stress-stretch relationships are well captured by the model. The values of the model parameters are reported in Table 3.1. The \(R^2\) was found to be 0.965. As expected, the axial stresses are found to be lower than the circumferential ones. This can be explained by the collagen fiber preferred orientation of 37° off the circumferential direction (see Table 3.1). That is to say, the tissue is anisotropic.
It is stiffer in the circumferential direction than in the axial direction.

3.5 Discussion

In recent years, mechanochemical constitutive models that account for the chemical states of myosin have been developed for SMC contraction based on the so-called four-state chemical model [13, 26, 1]. In these models, the active stress of biological tissues depends on the number of activated myosin heads and their stiffness. This number is obtained from chemical kinetics models. In the constitutive model proposed by Kroon [26], five chemical parameters were fixed based on the work by Hai and Murphy [10], and two additional chemical parameters were identified using experimental data published by Dillon and Murphy [9]. Four material parameters for the active mechanical response and five material parameters for the passive mechanical response were then determined by fitting mechanical experimental data. In the constitutive model proposed by Murtada et al. [1] two chemical parameters were estimated by the authors and five chemical parameters were fixed based on the work by Rembold and Murphy [27] and Hai and Murphy [10]. In addition, five material parameters for the active mechanical response and three material parameters for the passive mechanical response were also determined using mechanical experimental data. Like the above-cited mechanochemical models, the constitutive model we presented here fits the mechanical experimental data well but with fewer parameters.

In formulating the proposed constitutive model, we assumed that the active stress of the SMCs can be computed from the stress of their connected ACFs. This assumption was made since there is no information about the SMC deformation within the tissue during contraction. Under this assumption, the active response of the tissue can then be described without introducing a kinetics model for the interaction of the myosin heads and thin filaments. Instead, based on the sliding filament theory, we assumed that there are three forces that generate contraction: a motor force that initiates contraction, an elastic force for the cross-bridge deformation, and a viscous force for the filament sliding. The resulting evolution law expressed by Eq. (3.11) for an isometric contraction and Eq. (3.13) for an isotonic contraction defines the deformation of ACFs with only four parameters. Three additional parameters were needed to describe the active and passive collagen fibers’ mechanical behavior: two parameters for the recruitment of the fibers and one parameter for their elastic modulus. Overall, seven to eight parameters were needed to capture the results of uniaxial isotonic and isometric experiments on arteries.

The nonlinearity in the active stress stemmed from the overlap mechanisms between the actin and myosin filaments for isometric contractions in the structural constitutive model by Murtada et al. [1]. These authors introduced a homogenous parabolic function to describe this overlap mechanisms and, consequently, the active stress as a function of the tissue stretch had a parabolic profile. This led to underestimated values of the active stress when the tissue was stretched above the optimal stretch. In our study, the nonlinearity in the active stress
was assumed to be determined by the recruitment model that defined the stress of the ACFs. Because this stress was assumed to be equal to the stress of the SMCs (Fig. 3.1(c)), we did not need a description of the SMC deformation and, thus, of the overlap mechanism to compute the active stress. The recruitment model with a Weibull probability distribution function yielded a better fit of the active stress-stretch data following the optimal stretch. The parameters that were found by curve fitting the model to three sets of experimental data on arteries [1, 2, 3] are reported in Table 3.1. The value of the parameter $\alpha$ computed by using the data by Murtada et al. [1] was comparable to the value computed using the data by Chen et al. [3], but lower than the value computed using the data by Dillon et al. [2]. For a higher $\alpha$-value, the maximum stress during an isometric test was reached within a shorter interval of time. This can be observed from the experimental data in Figs. 3.2(a) and 3.3(a). As $\beta$ increased, the stretch of the ACFs in isometric contractions increased as one can see from Eq. (3.11) and, consequently, the active stress increased too. The high $\beta$-value obtained from the data by Dillon et al. [2] was due to the high value of the active stress obtained in their experiments. The elastic modulus of the collagen fibers, $K$, had highest value for the data published by by Dillon et al. [2]. This largest $K$-value can be explained by the highest active stress reported by Dillon et al. [2]. The value of $a$, which denotes the ratio of the initial axial displacement of the SMC contractile units to the axial tissue displacement, obtained from biaxial experimental data [3] was lower than that obtained from uniaxial experimental data [1, 2]. Thus, the initial axial displacement of the SMC contractile units given by Eq. (3.9) is much lower along the two preferred collagen fiber directions than along the circumferential direction of the arteries. Finally, the collagen fiber orientation angle $\psi$ was found to be similar to the $37^\circ$ angle reported by Chen et al. [3].

This study has several limitations that are worth mentioning. Thus far we have only tested the proposed constitutive model with uniaxial and biaxial experimental data considering specific deformations. We selected published data on arteries to identify the model parameters since the active and passive mechanical data on these biological tissues are the most complete sets of published data. Three dimensional data that characterize the active and passive mechanics of arteries and other biological tissues are needed to further evaluate the proposed model. Moreover, in the reference configuration, the collagen fibers and their connected SMCs have been assumed to be all aligned along one direction when testing the model with uniaxial data or two directions when testing the model with biaxial data. In fact, the collagen fibers and SMCs are oriented along different directions within the arteries and the probability density function $R(m)$ in Eq. (3.2) should be computed through techniques such as small angle light scattering [28] and histology [3]. Finally, the orientation of the contractile units within the SMCs should also be taken into consideration in the model development. Such orientation is important since the active force is generated along these units and transmitted, through filament anchor points on the cell membrane, to surrounding collagen fibers and ground substance. Information about the micro-structural changes of the contractile units during mechanical loading is crucial for developing robust micro-structural constitutive models. These models will provide a better understanding of the mechanism
of smooth muscle contraction and, ultimately, improve the treatment of medical disorders such as hypertension, asthma, and pelvic floor disorders caused, in part, by a mechanical dysfunction of the SMCs.

