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Experimental and numerical studies of soft biological tissues

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Abstract

The female pelvic region comprises one of the most complex regions of the human anatomy. However, this region remains understudied from a biomechanical perspective. The need to augment the available knowledge on this region of the human body becomes more pressing due to the tendency of life expectancy to increase (specially in the developed world). Age is an important issue on the etiology of pelvic floor disorders such as pelvic organ prolapse and urinary incontinence. This situation has led in the last few years to multidisciplinary efforts in the form of mixed collaborations of engineers and medical doctors. These contributions are split between major domains:

- Determination of the biomechanical and physical properties of the tissues, structures and prostheses (used in prolapse and incontinence surgeries) of the female pelvic cavity.
- Elaboration of material models (hyperelastic, hyper-viscoelastic), adequate to describe the nonlinear mechanical behavior of the soft tissues from the female pelvic cavity.
- Simulation of the geometry and static/dynamic behaviors of the pelvic components (including the effect of corrective surgeries) using numerical techniques.

Ideally this last component includes the knowledge produced with the previous two.

This thesis addresses an experimental and analytical work on soft tissues of the female pelvic cavity. An experimental set-up was built and an intensive testing program was conducted to obtain the mechanical properties of the female pelvic cavity. Based on the mechanical properties a model for soft vaginal tissue was developed, on the basis of an hyperelastic concept. The work aims to help practitioner medical doctors to choose prosthesis in order to match the real tissue properties.

Résumé

La cavité pelvienne de la femme est l'une des plus complexes régions de l'anatomie humaine. Cependant, c'est l'une des moins étudiées du point de vue biomécanique. La nécessité d'augmenter cette connaissance est plus urgente lorsque la population vieillit parce que l'âge est d'une importance particulière dans l'étiologie des affections telles que l'incontinence et le prolapsus, en particulier dans les pays plus développés. Cela a conduit à l'émergence d'équipes de recherche multidisciplinaires, composées de médecins et ingénieurs. Ces efforts ont des objectifs fondamentaux :

- Détermination des propriétés biomécaniques des structures de tissus et de prothèses (utilisées dans la correction chirurgicale de prolapsus et incontinence) associées à la cavité pelvienne de la femme.
 - Modélisation du comportement des matériaux (hyper, hyper-viscoélastique, etc.) appropriées à l'étude du comportement non linéaire des tissus de la cavité pelvienne.
 - Simulation de la géométrie et du comportement statique et dynamique des structures de la cavité pelvienne, à l'aide des éléments finis ou d'autres techniques appropriées.
- Idéalement, le dernier objectif réunira la connaissance des propriétés mécaniques bien comme les modèles constitutifs les plus appropriés pour les tissus à étudier.

Dans cette thèse on a effectué une étude expérimentale, numérique et analytique des tissus de la cavité pelvienne féminine. Un set-up expérimental a été construit et un extensif programme expérimental a été conduit pour obtenir les propriétés mécaniques de la cavité pelvienne féminine. Avec ces propriétés, on a développé un modèle pour les tissus souples vaginales, dans un environnement hyperélastique. Ce travail cherche l'aide aux médecins pour mieux choisir des prothèses plus adaptées aux tissus réels.

Resumo

A cavidade pélvica feminina é uma das regiões mais complexas da anatomia Humana, no entanto permanece como uma das menos estudadas do ponto de vista biomecânico. A necessidade de aumentar o corpo de conhecimento existente em relação à cavidade pélvica feminina torna-se mais evidente na medida em que o envelhecimento da população, factor de especial importância na etiologia de patologias como o prolapso e a incontinência urinária, se torna uma realidade, em especial nos países mais desenvolvidos. Esta situação levou ao aparecimento de equipas de investigação multidisciplinares, compostas por médicos e engenheiros. Estes esforços têm por objectivos fundamentais:

- a determinação das propriedades biomecânicas dos tecidos, estruturas e próteses (usadas em cirurgias de correcção de prolapso e incontinência) associados à cavidade pélvica feminina.
- a elaboração de modelos de comportamento material (hiperelásticos, hiper-viscoelásticos, etc.) adequados ao estudo do comportamento não linear dos tecidos da cavidade pélvica.
- a simulação da geometria e do comportamento estático e dinâmico das estruturas da cavidade pélvica (incluindo o efeito de cirurgias correctivas), recorrendo a técnicas numéricas.

Idealmente esta última componente reunirá tanto o conhecimento das propriedades mecânicas como os modelos constitutivos mais adequados aos tecidos a estudar.

Esta tese compreende um estudo experimental e analítico sobre tecidos moles da cavidade pélvica feminina. Foi desenvolvido um sistema experimental que permitiu realizar um programa de ensaios intensivo para obter as propriedades mecânicas dos tecidos que constituem a cavidade pélvica feminina. Com base nestas propriedades mecânicas foi desenvolvido um modelo analítico para tecidos vaginal prolapsados, no contexto de uma formulação hiperelástica. Para além da recolha de um extenso conjunto de dados sobre as propriedades mecânicas, este trabalho permitirá na prática clínica, uma melhor selecção das próteses para uso em cirurgias de correcção de prolapsos.

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Nomenclature

Δl	Elongation
λ_U	Ultimate stretch
$\mathbf{b}$	Left Cauchy-Green tensor
$\mathbf{C}$	Right Cauchy-Green tensor
$\mathbf{F}$	Deformation gradient tensor.
$\mathbf{F}$	Gradient of deformation tensor
ρ	Density
σ	Engineering stress
σ_Y	Yield strength
σ_{max}	Tensile strength
ε	Engineering strain
ε_n	Normalized mean square root error
d_U	Ultimate displacement

E	Modulus of elasticity, or Young's modulus
E_s	Secant modulus
E_t	Tangent modulus
L_U	Ultimate Load
R^2	Coefficient of determination
SEM	Standard Error of the Mean
U_s	Strain energy density
F	Load
BMI	Body mass index
DIC	Digital image correlation
FEM,FE	Finite element method
LM	Levenberg-Marquardt minimization algorithm
LVDT	linear variable differential transformer
POP	Pelvic organ prolapse
PP	Polypropylene
RL	Round ligaments
ROI	Region of Interest
SUI	Stress Urinary incontinence

UI	Urge Incontinence
USL	Uterosacral ligaments
VA	Vaginal Atrophy

Chapter 1

Introduction

1.1 Objectives

The female pelvic region comprises one of the most complex regions of the human anatomy. However, this region remains understudied from a biomechanical perspective [Ashton-Miller and DeLancey, 2007]. Dysfunctions in the genital and rectal support, as well as urinary and fecal continence and pelvic pain are common problems for adult women [Davila et al., 2006, Ch. 1-1]. The prevalence of pelvic floor disorders increases with age [Zimmern et al., 2006, Ch. 2]. Therefore, the tendency of life expectancy to increase (specially in the developed world) [Christensen et al., 2009], will mean that in a near future, many more women will suffer from pelvic disorders. Projections from the United States Census Bureau, claim the number of American women aged 60 and over will almost double between 2000 and 2030. In this context, pelvic organ prolapse is an important issue in women's health care, occurring in up to 50% of parous women [Maher et al., 2008; Olsen et al., 1997]. The pathophysiology is multifactorial and complex, with collagen levels, vaginal child birth, advancing age, and increasing body-mass index as the most consistent risk factors [Jelovsek et al., 2007; Swift et al., 2005]. It is known that anatomical integrity is critical to the maintenance of pelvic floor function. Any change to the normal anatomy, may be a multifactorial phenomena, adding contributions such as collagen deficiency or vaginal birth induced pelvic trauma

[Jelovsek et al., 2007]. Some of these factors may change the normal biomechanical properties of the affected structures. A common approach followed in the field of health sciences whenever in such cases, is the use of animal models. However, the feasible animal models (rabbit, guinea pigs, sheep, etc) are quadrupedal, factor which differentiates significantly the morphology and physiology of their pelvic cavity from the humans [Abramowitch et al., 2009].