3.6 Conclusions

We proposed a new structural constitutive model that characterizes the active and passive mechanical responses of biological tissues containing SMCs. The active response was attributed to the collagen fibers connected to the SMCs and the passive response was attributed to the remaining collagen fibers. A new evolution law for the collagen fibers that are connected to the SMCs and are thus activated with them was derived based on the sliding filament theory. The active force was assumed to be determined by an initial motor force, cross-bridge deformation, and filament sliding. The constitutive model was validated using uniaxial isometric and isotonic and biaxial isometric experimental data on arteries [2, 1, 3]. This study advanced our understanding of the active mechanical behavior of biological tissues containing SMCs.


4.1 Introduction

Every year millions of women are affected by pelvic floor disorders (PFDs) such as urinary incontinence, fecal incontinence, and pelvic organ prolapse. For example, in the United States alone, the number of women with at least one PFD is projected to significantly increase from 28.1 million in 2010 to 43.8 million in 2050 with 55%, 59%, and 46% increases in urinary incontinence, fecal incontinence, and pelvic organ prolapse, respectively [1]. The lifetime risk of surgery for PFDs is 11.1% with a 29.2% risk of an additional surgery [2]. The annual direct cost for pelvic organ prolapse surgeries alone is approximately one billion dollars [3]. Thus, given the projected increase in the number of affected women, PFDs are expected to place a significant burden on the quality of life of women and a financial strain on the health care system.

Pelvic floor muscles, fasciae, and ligaments are the main supportive structures of the pelvic organs. They maintain the organs in their anatomical positions allowing them to perform their normal physiological function. These supportive structures can be weakened or damaged by labor, delivery, menopause, aging, and obesity, leading to the development of PFDs [4, 5, 6]. For example, when the supportive function of the USL/CL complex is compromised, a uterine or vaginal prolapse may develop [7]. The USL and CL not only play a pivotal role in supporting the uterus, cervix, and vagina in healthy women but they also are extensively used as anchor structures in surgical procedures for pelvic organ prolapse [8, 9, 10, 11]. During these surgeries, the ligaments are often stretched and tensed in an *ad-hoc* manner. However, the amount of stretch and tension placed on them can seriously compromise the successful outcome of the surgeries. For instance, one study attributed post hysterectomy vaginal vault prolapse to excessive tension placed on these ligaments during surgery [8]. Artificial mesh materials have been also used in surgeries for PFDs. The mesh
products are expected to have appropriate biomechanical properties [12, 13], similar to those of healthy and strong suspensory tissues [14]. Therefore, investigating the mechanical properties of the USL/CL complex is critical for the development of new surgical reconstruction strategies and mesh materials for PFDs.

The mechanical properties of the USL and CL have only recently been investigated. Uniaxial force-displacement data have been obtained by performing tensile tests on ex-vivo specimens from patients undergoing hysterectomy [15] and by conducting tensile and stress-relaxation tests on in-vivo specimens from women affected by pelvic organ prolapse [16, 17]. Uniaxial force-displacement data were also collected on ex-vivo rat specimens from the vagina-ligament complex by means of tensile tests [18]. Although force-displacement data provide useful information on the mechanical behavior of the USL and CL, they are highly dependent on the dimensions of the tested specimens. Mechanical properties of these ligaments that are independent of the dimensions of the tested specimens have been determined from stress-strain data [19-23]. The mean strength and tensile modulus of the USL were reported for ex-vivo specimens from monkeys [19], female cadavers [20, 21], and swine [22]. The strain-dependent tensile modulus of the USL was found to increase in post-menopausal monkeys due to hormone replacement therapy [19]. The USL of nulliparous women was reported to have lower stiffness and strength than the USL of parous women [20]. Compared to the round and the broad ligaments, the USL was found to have higher tangent modulus at both low and high strain levels [21]. The tensile properties of the USL/CL specimens were highly dependent on their location relative to the uterus, cervix, and vagina [22]. Recently, our group has characterized the elastic and stress relaxation properties of these ligaments by performing planar biaxial tests [23]. The USL/CL complex was found to be stiffer along the main physiological loading direction but relaxed equally under equi-biaxial strain along such direction and the direction perpendicular to it, with higher relaxation occurring at lower strain.

In vivo, the USL and CL are subjected to nominally constant forces that are, for example, imposed by the weight of the pelvic organs. The deformation over time under constant loads, the so-called creep behavior, has yet to be characterized for these ligaments despite their physiological relevance. Creep has been studied for other biological soft tissues such as rat and rabbit medial collateral ligaments [13, 14, 26, 27, 28], porcine mitral and aortic valves [29, 30, 31], and human amnion [32] to name but a few. It was found that the creep strain and creep rate in rat medial collateral ligaments were highly dependent on the magnitude of the applied constant stress [13, 14]. In the rabbit medial collateral ligaments, creep was attributed to the straightening of collagen fibers under low stress and damage of such fibers at high stress [28]. The decrease in creep rate with the increase in stress was explained by fewer collagen fibers left to be recruited during creep as the stress increased [26, 28]. Creep in the aortic valve was found to be stress dependent and differed in the circumferential and radial directions [29]. In the human amnion, creep measured via uniaxial tests was significantly larger than creep measured via inflation tests at a comparable tension [32]. To date, the only planar biaxial creep studies on biological soft tissues have been conducted by Grashow.
et al. [30] and Stella et al. [31]. However, these authors reported negligible creep for mitral valves [30] and aortic valves [31].

In this study, we investigate the micro-structural and mechanical properties of the USL/CL complex in swine. By using scanning electron microscopy (SEM), we reveal the organization of collagen fibers, which represent the main structural components of these ligaments. More specifically, we quantify the straightness and the alignment of collagen fiber bundles. We determine the biaxial creep properties of the swine USL/CL complex by using a planar biaxial testing system in conjunction with the Digital Image Correlation (DIC) method. This method provides a more accurate strain measurement than previously used techniques such as those relying on the gauge length measurement [19, 20, 21] or on optically tracking a few markers [22, 23]. The DIC method is employed here to measure the deformation over time experienced by the USL/CL complex under constant equi-biaxial loads. Overall, this study advances our limited knowledge about the structural and mechanical properties of some components of the pelvic connective tissues that play a crucial role in the surgical treatment of PFDs.

4.2 Material and Methods

4.2.a Specimen Preparation

This study was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Virginia Tech. Five adult domestic swine were obtained from a slaughterhouse. The swine were 3 to 4 years old and had masses of approximately 200 kg. The harvesting technique for USL/CL complex was described in detail in our previous study [22]. The ligaments were carefully dissected, hydrated in phosphate-buffered saline solution (PBS, pH 7.4, Fisher Scientific, USA) and then frozen at -20°C. They were thawed at room temperature before being used for scanning electron microscopy (SEM) or creep tests.
4.2.b SEM Examination

Figure 4.1: (a) Schematic of the swine USL and its main in-vivo loading direction relative to the cervix, vagina, and rectum (transverse plane view). The main in-vivo loading direction of the ligaments (parallel direction) is denoted using an orange arrow and the direction that is perpendicular to it is (perpendicular direction) denoted using a green arrow. (b) Locations of the specimen sections used for SEM analysis.

A square-shaped specimen was collected from one USL/CL complex. More precisely, the specimen was isolated from the USL as shown in Figure 4.1 (a) and cut so that the sides were parallel and perpendicular to the main in-vivo loading direction. Hereafter, the direction that is parallel to the main in-vivo loading direction of the ligaments will be termed the parallel direction and the direction that is perpendicular to it will be termed the perpendicular direction. The specimen was then fixed overnight in 10% buffered formalin, washed in phosphate-buffered saline solution (PBS, pH 7.4, Fisher Scientific, USA), post-fixed in osmium tetraoxide, and dried in a critical point dryer (Model 28000, LADD Research Industries, Williston, Vermont, USA). It was then immersed in liquid nitrogen and fractured with a sharp razor blade in order to reveal three different sections: one planar section (section 1 in Figure 4.2 (b)) and two through-thickness sections (sections 2 and 3 in Figure 4.2 (b)). After being sputter coated with gold, these sections were examined using an SEM (EVO 40, Carl Zeiss, Jena, Germany) at 1,000× and 20,000× magnifications.
Figure 4.2: (a)-(b) SEM of the in-plane specimen cross-section (section 1 in Figure 4.1) at 1,000× and 20,000× magnifications, respectively. (c)-(d) SEM of the out-of-plane specimen cross-section (section 2 in Figure 4.1) at 1,000× and 20,000× magnifications, respectively. (e)-(f) SEM of the out-of-plane specimen cross-section (section 3 in Figure 4.1) at 1,000× and 20,000× magnifications, respectively. (Main *in-vivo* loading direction of the ligaments is denoted using an orange arrow).

Seven SEM images from section 1 of the specimen (Figure 4.2 (b)) at 1,000× magnification were analyzed to measure collagen fiber bundle straightness and global and local orientation angles relative to the parallel direction. Fiber bundles on these images were traced and their length and end point coordinates were determined using NeuronJ [33], a plugin of the ImageJ software (v1.48, NIH, Bethesda, Maryland, USA). A total of 142 fiber bundles were detected. From the coordinates of the end points, the end-to-end distance, $L_{0}^{(i)}$, of the $i$-th fiber bundle
and the global orientation angle, $\theta^{(i)}$, with respect to the parallel direction were calculated (Figure 4.3). The straightness parameter of the $i$-th fiber bundle was defined as $L_0^{(i)}/L_f^{(i)}$ where $L_f^{(i)}$ is the length of $i$-th fiber bundle (Figure 4.3). The mean thickness, $t^{(i)}$, was computed by averaging the thickness measurements from three randomly chosen locations along the length of the fiber bundle using ImageJ. The area of the $i$-th fiber bundle, $S^{(i)}$, was then calculated as the product of its length $L_f^{(i)}$ and mean thickness $t^{(i)}$. The fraction, $f^{(i,j)}$, of fiber bundles with a given straightness parameter $L_0^{(i)}/L_f^{(i)}$ and global orientation angle $\theta^{(j)}$ was computed as $f^{(i,j)} = \frac{\sum_{k=1}^{m} S^{(k)}}{\sum_{k=1}^{n} S^{(k)}}$, where $m$ denotes the number of fiber bundles with straightness parameter $L_0^{(i)}/L_f^{(i)}$ and global orientation angle $\theta^{(j)}$ and $n = 142$ denotes the total number of fiber bundles detected in the seven SEM images that were analyzed. The fraction of fiber bundles with different straightness parameters was computed as $g^{(i)} = \frac{\sum_{k=1}^{p} S^{(k)}}{\sum_{k=1}^{n} S^{(k)}}$, where $p$ denotes the number of fiber bundles with straightness parameter $L_0^{(i)}/L_f^{(i)}$. The fraction of fiber bundles with different global orientation angles with respect to the parallel direction was computed as $h^{(j)} = \frac{\sum_{k=1}^{q} S^{(k)}}{\sum_{k=1}^{n} S^{(k)}}$, where $q$ denotes the number of fiber bundles with global orientation angle $\theta^{(j)}$. The local collagen fiber bundle orientation angles and the fractions of fiber bundle with various local orientation angles were determined from the same SEM images using OrientationJ [33], also a plugin of ImageJ. This determination was accomplished using the Gaussian gradient interpolation method described in detail elsewhere [33].