The need to augment the available knowledge on this region of the human body becomes more pressing as the costs involved are estimated in billions of dollars in developed countries: In the United States \$12.4 billion (1995 values) [Wilson et al., 2001]; In 1997, direct costs of pelvic organ prolapse surgery (only) were \$1012 million [Subak et al., 2001].

This situation has led in the last few years to multidisciplinary efforts in the form of mixed collaborations of engineers and medical doctors. These contributions are split between three major domains:

- I** Determination of the biomechanical and physical properties of the tissues, structures and prostheses (used in prolapse and incontinence surgeries) of the female pelvic cavity [Goh, 2002, 2003; Kauer, 2001; Epstein et al., 2007; Mazza et al., 2006; Epstein et al., 2008b; Cosson et al., 2003a, 2004; Boreham et al., 2002a; Boukerrou et al., 2004; Fu et al., 1995; Dietz et al., 2003; Cosson et al., 2003b; Afonso et al., 2008, 2009; Calvo et al., 2009; Martins et al., 2010b; Peña et al., 2010].
- II** Elaboration of material models (hyperelastic, hyper-viscoelastic), adequate to describe the nonlinear mechanical behavior of the soft tissues from the female pelvic cavity [Aulignac et al., 2005; Calvo et al., 2009; Martins et al., 2010b; Peña et al., 2010].
- III** Simulation of the geometry and static/dynamic behaviors of the pelvic components mostly by means of Finite element simulations [Chen et al., 2006; Ashton-Miller and Delancey, 2009; Parente et al., 2008; Parente, 2008; Zhang et al., 2009a,b; Martins et al., 2006a; Constantinou, 2008; Haridas et al., 2006].

Despite the efforts regarding the determination of biomechanical and physical properties of the tissues from the pelvic cavity, there are not enough experimental results to produce a steady and complete knowledge of the mechanical behavior. Also a reliable relation between the constitutive models and experimental results is missing. These two issues have a significant impact in the accuracy and consequently on the practical usability of simulations (by Finite Elements or other means) for the clinical practice. To overcome these problems, it is very important to build a database of experimental results on the tissues from the female pelvic cavity, allowing researchers to:

- (a) Use accurate material properties of soft tissues during biomechanical simulation.
- (b) Build material models capable of mimic the nonlinear mechanical behavior of the soft tissues under a wide range of loads and stretches (ex.: during the birth or while coughing).
- (c) If strong correlations between the biomechanical properties and a particular pelvic problem can be established, use these relations as diagnostic tools in clinical practice.
- (d) Improve the usability, quality, comfort and success rates of the polypropylene meshes (or future prostheses) used in pelvic floor repair.

The main purpose of this thesis was to contribute for the body of knowledge on the female pelvic cavity, by filling some gaps on the biomechanical properties of soft tissues and polypropylene (PP) meshes (domain **I**) and on the understanding of their nonlinear mechanical behavior (domain **II**).

To achieve meaningful contributions on this domain, required a multidisciplinary effort between engineers and medical doctors. The partnerships established will be detailed next, in chronological order.

The first collaboration, which marks the beginning of the research, was established with Dr. Teresa Mascarenhas¹ (medical doctor). This collaboration included a research protocol with ethics committee approval and allowed our initial studies on the

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mechanical behavior of prolapsed vaginal tissue.

To model the nonlinear (hyperelastic and visco-hyperelastic) mechanical behavior, as well as the damage phenomena of vaginal tissues, it was fundamental the partnership established with Dr. Begoña Calvo and Dr. Estefanía Peña (engineers) from University of Zaragoza².

The study of the mechanical and physical properties of prostheses used in pelvic surgery (PP meshes), was proposed by Dr. José Sebastião Afonso³ (medical doctor).

For the study of pelvic tissues from cadavers it was established a collaboration (and a research protocol with ethics committee approval) with Dr. Agostinho Santos and Dr. Liliana Santos⁴ (medical doctors).

The last partnership was established with Dr. Agnaldo Silva Filho⁵ (medical doctor), whose insight on pelvic floor problems and on the statistical interpretation of medical data can be appreciated in Chapter 5.

The most relevant contributions of this Ibero-American effort can be summarized as follows:

1. It was produced an extensive set of experimental results on soft biological tissues under mechanical loads (Chapter 5).
2. A very large number of tests on cadaver tissues was performed, in order to consistently characterize the tissues from the female pelvic cavity (Chapter 5).
3. A special device for the experimental set-up was developed to carry on all uniaxial tests on a wide range of biological soft tissues (Chapter 4).
4. A test protocol for the mechanical testing of biological soft tissues was created (Chapter 4).

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5. Non-contact imaging techniques were used to assess the evolution of strains, allowing a better understanding of the deformation phenomenon (Chapter 4).
6. The influence of the PP mesh on pelvic floor repair was investigated, by testing the mechanical and physical properties of various types of meshes (Chapter 5).
7. All experimental data presented in the current thesis was analyzed using custom software written by the author (Chapters 5).
8. Experimental results were analyzed using the Levenberg-Marquadt algorithm to fit nonlinear hyperelastic models (Chapter 6).
9. It was proposed an hyperelastic model to address the specificity and complexity of prolapsed vaginal tissue, based on the previous works of Dr. Peña and Dr. Calvo. The correlation between models and experimental results proved to be adequate (Chapter 6).
10. A visco-hyperelastic model for human prolapsed vaginal tissue was proposed. The correlation between models and experimental results proved to be adequate.
11. A damage model by Dr. Peña and Dr. Calvo for soft tissues was adapted for the study of prolapsed vaginal tissue. The correlation between models and experimental results proved to be adequate.

On the scope of this thesis, the following papers were published by the author and his colleagues in international peer reviewed journals:

1. Martins, P., Peña, E., Calvo, B., Mascarenhas, T., Doblaré, M., Natal Jorge, R., and Ferreira, A. (2010b). Prediction of nonlinear elastic behaviour of vaginal tissue: experimental results and model formulation. *Computer Methods in Biomechanics and Biomedical Engineering*, (Doi: 10.1080/10255840903208197)
2. Martins, P., Natal Jorge, R., Salema, C., Roza, T., Ferreira, A., Parente, M., Pinotti, M., Mascarenhas, T., Santos, A., Santos, L., and Silva-Filho, A. (2010a). Vaginal tissue properties versus increased intra-abdominal pressure: a biomechanical study. *Gyneco-*

logic and Obstetric Investigation, (Accepted)

3. Silva-Filho, A., Martins, P., Parente, M., Salema, C., Roza, T., Pinotti, M., Mascarenhas, T., and Natal Jorge, R. (2010). Translation of biomechanics research to urogynecology. *Archives of Gynecology and Obstetrics*, (Doi: 10.1007/s00404-010-1396-2)
4. Jorge, R. N., Ferreira, A., ao Manuel R. S. Tavares, J., Garbe, C., Gentil, F., Parente, M., and Martins, P. (2010). On the influence of mechanical behavior of the middle ear ligaments: A finite element analysis. *Journal of Engineering in Medicine*, (Accepted)
5. Peña, E., Calvo, B., Martínez, M., Martins, P., Mascarenhas, T., Jorge, R., Ferreira, A., and Doblaré, M. (2010). Experimental study and constitutive modeling of the viscoelastic mechanical properties of the human prolapsed vaginal tissue. *Biomechanics and Modeling in Mechanobiology*, 9(1):35–44
6. Calvo, B., Peña, E., Martins, P., Mascarenhas, T., Doblaré, M., Natal Jorge, R., and Ferreira, A. (2009). On modelling damage process in vaginal tissue. *Journal of Biomechanics*, 42(5):642–651
7. Afonso, J. S., Jorge, R. M. N., Martins, P. S., Soldi, M., Alves, O. L., Patricio, B., Mascarenhas, T., Sartori, M., and Girao, M. (2009). Structural and thermal properties of polypropylene mesh used in treatment of stress urinary incontinence. *Acta of Bioengineering and Biomechanics*, 11(3):27–33
8. Afonso, J., Martins, P., Girao, M., Natal Jorge, R., Ferreira, A., Mascarenhas, T., Fernandes, A., Bernardes, J., Baracat, E., Rodrigues de Lima, G., and Patricio, B. (2008). Mechanical properties of polypropylene mesh used in pelvic floor repair. *International Urogynecology Journal*, 19(3):375–380
9. Martins, P. A. L. S., Jorge, R. M. N., and Ferreira, A. J. M. (2006b). A comparative study of several material models for prediction of hyperelastic properties: Application to silicone-rubber and soft tissues. *Strain*, 42(3):135–147

Some of the work published does not appear explicitly in the thesis, because it falls outside its main core. There are also other works with animal (middle ear ossicles and ligaments), and human tissues (knee ligaments and oral mucosa) left outside this document. The author's criteria in this respect was the completeness and representativeness of those works.