![Figure 4.3: Measured parameters: fiber bundle length $L_f^{(i)}$, end-to-end fiber bundle distance $L_0^{(i)}$, and global fiber orientation angle $\theta^{(i)}$ computed with respect to the parallel direction.](image-url)
4.2.c Biaxial Creep Testing

Specimens \((n = 25)\) from four sows were cut into squares having side of length 3 cm parallel and perpendicular to the main *in-vivo* loading direction. The thickness of each specimen was measured by means of a digital caliper (accuracy \(\pm 0.05\) mm, Series 573, Mitutoyo, Japan) under a 50 g compressive load. For mechanical testing, each specimen was then immersed in methylene blue, 1% aqueous solution (Fisher Science Education, USA), and gripped by four safety pins on each side. A high-contrast speckle pattern was then created on the surface of the specimen with aerosol fast dry gloss white paint (McMaster-Carr, USA) [34]. Two CCD cameras (Prosilica GX 1660, Allied Vision Technologies, Exton, Pennsylvania, USA) equipped with macro lenses (AT-X 100mm F2.8 AT-X M100 Pro D Macro Lens, Tokina, Tokyo, Japan) were employed to capture high resolution \((1600 \times 1200\) pixel\) images of a 12 mm\(\times\)9 mm plastic grid with 4 mm spacing. These images were used to calibrate a 3-D DIC (VIC-3D, Correlated Solutions, Columbia, South Carolina, USA) for non-contact strain measurement. After calibration, the safety pins on each side of the specimen were connected to four pulleys attached to four actuators (accuracy: 5 \(\mu\)m) of a planar biaxial testing system (Instron, UK). The capacity of the four load cells of the planar biaxial testing system was 20 N (accuracy: 0.02 N). The specimen was then immersed into an enclosed bath made of acrylic glass (Perspex, UK) that was fully filled with PBS at room temperature \((21^\circ C)\).

Starting from zero displacement, the specimen was preloaded to 0.1 N and preconditioned from 0.1 N to 0.6 N at 0.1 mm/s displacement rate for 10 cycles. Following preconditioning, the specimen was unloaded and allowed to recover for 10 min. Each specimen was then stretched at 0.1 mm/s displacement rate until equi-biaxial loads of 2 N \((n = 15\) specimens\) or 4 N \((n = 10\) specimens\) were detected. These equi-biaxial loads were kept constant for 120 min. The full field displacement and Lagrangian strain were determined by postprocessing the specimen images obtained during equi-biaxial tests using the VIC-3D software (v7, VIC-3D, Correlated Solutions, Columbia, South Carolina, USA) after these images underwent background subtraction for the presence of the plastic cover and PBS. We note that additional specimens were subjected to equibiaxial loads of 4 N. However, these specimens failed at the grips and thus the data collected from these samples were excluded from this study.

For each specimen, nominal axial stresses in the parallel and perpendicular directions were calculated by dividing the axial loads in the corresponding directions by the specimen undeformed cross-sectional area. The specimen cross-sectional area was assumed to be rectangular. Lagrangian axial strains in the parallel and perpendicular directions were computed by averaging the local Lagrangian axial strains in the corresponding directions computed over a 1 cm\(\times\)1 cm central region of the specimen using the 3D-DIC methods (Figure 4.4). Normalized Lagrangian axial strains in the parallel and perpendicular directions were determined by dividing the Lagrangian axial strains by the initial Lagrangian axial strain (i.e., the Lagrangian axial strain at beginning of the creep test) in the corresponding directions. Hereafter and throughout this manuscript, *stress* will be used to denote nominal axial stress, *strain* to denote Lagrangian axial strain, and *normalized strain* to denote normalized La-
grangian axial strain. For each tested specimen, creep rates in the parallel and perpendicular directions were computed as the slope of the linear regression lines of the normalized strain data in the corresponding directions versus time data using logarithmic scales [14].

Figure 4.4: Area selected on the specimen surface for the measurement of local Lagrangian axial strains via the DIC method. Shown here is the strain map in the parallel direction.

4.2.d Statistical Analysis

Means and standard deviations were calculated for the axial loads and corresponding stresses, initial strains (i.e., strain at the beginning of the creep tests), and normalized strains over time in both parallel and perpendicular directions for two groups of specimens: one group of $n = 15$ specimens subjected to equi-biaxial loads of 2 N and one group of $n = 10$ specimens subjected to equi-biaxial loads of 4 N. The Student’s $t$-test was used to compare the means of the normalized strain at 120 min and the creep rate between the parallel and perpendicular directions and between equi-biaxial loads of 2 N and 4 N. The threshold chosen for statistical significance was 0.05. Data were analyzed using the JMP statistical software (JMP 10, SAS Institute Inc.).

4.3 Results

4.3.a SEM Examination

Representative scanning electron micrographs of the USL/CL complex are shown in Figure 4.2. Most collagen fibers were oriented along the main in-vivo loading direction of the ligaments, the parallel direction. However, some fibers were oriented at small angles off
such direction (Figure 4.2 (a)-(d)). The collagen fibers were found to be arranged into layers (Figure 4.2 (e) and (f)). Some layers contained more collagen fibers than others and, in these layers, the collagen fibers were organized into bundles (Figure 4.2 (f)). The collagen fiber bundles (Figure 4.2 (a) and (c)) and collagen fibers (Figure 4.2 (b) and (d)) were observed to have different straightness levels.

Figure 4.5: Fractions of collagen fiber bundles with different straightness parameters and global orientation angles (with respect to the parallel direction). The reported data are obtained by analyzing $n = 7$ SEM images.
Figure 4.6: Fractions of collagen fiber bundles with global angle oriented between -10° and 10° with straightness parameters that differ by 0.02. The reported data were obtained by analyzing $n = 7$ SEM images.

The fractions of fiber bundles with different straightness parameters and global orientation angles relative to the parallel direction are presented in a three-dimensional bar plot in Figure 4.5. It can be seen that 90.6% fiber bundles had a straightness parameter between 0.70 and 0.94, and 92.4% fiber bundles had a global orientation angle between -50° and +50°. The fractions of fiber bundles with different straightness parameters aligned near the parallel direction, with global orientation angles between -10° and +10°, are shown in Figure 4.6. The straightness parameters for these fiber bundles were between 0.70 and 0.94 with the largest fraction of fiber bundle having a straightness parameter between 0.86 and 0.87. The fractions of fiber bundles with different straightness parameters (regardless of global orientation) are shown in Figure 4.7. The empirical data revealed that the highest fraction of collagen fiber bundles (approximately 14%) had a straightness parameter between 0.90 and 0.92.
Figure 4.7: Fractions of collagen fiber bundles with straightness parameters that differ by 0.02. The reported data were obtained by analyzing \( n = 7 \) SEM images.

The histogram of fiber bundle orientations is presented in Figure 4.8. We recall that the global orientation angle was defined as the angle formed by a straight line connecting the end-to-end points of a fiber bundle and the parallel direction while the local orientation angle was defined as the angle formed by segments of a fiber bundle and the parallel direction. The highest fraction of fiber bundles was between -10° and +10°, that is, around the main in-vivo loading direction, while a few fiber bundles were oriented perpendicular to such direction. The fractions of collagen fiber bundles with different local orientation angles are reported in Figure 4.9. Again, one can note that the collagen fiber bundles were aligned along the main in-vivo loading direction with the highest fraction of fiber bundles between -10° and +10°. Fewer fiber bundles were oriented in the perpendicular direction. However, the results of the local angle analysis in Figure 4.9 showed that more fiber bundles than those detected by the global angle analysis were aligned along this direction.
4.3.b Biaxial Creep

The axial loads, corresponding stresses, and initial strains (mean±standard deviation) used during the creep tests at constant equi-biaxial loads of 2 N and 4 N are reported in Table 4.1 and Table 4.2, respectively. For both loads, the mean stresses in the parallel and perpendicular directions were nearly identical, while the mean initial strain measured in the perpendicular direction was always larger than the mean initial strain in the parallel direction (Table 4.1 and Table 4.2). We note that mean initial strains for the equi-biaxial loads of 2 N are 0.116 and 0.152 in the parallel and perpendicular directions, respectively, and that the mean initial strains for the equi-biaxial loads of 4 N are 0.216 and 0.263 in the parallel and perpendicular directions, respectively. The mean and standard deviation of the normalized strain data versus time obtained from creep tests at constant equi-biaxial loads of 2 N and 4 N are presented in Figure 4.10. Despite the large variation, the creep of all the specimens in both loading directions had a similar trend over the 120 min duration of the tests: the normalized strain increased quickly over the first 18 min and very slowly over the last 35 min (Figure 4.10). At the constant equi-biaxial load of 2 N, the creep at 18 min (the difference between the mean normalized strain at 18 min and the mean normalized strain at 0 min) was approximately 64% and 71% of the creep at 120 min (the difference
between the mean normalized strain at 120 min and the mean normalized strain at 0 min) in the parallel and perpendicular directions, respectively (Figure 4.10 (a)). At the constant equi-biaxial load of 4 N, the creep at 18 min was approximately 50% and 77% of the creep measured at 120 min in the parallel and perpendicular directions, respectively (Figure 4.10 (b)). The mean normalized strain of the ligaments over time appeared to be different in the two loading directions.

The mean and standard deviation of the normalized strain data at 120 min in the parallel and perpendicular directions computed from creep tests at constant equi-biaxial loads of 2 N and 4 N are shown in Figure 4.11. The mean strain at 120 min from creep tests at constant equi-biaxial loads of 2 N was found to be 1.41 and 1.32 times the initial mean strain in the perpendicular and parallel directions, respectively. The mean strain at 120 min from creep tests at constant equi-biaxial loads of 4 N was found to be 1.30 and 1.20 times the initial mean strain in the perpendicular and parallel directions, respectively. In the perpendicular direction, the mean normalized strain obtained from creep tests at constant equi-biaxial loads of 2 N was slightly larger than the mean normalized strain obtained from creep tests at constant equi-biaxial loads of 4 N ($p = 0.2541$). In the parallel direction, the mean normalized strain obtained from creep tests at constant equi-biaxial loads of 2 N was
much larger, but still not significantly larger, than the mean normalized strain obtained from
creep tests at constant equi-biaxial loads of 4 N ($p = 0.0662$). In other words, the ligaments
experienced more creep under constant equi-biaxial loads of 2 N than under constant equi-
biaxial loads of 4 N. By comparing the mean strain values at 120 min, one can observe
that the ligaments exhibited more creep in the perpendicular direction than in the parallel
direction. However, our statistical analysis showed that, at 120 min, there was no significant
difference between the mean normalized strains in the parallel and perpendicular directions
for both constant equi-biaxial loads of 2 N ($p = 0.2690$) and 4 N ($p = 0.0858$) (Figure
4.11).

Table 4.1: Creep test parameters for $n = 15$ specimens (thickness: 0.691±0.226 mm) sub-
jected to constant equi-biaxial loads of 2 N.

<table>
<thead>
<tr>
<th>Direction</th>
<th>Mechanical Quantity</th>
<th>Value (Mean±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel</td>
<td>Load (N)</td>
<td>2.09±0.19</td>
</tr>
<tr>
<td></td>
<td>Stress (MPa)</td>
<td>0.153±0.066</td>
</tr>
<tr>
<td></td>
<td>Initial strain</td>
<td>0.116±0.065</td>
</tr>
<tr>
<td>Perpendicular</td>
<td>Load (N)</td>
<td>2.00±0.07</td>
</tr>
<tr>
<td></td>
<td>Stress (MPa)</td>
<td>0.142±0.061</td>
</tr>
<tr>
<td></td>
<td>Initial strain</td>
<td>0.152±0.076</td>
</tr>
</tbody>
</table>

Table 4.2: Creep test parameters for $n = 10$ specimens (thickness: 0.652±0.152 mm) sub-
jected to constant equi-biaxial loads of 4 N.

<table>
<thead>
<tr>
<th>Direction</th>
<th>Mechanical Quantity</th>
<th>Value (Mean±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel</td>
<td>Load (N)</td>
<td>3.87±0.12</td>
</tr>
<tr>
<td></td>
<td>Stress (MPa)</td>
<td>0.284±0.080</td>
</tr>
<tr>
<td></td>
<td>Initial strain</td>
<td>0.216±0.125</td>
</tr>
<tr>
<td>Perpendicular</td>
<td>Load (N)</td>
<td>4.02±0.08</td>
</tr>
<tr>
<td></td>
<td>Stress (MPa)</td>
<td>0.289±0.080</td>
</tr>
<tr>
<td></td>
<td>Initial strain</td>
<td>0.263 ±0.218</td>
</tr>
</tbody>
</table>
Figure 4.10: Mean and standard deviation of the normalized strain over time in the parallel and perpendicular directions for (a) \( n = 15 \) specimens subjected to constant equi-biaxial loads of 2 N and (b) for \( n = 10 \) specimens subjected to constant equi-biaxial loads of 4 N.
Figure 4.11: Mean and standard deviation of normalized strain at $t = 120$ min for $n = 15$ specimens subjected to constant equibiaxial loads of 2 N and for $n = 10$ specimens subjected to constant equibiaxial loads of 4 N in the parallel and perpendicular directions.