1.2 Background

There's not much doubt that an "ideal" measurement of the biomechanical properties would be done in-vivo conditions. However, these techniques provide only indirect quantifications and inferences of the biomechanical properties. Therefore, the existing experimental works on the biomechanical properties of human female pelvic tissues, may be split in two major categories:

Cat. I Destructive techniques, that normally require a sample of the tissue, organ or structure to be severed from the patient [Goh, 2002, 2003; Cosson et al., 2003a, 2004; Boukerrou et al., 2004; Lei et al., 2007; Rubod et al., 2008]. These techniques include uniaxial and biaxial tensile tests, compression tests, fatigue tests, etc.

Cat. II Nondestructive techniques, that try to infer the mechanical properties *in situ*, using techniques such as imaging, suction or microindentation, that are in general less invasive [Constantinou, 2008; Epstein et al., 2008b,a; Mazza et al., 2006; Tay et al., 2006].

The mechanical properties of the modeled tissues constitute key ingredients to achieve realistic simulations [Peña et al., 2010]. To obtain these mechanical properties, tissue excised during surgery or from cadaver has been tested using several techniques: uniaxial tensile tests either at constant [Boukerrou et al., 2004] or variable strain rates [Ettema et al., 1998] and also biaxial (equibiaxial) tension tests [Janda, 2006]. All these contributions do not imply that the subject, the biomechanical properties of the female pelvic cavity tissues, has been extensively studied. It is still true the consideration from Ashton-Miller and DeLancey [2007] that "The female pelvic floor is an understudied region of the body from a biomechanical perspective."

From all the research papers cited, the work from Lei et al. [2007] is the most complete in terms of the studied population and the biomechanical properties presented. However it only considers prolapsed and non-prolapsed vaginal tissue. In terms of the variety of structures studied, the research of Cosson et al. [2003a] on pelvic ligament

strength (iliopectineal ligament, sacrospinous ligament and arcus tendineus of pelvic fascia) is an important contribution. However, this work only considers pelvic ligaments. The only experimental study found concerning a direct measurement (uniaxial test) of biomechanical properties in human bladder was from Susset and Regnier [1981]. These limitations are common to all research efforts prior to this thesis.

To achieve a deeper characterization of the pelvic cavity tissues in terms of biomechanical properties, on the scope of this thesis, seven different tissues were tested:

- Vagina - anterior and posterior wall. From cadavers, patients without known pelvic pathologies and from patients with pelvic organ prolapse (POP).
- Bladder wall. From cadavers.
- *Levator ani* muscle. From cadavers.
- Ligaments, Round and Uterosacral. From cadavers.
- *Scarpa's* fascia (anterior abdominal wall). From cadavers.

The supports (ligaments), organs (vagina, bladder) and musculature (*levator ani*) of the pelvic cavity are studied simultaneously. In this respect the thesis can be considered ground breaking, since all (as far as the MedLine search went) previous research lacked this integrated perspective.

1.3 Thesis outline

This thesis is organized in 7 Chapters and 3 Appendices. Each Chapter was intended to allow the reader a comprehension of its core subject, having in mind its contributions to the thesis overall understanding. Some technical subjects required for the thesis comprehension, but not belonging to the body of knowledge produced during the thesis preparation were included as Appendices.

Chapters 1 to 3 give the reader an overview of the materials studied and the problems involving those materials.

Chapters 4 to 7 concern the methods and strategies employed, as well as the results and conclusions achieved.

Chapter 1 introduces the thesis subject. The problem studied is presented in the context of its broader domain i.e., in the body of knowledge of the Biomechanical Properties of Soft Biological Tissues. Some of the issues concerning the soft tissues of the Female Pelvic Cavity, the materials studied in the thesis, are presented to the reader in this Chapter. The last Section presents the thesis outline with a brief explanation of its structure.

Chapter 2 provides the reader with a background on the Anatomy, function and problematics (Section Pathologies) associated with female pelvic cavity tissues. The internal anatomy of the pelvis and its major functions are presented in Section 2.2. The pathologies associated to the pelvic cavity, in particular those related with pelvic organ prolapse (POP) are discussed in Section 2.3.

Chapter 3 introduces the 4 basic types of tissue found in the human body. These are the fundamental building blocks of the tissues and organs referred throughout the thesis. The structure of the tissues tested (Bladder wall, *Scarpa's* fascia, Round ligaments, Uterosacral ligaments, *Levator ani* muscle, Anterior and Posterior vaginal wall) is then presented. In the case of vaginal tissue, the morphological data collected (from histologies) is presented as well.

Chapter 4 concerns the Mechanical testing of Soft Biological Tissues. The Materials Science concepts and definitions considered fundamental to explain the processing of experimental data, directly from the sensor output logs (load and displacement), are explained. These concepts are applied to the experimental results of the Uniaxial Tensile test (Section 4.2). The experimental techniques most suited for strain measurement in highly deformable materials are discussed, as well as the technical problems arising from their application (Section 4.3). The Mechanical Test solutions applied in the thesis are explained in detail (Section 4.4), stressing the adaptations required for the study of the tissues under consideration. The chapter ends with a qualitative discussion, based on the analysis of histologies, on the effects of the uniaxial tensile test to the micro-structure of some of the tested tissues (Section 4.5).

Chapter 5 contains the experimental data (Tables 5.9 to A.1). This data may be analyzed from different perspectives, given the multidisciplinary scope of this thesis. Therefore, engineering and in some cases clinical analyses were conducted.

The first study is “The influence of POP (Pelvic Organ Prolapse) on the biomechanical properties of vaginal tissue” (Section 5.2). The variations of the biomechanical properties (Section 4.2.1 and Table 5.1) in cadaver (“normal” vaginal tissue) and vivo (prolapsed vaginal tissue) populations were investigated. A clinical interpretation of the results was proposed. The second study is the “Uniaxial mechanical behavior of Human female bladder” (Section 5.3). It was not possible to obtain bladder samples from living patients. Therefore, thirteen bladder tissue samples from female cadavers without known pelvic disorders were tested. The mechanical tests were analyzed and a clinical interpretation of the results was produced. The third study “Round and Uterosacral Ligaments Strength” investigates the tensile biomechanical properties of Round and Uterosacral ligaments. The ligament samples were obtained from fifteen female cadavers without known pelvic disorders. The mechanical tests were analyzed and a clinical interpretation of the results was produced. The fourth study considered [Afonso et al., 2008], compares the biomechanical properties (tensile stiffness and flexural stiffness) of different meshes used in urogynecological surgery, and one standard mesh. Two mechanical tests were carried out. Five different commercial meshes were considered. A clinical interpretation of the experimental results was presented.

Chapter 6 presents a path from the nonlinear theory of elasticity to the constitutive modeling of biological soft tissues [Martins et al., 2006b]. Both isotropic and transversely isotropic hyperelastic materials, under uniaxial and biaxial loading conditions are considered.

The numerical results of the hyperelastic model proposed [Martins et al., 2010b] are presented. The ability of the model to predict the nonlinear mechanical behavior of prolapsed vaginal tissue was discussed. The prospective impact of the model on the study of the female pelvic cavity was discussed.

Chapter 7 contains the general conclusions extracted from the thesis and possible pathways for future research.

Chapter 2

Anatomy and pathologies of the female pelvic cavity.

2.1 Introduction

This chapter provides to the reader a background on the anatomy, function and pathologies associated with the female pelvic cavity. These notions may allow a better understanding of the next chapters as they contextualize the problems addressed in the thesis on a broader perspective.

In Section 2.2 the internal anatomy of the pelvic cavity is presented to the reader. The organs and tissues of the internal pelvic cavity have special relevance on the thesis context. The *perinium* and the external *genitalia*, were not considered in this discussion. Some of the tissues and organs discussed were mechanically tested. Those tissues were: Uterosacral and Round ligaments, the vagina, the bladder and the levator ani muscle. The pathologies associated to the pelvic cavity, in particular those related with pelvic organ prolapse (POP) are discussed in Section 2.3.

2.2 Anatomy of the female pelvic cavity

An understanding of the normal anatomy of the pelvis provides a basis for understanding the function and support of pelvic organs [Ghoniem and Davila, 2006, Ch. I]. The general designation pelvis refers to several structures, including the pelvic girdle (bony pelvis) and the pelvic cavity. The pelvic cavity is the intra-abdominal cavity extending from the diaphragm cranially ¹ to the pelvic floor caudally².

2.2.1 Pelvic girdle

The three bones composing the pelvic girdle are the pubis, the ischium, and the ilium (Figure 2.1). There are differences between the pelvis of an adult male and that of an adult female (Figure 2.2). These differences reflecting mainly female's role in pregnancy and parturition, affect the morphology of the pelvic girdle in several ways. The major morphologic changes are summarized in Table 2.1. The pelvic girdle supports the

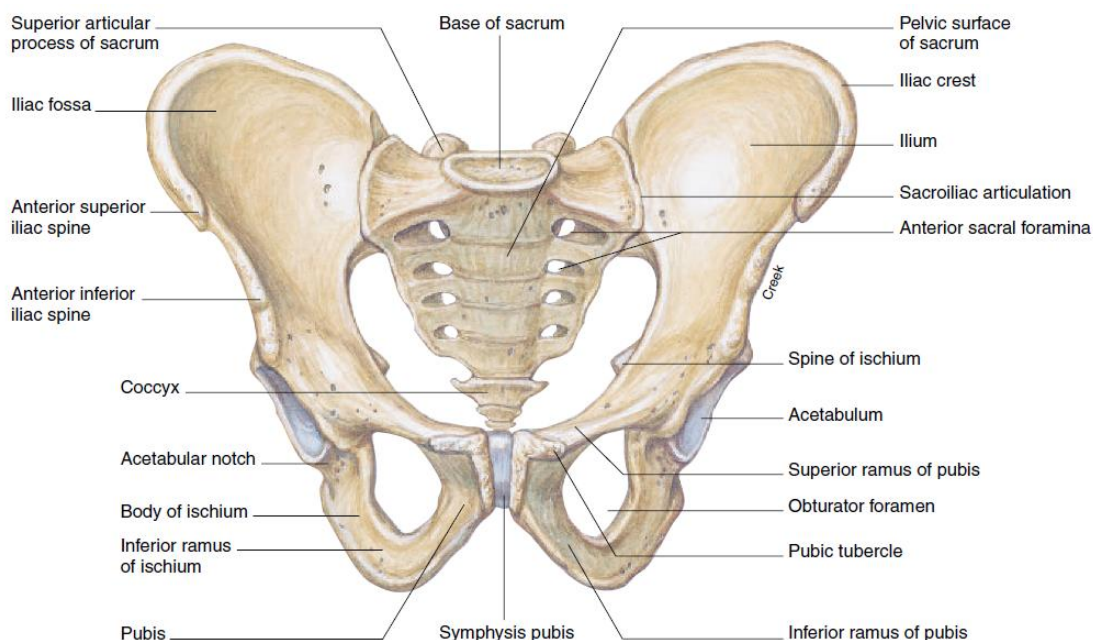


Figure 2.1: Anterior view of the pelvic girdle. Adapted from [Van de Graaff, 2001, Ch. 7]

¹Toward the head.

Dorland's Medical Dictionary for Healthcare Consumers. Elsevier. 2007. Retrieved 2010-04-30.

²Situated more toward the cauda, or tail, than some specified reference point; toward the inferior (in humans)

weight of the body through the vertebral column and protects the viscera within the pelvic cavity [Van de Graaff, 2001].

As seen in Figure 2.2, the pelvis is divided into a greater (false) pelvis and a lesser (true) pelvis. The greater pelvis protects the inferior abdominal viscera. The lesser pelvis, forms the walls of the birth canal. In a vaginal delivery, a full-term fetus must pass through its mother's lesser pelvis.

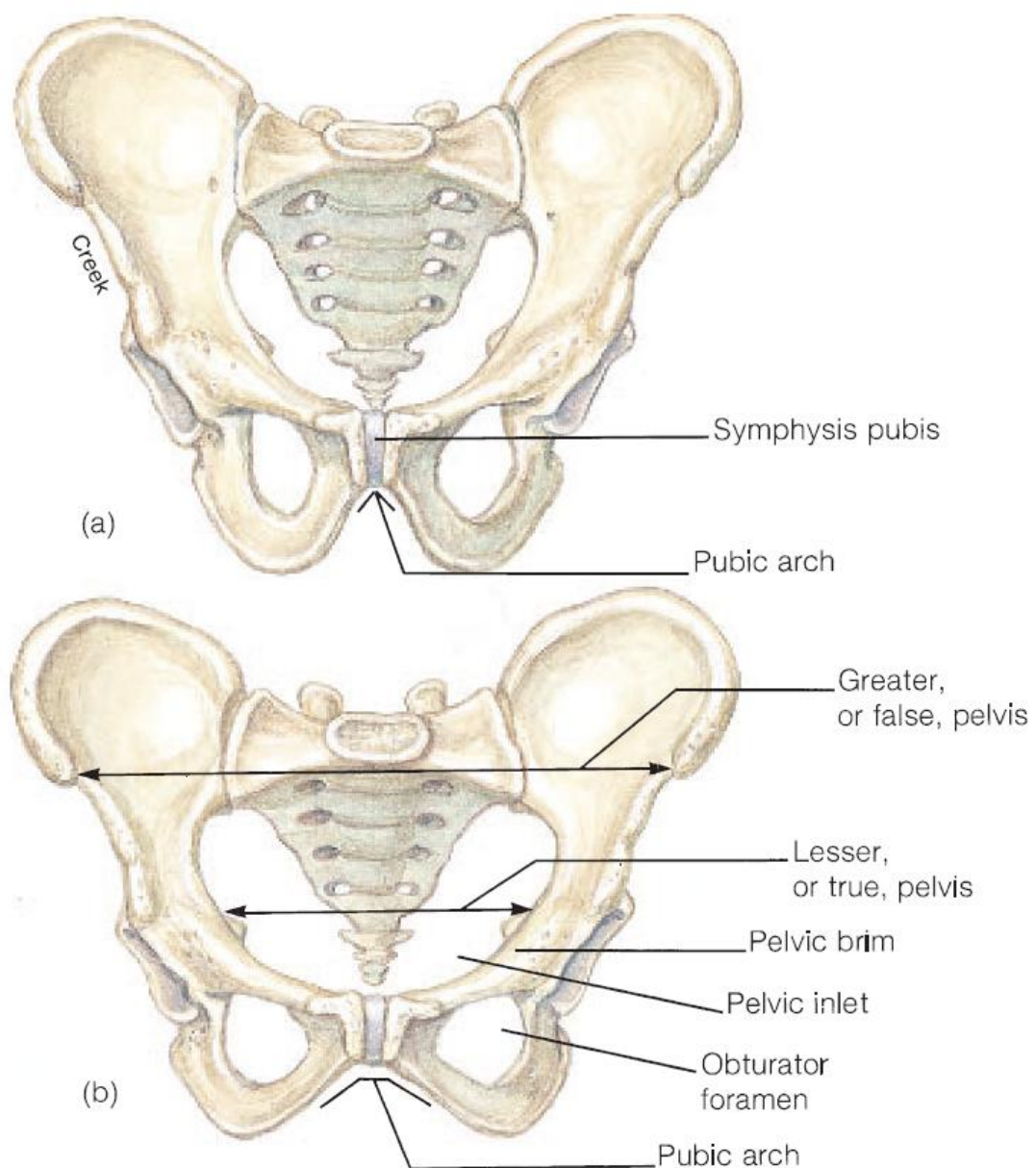


Figure 2.2: Differences between the (a) male and (b) female pelvic girdle. Adapted from [Van de Graaff, 2001, Ch. 7]