The mean and standard deviation of the creep rate at equibiaxial loads of 2 N and 4 N in the parallel and perpendicular directions were also determined (Figure 5.2). The mean creep rate at equibiaxial loads of 2 N was found to be 0.050 and 0.054 in the perpendicular and parallel directions, respectively, while the mean creep rate at equibiaxial loads of 4 N was found to be 0.041 and 0.031 in the perpendicular and parallel directions, respectively. In the ligaments, creep proceeded significantly faster at equibiaxial loads of 2 N than at equibiaxial loads of 4 N in the parallel direction ($p = 0.0284$). It proceeded slightly faster at equibiaxial loads of 2 N than at equibiaxial loads of 4 N in the perpendicular direction ($p = 0.3696$). Additionally, no significant difference between the mean creep rate of the ligaments in the parallel and perpendicular directions at both equibiaxial loads of 2 N ($p = 0.5709$) and 4 N ($p = 0.3542$) was noted.
To investigate the effect of stress on the creep behavior of the USL/CL complex, the normalized strain at three chosen time points \( t = 2.5, t = 12.5, \) and \( t = 120 \) min) in the parallel and perpendicular directions were analyzed with the corresponding stress (Figure 4.13). Two of the three time points were chosen within the first 20 min of the creep tests when a sharp increase in normalized strain was detected (i.e., \( t = 2.5 \) min and \( t = 12.5 \) min), and one time point was selected toward the end of the creep tests (\( t = 120 \) min). Linear regression lines for the three time points are plotted with the normalized strain-stress data (Figure 4.13). These lines indicate that the ligaments experienced slightly less creep under high stress levels. A more clear decrease in normalized strain with stress can be observed at \( t = 120 \) min. The creep rate versus stress data in the parallel and perpendicular directions are also reported with linear regression lines in Figure 4.14. Although there was a large variation in the experimental data, a trend of decreasing creep rate with increasing stress was noted in both the parallel and perpendicular directions. The creep rate of the USL/CL complex appeared to be dependent on stress.
Figure 4.13: Normalized strain at $t = 2.5$, $t = 12.5$, $t = 120$ min versus stress for $n = 25$ specimens along (a) the parallel direction and (b) the perpendicular direction.
Figure 4.14: Creep rate versus stress for $n = 25$ specimens along (a) the parallel direction and (b) the perpendicular direction.
4.4 Discussion

Collagen fibers were observed on three different sections of the USL/CL complex using SEM (Figure 4.1 (a)-(b)). The ligaments were found to be composed of layers of collagen fibers (Figure 4.2 (a)) and, in each layer, the collagen fibers were arranged into bundles that were mainly oriented along the \textit{in-vivo} loading direction (Figure 4.2). We obtained consistent results with our previous study \cite{22}, where we visualized \textit{only} the cross-section of the USL/CL that was perpendicular to the main \textit{in-vivo} loading direction. The fractions of fiber bundles with various straightness parameters and orientation angles (Figures 4.5-4.9) were quantified from the SEM images of the in-plane specimen section (section 3 in Figure 4.1 (b)) to aid the interpretation of the results of the mechanical experiments.

We determined a discrete distribution for the fractions of fiber bundles with different global orientation angles (Figure 4.8) and a continuous distribution for the fractions of fiber bundles with different local orientation angles (Figure 4.9). According to global orientation angle measurements, more fiber bundles were aligned in the parallel direction than in the perpendicular direction (Figure 4.8). Both the global and local measurements of orientation indicated that the fibers were preferentially oriented along the parallel direction. These results are to be expected since the collagen fibers must provide structural support to the ligaments mainly along their \textit{in-vivo} loading direction. They also explain the previously observed elastic anisotropy of these ligaments \cite{23}.

The measurement of the straightness parameters of the collagen fiber bundles helped us shed some light on the relationship between the un-crimping process and the creep phenomenon. Hingorani et al. \cite{14} speculated that fiber un-crimping was the creep mechanism in rabbit medial collateral ligament. In our study, fiber bundles were observed to have higher straightness parameters in the parallel direction than in the perpendicular direction (Figure 4.5). In agreement with previous speculations \cite{14}, these results may explain the reduced creep reported for the parallel direction (Figure 4.10). For most fiber bundles, the straightness parameter was between 0.7 and 0.94 (Figures 4.6-4.7). This means that 30\% to 16\% of the fiber bundle could un-crimp before being stretched. We note that the straightness parameters of the USL/CL complex were, however, underestimated since the out-of-plane waviness of the fiber bundles was not captured from the two dimensional SEM images used for our analysis.

Over the past years, the mechanical properties of USLs have been characterized by quantifying their deformation using engineering strain \cite{19, 20, 21}. In this study, the USL/CL specimens underwent large deformation with strains up to 0.846 during creep and, for this reason, the use of the engineering strain was deemed inappropriate. The Lagrangian strain was determined instead and was measured using the 3-D DIC method. This method accounted for the inhomogeneities in strain of the USL/CL complex that were neglected in our previous studies \cite{23, 22}. It must be noted that, inevitably, the safety pins used for gripping the specimens caused stress concentration. By using the 3-D DIC, we were able to computed
the average strain during creep in the central region of the specimens located away from the safety pins, where the effects of stress concentration were absent. The 3-D DIC also excluded from the strain measurement possible artifacts due to the out-of-plane motion of the specimens during testing. Although the surface strain was accurately measured using the 3-D DIC, relative sliding of the thin layers that make the ligaments (Figure 4.2 (e)) may also have occurred, but was not captured. This speculation is based on the observed layer debonding that preceded complete failure during uniaxial tensile tests of the USL/CL [22].

While negligible biaxial creep was reported over a three-hour period for the porcine mitral valve (mean radial stretch increased from 1.36 to 1.38 and mean circumferential stretch increased from 1.05 to 1.06 at an equi-biaxial tension of 90 N/m) [30] and porcine aortic valve (mean radial stretch increased from 1.43 to 1.46 and mean circumferential stretch increased from 1.04 to 1.05 at an equi-biaxial tension of 60 N/m) [31], noticeable biaxial creep was found over a two-hour period for the swine USL/CL complex. We recorded a mean normalized strain of 1.34 and 1.41 in the parallel and perpendicular directions, respectively, at constant equi-biaxial loads of 2 N and a mean normalized strain of 1.20 and 1.33 in the parallel and perpendicular directions, respectively, at constant equi-biaxial loads of 4 N (Figure 4.10). The difference in the amount of biaxial creep between the heart valves and USL/CL complex is very likely due to the difference in structure, composition, and physiological function between these tissues. Creep of the USL/CL complex is significant and surgeons should consider possible changes in the length of these ligaments in reconstructive surgeries for the utero-vaginal prolapse (e.g., uterosacral ligament fixation). Of course, mechanical experiments need to be conducted on human ligaments to determine whether a comparable increase in strain over time under constant equi-biaxial loads can be observed. Nevertheless, given the similarities that exist between the human and swine USL and CL [22], we believe that the data collected in this study indicate that creep may alter the supportive role of the USL and CL.

Equi-biaxial loads of 2 N and 4 N were used on the swine USL/CL complex to generate relatively low and high stresses (Table 4.1). Higher normalized strain and creep rate were found at lower equi-biaxial loads in both loading directions (Figures 4.11 and 5.2). The normalized strain, especially at 115 min (Figure 4.13), and creep rate decreased with increasing stress, as reported for rat and rabbit medial collateral ligaments [13, 14]. Such decrease in creep and creep rate with an increase in stress can be explained by the fact that, as the stress increases, more fibers become straight and fewer fibers remain crimped, thus reducing the overall tissue’s deformation [28]. The difference between the normalized strain at 120 min at equi-biaxial loads of 2 N and the normalized strain at 120 min at equi-biaxial loads of 4 N was much larger for the parallel direction than the perpendicular one. A similar result was observed for the difference between the creep rate at equi-biaxial loads of 2 N and 4 N. Again, this could be due to the presence of more, straighter fibers in the parallel direction (Figures 4.6, 4.8, 4.9) and, hence, less fiber uncrimping at higher stresses.

We compared the creep behavior of the swine USL/CL complex under the same equi-biaxial
loads in the parallel and the perpendicular directions. The mean normalized strain at 120 min in the parallel direction was less than that in the perpendicular direction at both constant equi-biaxial loads of 2 N and 4 N. However, no significant difference in the mean normalized strain at 120 min and creep rate were detected between the two loading directions at both equi-biaxial loads. It is possible that, during equi-biaxial loading, some of the collagen fibers reorient making the difference in mechanical properties along the two loading direction insignificant. The slight increase in creep in the perpendicular direction may be explained by fewer and wavier fibers and, thus, more fluid movement in such direction.

The large variation reported for the normalized strain and creep rate data of the ligaments can be attributed to inter-animal and intra-animal variations (Figures 4.10-4.14). Indeed, in this study, the specimens for mechanical testing were collected from four different sows. Moreover, even in one of our previous studies [22], where the specimens were collected from a single sow, the uniaxial elastic properties of the USL and CL were found to be location-dependent. Unlike the creep tests, the SEM analysis was conducted on specimens isolated from a single sow and location within the USL/CL complex. This eliminated the intra-animal variation but specimens from several sows/locations need to be analyzed to obtain a more complete and definitive representation of the collagen fiber architecture.

4.5 Conclusions

This study presents the first characterization of the collagen fiber organization and biaxial creep properties of the swine USL/CL complex. In this ligamentous complex there were more and straighter collagen fiber bundles oriented along the main \textit{in-vivo} loading direction (the parallel direction) than along the direction perpendicular to it (the perpendicular direction). In both loading directions, the USL and CL specimens deforms substantially over time under constant equi-biaxial load. A difference was noted between creep in the parallel and perpendicular directions: creep was greater in the perpendicular direction than in the parallel direction. Moreover, creep proceeded significantly faster in the parallel direction, but not significantly faster in the perpendicular direction, for ligaments subjected to lower equi-biaxial loads versus higher equi-biaxial loads. Based on our micro-structural analysis, we speculated that fluid movement and fiber un-crimping determine the larger creep observed along the perpendicular direction and the higher creep rate at lower loads in the parallel direction. The creep properties of the USL/CL complex should be taken into account in the development of new surgical reconstruction methods, including mesh materials, for the treatment of PFDs.


[9] M.D. Barber, A.G. Visco, A.C. Weidner, C.L. Amundsen, R.C. Bump, Bilateral uterosacral ligament vaginal vault suspension with site-specific endopelvic fascia de-


5.1 Conclusions

The structural and tensile properties of two major ligaments, the CL and USL, of the uterus-cervix-vagina complex using the swine as an animal model were presented in Chapter 2. Our results suggest that the histology and passive mechanical properties of the swine CL and USL are very similar to those in humans. In light of this evidence, we believe that the swine may be a suitable animal model for pelvic floor research. This investigation provides crucial information on the material behavior of the supportive structures of the female pelvic floor.

The USL and CL are biological soft tissues containing SMCs. To characterize the active and passive mechanical responses of such tissues, a novel structural constitutive model was proposed in Chapter 3. The active response was attributed to the collagen fibers connected to the SMCs and the passive response was attributed to the remaining collagen fibers. The constitutive model was validated for now using uniaxial isometric and isotonic and biaxial isometric experimental data on arteries due to the lack of active data on the USL and CL. This study advanced our understanding of the active mechanical behavior of biological tissues containing SMCs.