(a) Male Pelvis	(b) Female Pelvis
General Structure	
more massive	more delicate
Pelvic inlet	
heart-shaped	round or oval
Pelvic outlet	
narrower	wider
Pubic symphysis	
deeper, longer	shallower, shorter
Pubic arch	
angle less than 90 °	angle greater than 90 °

Table 2.1: Main differences between male and female pelvis. Adapted from [Van de Graaff, 2001, Ch. 7].

2.2.2 Pelvic cavity

The pelvic cavity contains some urinary and reproductive organs, terminal portion of digestive tract (intestines) [Van de Graaff, 2001]. The smooth anterior surface of the sacrum forms the posterior surface of the pelvic cavity (Figure 2.4). The inferior limit of the pelvic cavity (Figure 2.3) is composed by the musculofascial pelvic diaphragm, which is the boundary between the pelvis and the perineum. The structure assumes the shape of a basin and is called the pelvic floor. It is at the same time the roof of the perineum which includes the anus and external genitalia. This *floor* has to support the loads of the internal viscera i.e., the gravitational forces acting on the internal viscera responsible for the intra-abdominal pressure. In quadrupeds this pressure is supported mainly by the abdominal region. As human beings adopted a standing position evolving from quadrupeds to bipeds, the intra-abdominal pressure from the abdominal region was progressively transferred to the pelvic floor muscles and fascias [Ghoniem and Davila, 2006]. The muscles responsible for tail movement in quadrupeds are activated under voluntary contraction. Those same muscles turned into supportive structures for bipeds and they require a continuous involuntary tonic contraction³. For bipeds, the pelvic floor muscles are also required to provide rapid voluntary contraction which is responsible for the bladder (urinary) and bowel (fecal) continence mechanisms. The pelvic cavity is delimited to the right and to the left (anterior view) by the hip bones (See Illium in Figure 2.1). These bones constitute the lateral walls of the cavity.

³The sustained contraction of a muscle, as is necessary for maintaining posture.

Pelvic floor

The pelvic floor is composed by the coccygeus and *levator ani* muscles (Figure 2.3). The *levator ani* is a thin sheet of muscle. It is composed of slow-twitch⁴, fatigue-resistant fibers that maintain constant tone and fast-twitch⁵ fibers that allow rapid contraction with sudden rises in intra-abdominal pressure [Ghoniem and Davila, 2006, Ch. 1]. The *levator ani* provides support for the pelvic viscera. It also constricts the lower part of the rectum aiding defecation. The coccygeus muscle aids the *levator ani* in its functions.

The *levator ani* is subdivided into three muscles: *puborectalis*, *pubococcygeus* and *iliococcygeus*. The criteria for this division is based on the muscle fiber directions and their respective attachments to the surrounding structures (Figure 2.3). The *puborectalis* represents the medial and inferior fibers (also the thicker region) of the *levator ani* muscle that arise on either side from the pubic bone and form a U-shaped sling [Schorge et al., 2008, Ch. 38]. It is considered part of the anal sphincter complex meaning that it plays a role in fecal continence. The *puborectalis* can be voluntarily activated during “squeezing”⁶ [Petros, 2007, Ch. 2].

The *pubococcygeus* is attached to the walls of the pelvic viscera, therefore it is also called pubovisceral muscle. The portion of the fibers attached to the lateral walls of the vagina (called *pubovaginalis* muscle) contribute for the elevation of the urethra during a pelvic muscle contraction. This contribution has a potential role on the urinary continence mechanisms [DeLancey and Starr, 1990].

The *iliococcygeus* is attached laterally to the arcus tendineus and the ischial spines. It is the thinnest part of the *levator ani* muscle and also the most posterior (Figure 2.3). It is an important component of the *levator plate*⁷. The main role of the *iliococcygeus* in the pelvic cavity is to give support to the rectum, upper vagina, and uterus.

⁴Slow-twitch or Type I fibers. Because of their high myoglobin content, slowtwitch fibers are also called red fibers. These fibers are common in muscles able to sustain a contraction for a long period of time without fatigue [Fox, 2003, Ch. 12].

⁵Fast-twitch or Type II fibers, have fewer capillaries and mitochondria than slow-twitch fibers and not as much myoglobin; hence, these fibers are also called white fibers [Fox, 2003, Ch. 12].

⁶“Squeezing” refers to the action that occurs when a patient crosses her legs and contracts the pelvic muscles upwards

⁷Region between the anus and the coccyx formed primarily by the insertion of the *iliococcygeus* muscles [Schorge et al., 2008, Ch. 38].

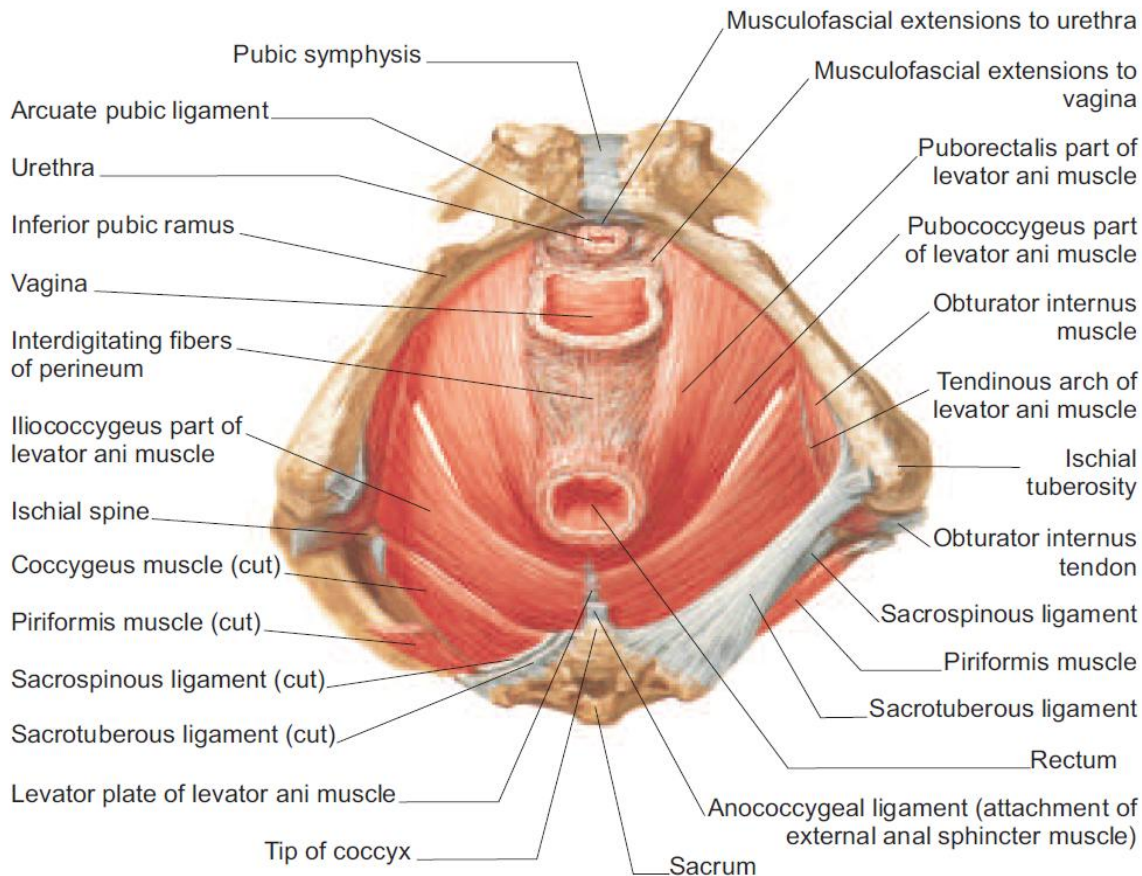


Figure 2.3: Pelvic floor diaphragm, inferior view. Adapted from [Netter, 2002]

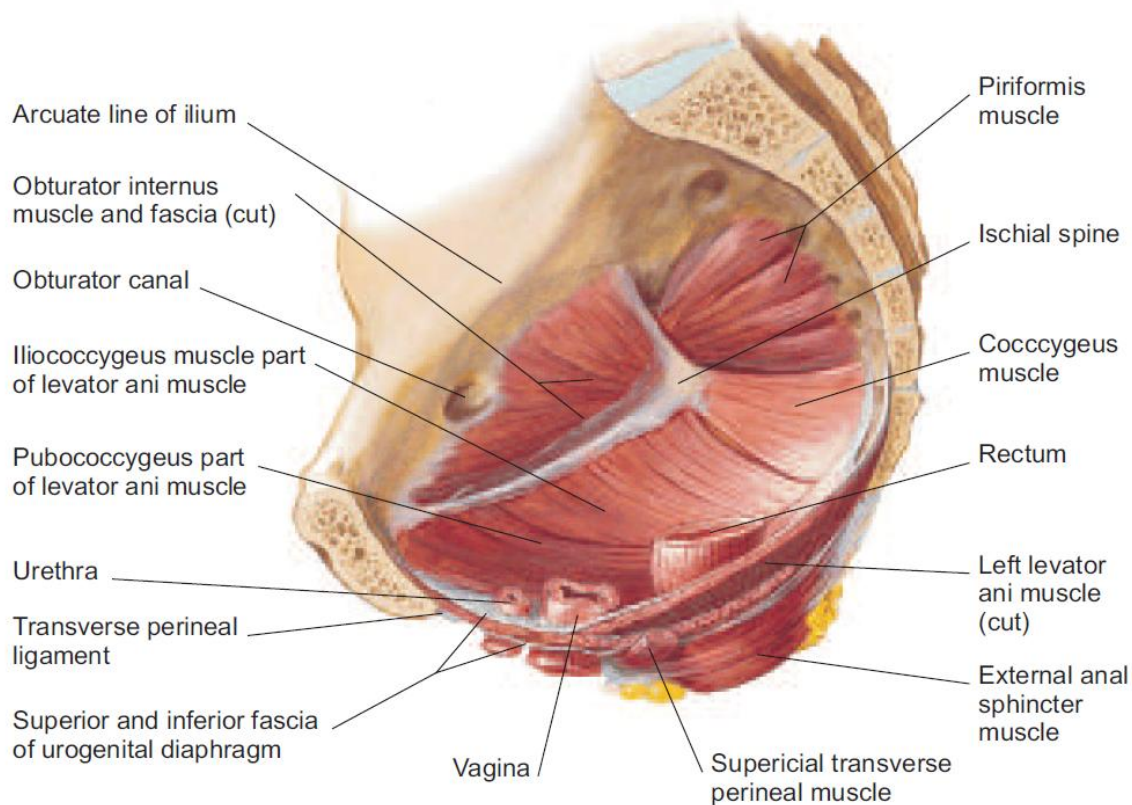


Figure 2.4: Pelvic floor diaphragm, medial view. Adapted from [Netter, 2002]

2.2.3 Pelvic connective tissue

The bladder, uterus, vagina, and rectum are attached to the lateral pelvic walls by a network of connective tissue (Figure 2.5), which is collectively called the endopelvic fascia [Davila et al., 2006, Ch. 4]. The endopelvic fascia is a visceral fascia i.e, it is in contact with the pelvic viscera and is anatomically and histologically different from parietal fascia, which invests most striated muscles [Schorge et al., 2008, Ch. 38]. The main composition of the endopelvic fascia are fibrocytes and fibroblasts, smooth muscle cells, collagen (mainly type I and III), elastin fibres, nerves, and blood vessels. It gives support to the pelvic organs, attaching them to the pelvic sidewalls [Ghoniem and Davila, 2006, Ch. II]. The endopelvic fascia represents the top layer of the pelvic floor.

The pelvis has a diversity of ligaments, with variable composition and function. These ligaments can range from dense connective tissue (those that support the bony pelvis and pelvic organs) with a significant support role, to smooth muscle, with small relevance for pelvic organ support [Schorge et al., 2008, Ch. 38]. The Uterosacral ligaments (USL) and the Round Ligaments (RL) are two of these ligamentous support structures. Since these anatomical structures were object of mechanical testing, they will be considered here. The uterus and cervix (Figure 2.6) are supported by the *levator ani* muscle and by Uterosacral ligaments and Cardinal Ligaments (Figure 2.5). These ligaments attach the walls of the cervix to the pelvic walls, in particular the cardinal ligaments which are bands of dense connective tissue. The direction of USL is vertical and provides a suspension of the upper vagina and cervix. USL are mainly composed of smooth muscle and connective tissue. USL attach to the sacrum posteriorly and are lateral boundaries for the *cul-de-sac* of Douglas (Rectouterine pouch of Douglas in Figure 2.6).

The round ligaments of the uterus (RL) (Figure 2.6) are smooth muscle extensions of the uterine body [Schorge et al., 2008, Ch. 38], which do not contribute significantly to uterine support. They emerge laterally from the uterine body and anterior to the origin of the fallopian tubes. RL extend laterally to the pelvic sidewall.