The first characterization of the collagen fiber organization and biaxial creep properties of the swine USL/CL complex was presented in Chapter 4. Based on our micro-structural analysis, we speculated that fluid movement and fiber un-crimping determine the higher creep rate at lower loads in the main physiological loading direction. The creep properties of the USL/CL complex should be taken into account in the development of new surgical reconstruction methods, including mesh materials, for the treatment of PFDs.
5.2 Future Work

5.2.a Improve three-dimensional structural constitutive model for capturing the active and passive mechanics of the USL and CL

In the proposed structural constitutive model (Chapter 3), the collagen fibers and their connected SMCs in the arteries have been assumed to be all aligned along one direction when testing the model with uniaxial data or two symmetric directions when testing the model with biaxial data. In fact, the collagen fibers and SMCs are continuously distributed in multi-directions. Moreover, the orientation of the contractile units within the SMCs should also be taken into consideration in the model development [1]. Such orientation is important since the active force is generated along these units and transmitted, through filament anchor points on the cell membrane, to surrounding collagen fibers and ground substance [2]. To examine the orientations of the collagen fibers in the adventitia of carotid arteries, fluorescence collagen marker CNA38-OG488 and confocal laser scanning microscopy have been used [3]. In aortic medial lamellar unit, orientation of collagen bundles, interlamellar elastin fibers and SMCs were quantified by confocal laser scanning microscopy with naturally autofluoresce for collagen and elastin and fluorescence marker propidium iodide for SMCs [4]. Our future study will use confocal laser scanning microscopy with fluorescence dyes to investigate the micro-structure of the USL and CL, including the orientation of the collagen fibers and SMCs as well as the contractile units within the SMCs in these ligaments, for developing robust microstructural constitutive models.

In the proposed model, we developed an evolution law for the deformation of SMCs and connected collagen fibers. Following the sliding filament theory, in this evolution law the contraction force is the sum of a motor force that initiates contraction, a viscous force that describes the actin-myosin filament sliding, and an elastic force that accounts for the cross-bridge deformation. It is also interesting to study the mechanical response of a single isolated SMC, specifically, the relation of the contraction force of an isolated SMC and its deformation [5, 6, 7, 8]. Knowledge about the microscopic mechanics of SMCs will help understanding the interaction between the SMCs and their surrounding fibers, and ultimately the macroscopic mechanics of biological soft tissues containing SMCs [2].

We have only tested the proposed model with active experimental data published on arteries. To the authors’ knowledge, no active mechanical data have been published on the USL and CL. In future, our lab plans to conduct isometric and isotonic tests on these ligaments. Isometric experiments will be conducted at constant stretches, recording the total stress and passive stress varying with time. Isotonic experiments will be performed at constant afterloads, collecting velocity changing with the total stress and stretch varying with time. These data will be used to assess the new structural constitutive model.
5.2.b Propose three-dimensional nonlinear viscoelastic model for anisotropic ligaments with large deformation

For capturing the viscoelastic response of biological soft materials, the quasi-linear viscoelastic (QLV) theory based on the linear superposition of the effects of the instantaneous elastic stresses (for stress relaxation) or strains (for creep) at all previous times was proposed [10]. In this theory, the stress-strain relationship was nonlinear. For creep, the dependence of the creep compliance on the stress and time was assumed to be separable, thus this theory is only appropriate for materials whose creep rates are weakly dependent on the stress. However, the creep rate was found to be dependent on the stress levels in some biological collagenous tissues such as mice tail tendon bundles [11], human digital tendon [12], rat and rabbit medial collateral ligaments [13, 14].

In order to determine the creep nonlinearity of the USL and CL, isochronal stress-strain curves at different times in the two loading directions using biaxial creep data in Chapter 4 are plotted in Figure 5.1. Indeed, the tangent modulus of the isochronal stress-strain curves was dependent on the stress in both loading directions. This is more clear in the perpendicular loading direction. For investigating the stress-dependency of the creep rate, the mean normalized strain and time data were plotted using logarithmic scales in Figure 5.2. The slopes of the resulting curves were different at different stresses, indicating that the creep rate was dependent on the stress. More specifically, the creep rate decreased as the stress increased. However, large inter-specimen variation in strains was noticed. Therefore, to better characterize the creep nonlinearity of these ligaments, creep data under more stress levels should be collected on the same specimens. For reducing loading history effects on each specimen, the ligaments will be allowed to recover and strain measure will be performed between consecutive creep tests.

In future, we will conduct nonlinear analysis of the creep in the USL and CL, as done in Figure 5.1 and Figure 5.2, using additional creep data. Microstructural data showing the collagen fiber distribution in these ligaments will be collected in future using the confocal laser scanning microscopy. We will develop a three-dimensional nonlinear viscoelastic structural constitutive model based on the nonlinear characterization and microstructural data for anisotropic biological soft tissues with large deformations. Biaxial creep data of specimens subjected to various stress levels will be used to assess the new model.
Figure 5.1: Mean isochronal stress-strain data at $t = 0$, $t = 2.5$, $t = 12.5$, $t = 115$ min for $n = 25$ specimens (a) along to the main in-vivo loading direction and (b) perpendicular to the main in-vivo loading direction.
Figure 5.2: (a) Mean normalized strain over time computed over \( n = 5 \) specimens for a stress (mean±S.D.) of 0.105±0.013 MPa, \( n = 8 \) specimens for a stress of 0.132±0.014 MPa, \( n = 8 \) specimens for stress of 0.244±0.068 MPa, and \( n = 4 \) specimens for a stress of 0.356±0.038 MPa along the main in-vivo loading direction. (b) Mean normalized strain over time computed over \( n = 6 \) specimens for a stress of 0.102±0.016 MPa, \( n = 6 \) specimens for a stress of 0.130±0.010 MPa, \( n = 8 \) specimens for a stress of 0.238±0.047 MPa, and \( n = 4 \) specimens for a stress of 0.361±0.046 MPa along the direction that is perpendicular to the main in-vivo loading direction.