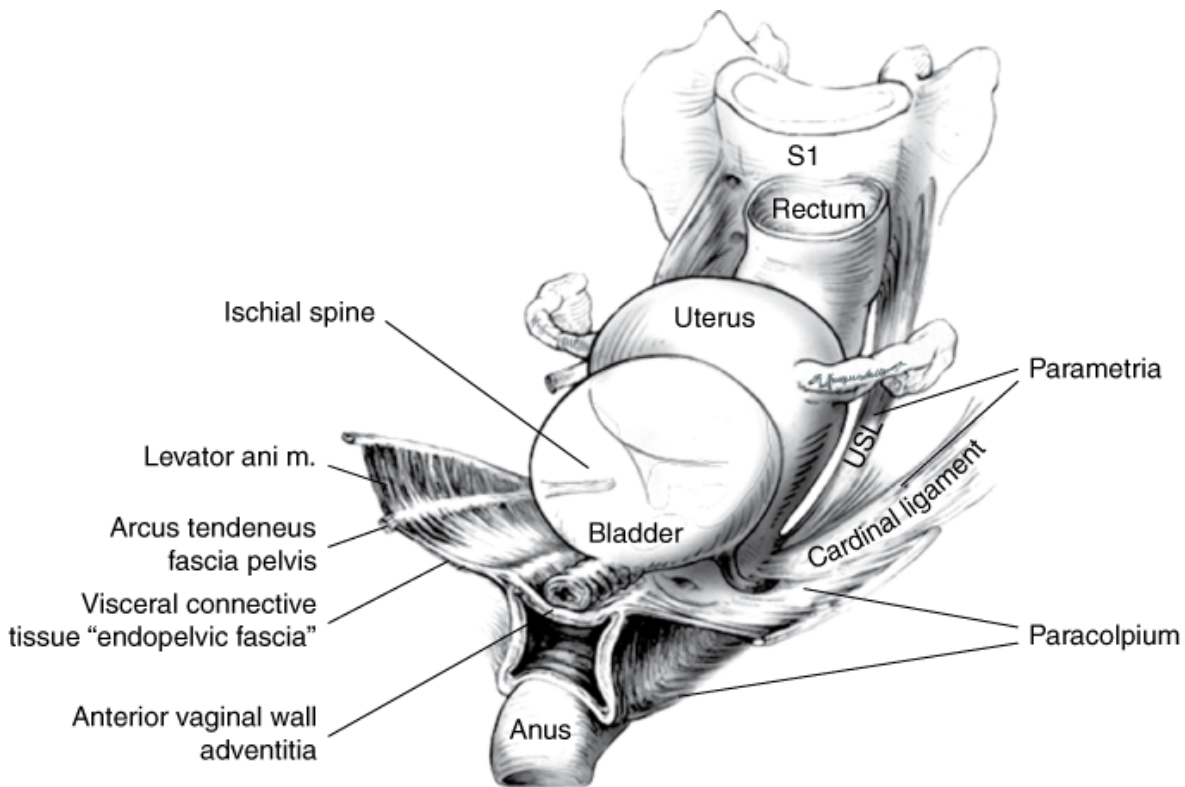


Figure 2.5: Endopelvic fascia and Uterosacral Ligament (USL); Pelvic viscera and their support. Adapted from [Schorge et al., 2008, Ch. 38]

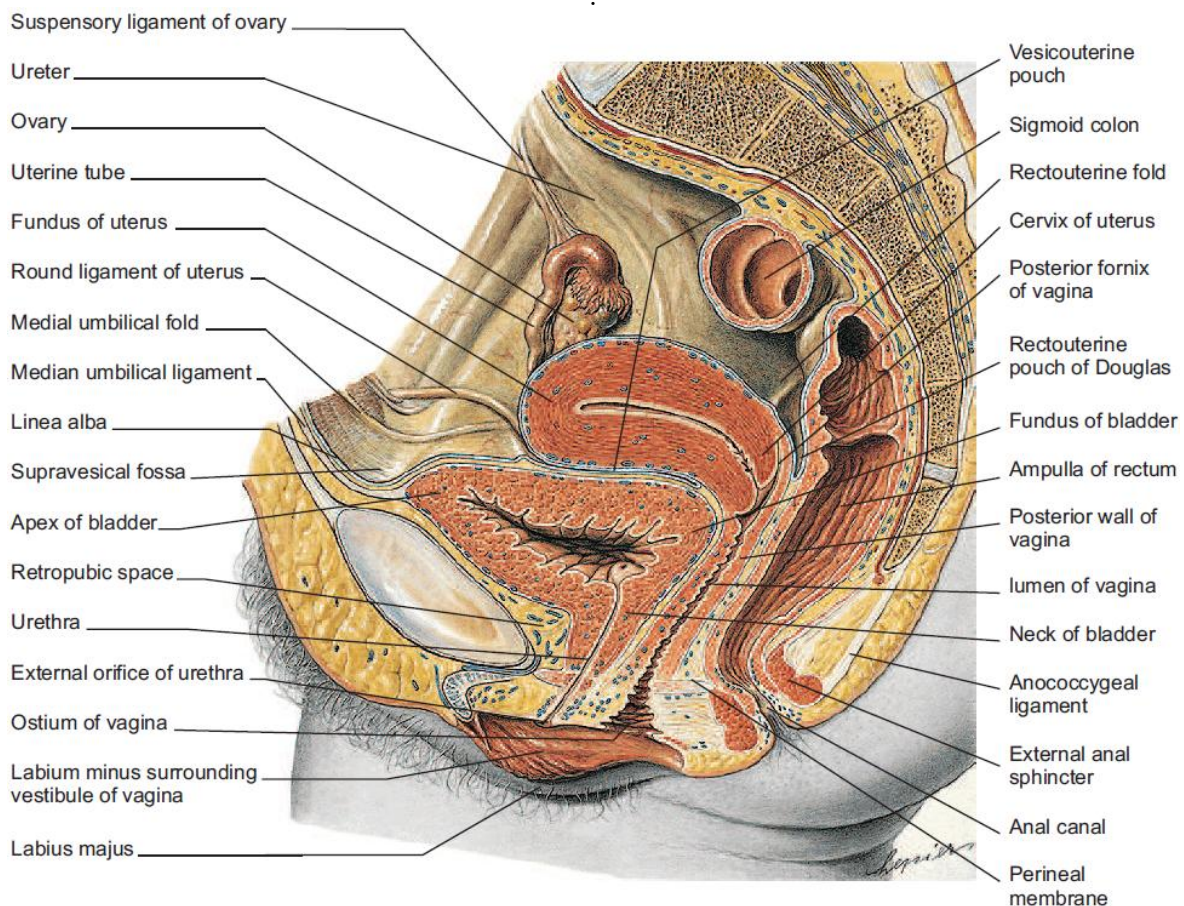


Figure 2.6: Genital viscera. Adapted from [Putz and Pabst, 2001]

2.2.4 Pelvic organs

The pelvic viscera include the urinary bladder and parts of the ureters, the reproductive system, the rectum, and the distal part of the alimentary tract. The sigmoid colon and parts of the small bowel also extend into the pelvic cavity. These structures are “compacted” inside the pelvic cavity. The understanding of their relations and interdependence in complex biological functions like the continence mechanisms is not fully understood.

Urinary organs

The components of the urinary system found in the pelvic cavity are the ureters, the bladder and the urethra (Figure 2.5↔2.7). Urine produced in the kidneys passes to the bladder through the ureters, to be expelled to the exterior through the urethra. **Ureters** are muscular tubes connecting the kidneys to the bladder. In the pelvic cavity, the ureter is closest to the uterosacral ligament at its distal end (approx. 1 cm). The ureter diverges laterally as the uterosacral ligament is traced toward the sacrum [Davila et al., 2006, Ch. 4-2].

Bladder is a hollow organ. Its main function is the storage and evacuation of urine. In the anterior region, the bladder rests against the anterior abdominal wall. In the posterior region, it rests against the vagina and cervix. In the inferior and lateral regions the bladder is in contact with the inner surface of the pubic bones. It is composed of smooth muscle (detrusor muscle), covered on its dome by a serosal layer and lined by a submucosa and transitional cell epithelium.

Urethra is a fibromuscular tube measuring 3↔4 cm. Posteriorly (distal⁸ part), the urethra is supported by the anterior vaginal wall, anteriorly it is fused with it (Figure 2.6). In the anterior region, the urethra is kept in suspension between the pubic bone and the anterior vaginal wall by the pubourethral ligaments. The endopelvic fascia connects it laterally to the pubovisceralis muscle. This suspension mechanism is a way of preventing urethral opening due to a surge of intra-abdominal pressure.

⁸The more (or most) distant of two (or more) things.

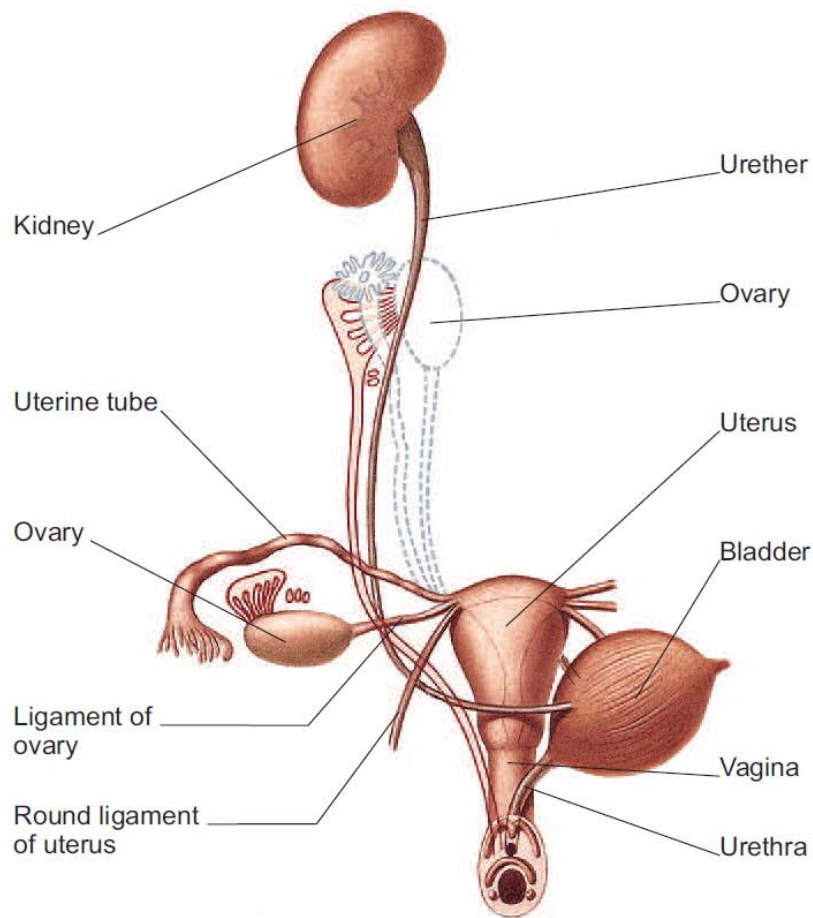


Figure 2.7: Urogenital viscera. Adapted from [Putz and Pabst, 2001].

Internal genital organs

The female internal genital organs (Figure 2.7) are the Ovaries and Fallopian Tubes, the vagina and the uterus. These organs form the genital tract where the process of reproduction as whole takes place. The Ovaries produce and release the eggs that can move down the genital tract. The Fallopian tubes, also called oviducts is where the sperm can fertilize an egg (fertilization). The vagina, is where sperm are deposited during sexual intercourse and starts to travel up the genital tract. It is also part of the birth canal. Finally the Uterus is where an embryo develops into a fetus until the pregnancy term.

Ovaries and Fallopian Tubes are known as uterine *adnexa*. The Ovaries suffer significant changes both in size and hormonal activity with age and menstrual cycle. The suspensory ligament of the ovary provides the lateral attachment to the pelvic

wall, and the utero-ovarian ligament attaches the medial portions of the ovaries to the uterus.

The fallopian tubes are tubular structures extending from the ovaries to the uterus. They are active organs able to move the egg from the ovary to the uterine cavity. If the egg is fertilized by sperm it travels through the tube until it is ready for implantation in the uterus.

The **Vagina** is a muscular organ, tubular in shape. The average length (there is some variability) of the anterior and posterior vaginal walls are 7 and 9 cm respectively. It lies in a central position on the female pelvis and its anterior and posterior walls are in contact (except at its superior end) so that a vaginal cross section is H shaped as shown in Figure 2.5. The anterior vaginal wall lies posterior to the urethra and urinary bladder. It is anterior to the rectum. The structure of the vaginal walls is discussed in the next chapter.

The **Uterus** can be divided into a pearshaped body and cervix. It is a fibromuscular hollow organ situated between the bladder and the rectum. The uterine body narrows from the *fundus*, which is the portion of the uterine body (or *corpus*) that extends above the level of entry of the fallopian tubes to the endometrial cavity. The Fallopian (or uterine) tubes carry the eggs from the ovaries to the uterine cavity on both sides of the *fundus*. Parity and estrogen stimulation have a significant influence on the dimensions, shape and weight of the uterus. As mentioned before, the cardinal ligaments and the uterosacral ligaments are responsible to attach the uterus to the pelvic walls. The inner layer of the uterus is a mucosa called the endometrium. This layer forms the inner cavity of the uterus (endometrial cavity) and changes with the menstrual cycle. The external thick muscular wall is called the myometrium.

Rectum

The **rectum** (Figure 2.4) and the anal canal constitute the terminal digestive tract. The rectum accumulates and stores the feces temporarily, while the anal canal (approximately 4 cm long) maintains continence. The success of the anal continence process depends largely upon the anal sphincter. Its inner part, the internal anal sphincter, is responsible for 80% of resting pressure. On the external anal sphincter rely the other

20%, as well as the generation of rapid voluntary contraction. The upper part of the external anal sphincter is fused with the puborectalis muscle. The superficial region of the external anal sphincter (Figure 2.4) is a ring wrapped around the terminus of the internal anal sphincter.

2.3 Pathologies related with female pelvic floor

Dysfunctions in the genital and rectal support, as well as urinary and fecal continence and pelvic pain are common problems for adult women [Davila et al., 2006, Ch. 1-1]. The prevalence of pelvic floor disorders increases with age [Zimmern et al., 2006, Ch. 2]. Therefore, the tendency of life expectancy to increase (specially in the developed world) [Christensen et al., 2009], will mean that in a near future, many more women will suffer from pelvic disorders. It was estimated by the United States Census Bureau that the number of American women aged 60 and over will almost double between 2000 and 2030. Much of the information related to urinary incontinence (Section 2.3.1) is extended to pelvic organ prolapse (Section 2.3.2), as the former is often a consequence of the later.

Pelvic floor disorders constitute the driving force behind most of the research efforts towards a better understanding of this body region. In this sense, a basic knowledge on the subject may prove useful to researchers.

All pelvic disorders covered, were selected according to their relevance on the thesis context. Urinary incontinence and pelvic organ prolapse were discussed with this purpose. Other female pelvic disorders such as,

- Frequency of Urination. More than eight times per day.
- Nocturia. More than two times per night.
- Abnormal Bladder Emptying. Difficulty in bladder evacuation.
- Chronic Pelvic Pain. Pain of varying severity.

although important in terms of their prevalence and quality of life impact, fall outside the scope of the thesis.

2.3.1 Urinary incontinence

Among the pathologies (or dysfunctions) commonly associated to the female pelvic floor, urinary incontinence⁹ is the best known [Petros, 2007, Ch. 1]. The reports on the prevalence of urinary incontinence in women vary between 10 and 60%. Urinary incontinence rates are higher in older women and in those confined to nursing homes [Vasavada et al., 2004, Ch. 3]. In the USA alone, the annual direct cost associated with female urinary incontinence was estimated in \$12.4 billion (1995 values) [Wilson et al., 2001]. There are two main types of urinary incontinence, Stress Urinary Incontinence (SUI) and Urge Incontinence (UI). There are many risk factors identified with urinary incontinence [Vasavada et al., 2004, Ch. 3]. These risk factors in some cases are also associated with the prevalence of pelvic organ prolapse (POP).

Increasing age: There is an increasing prevalence and severity of urinary incontinence with advanced age. This may be due to age-related laxity of pelvic musculature and connective tissue supporting the urethra.

Obesity: High body mass indices (BMIs) have been correlated with an increased risk for urinary incontinence in women [Moalli et al., 2003].

Hysterectomy: According to Moalli et al. [2003], gynecologic surgery, especially hysterectomy and previous prolapse surgery, predispose a woman to develop incontinence and/or prolapse. In other study, Swift et al. [2001] quantify the risk increase of developing further prolapse after a prior prolapse surgery as 500% greater than in the general population. However, data on the risk hysterectomy confers on the development of subsequent urinary incontinence are conflicting. There has not been established a definitive and completely clear connection between incontinence and hysterectomy.

Pregnancy and Parity: Some studies suggest that parity is a risk factor for urinary incontinence [Dietz, 2006]. However, according with Lukacz et al. [2006], “The risk of pelvic floor disorders is independently associated with vaginal delivery but not with

⁹Definition: involuntary urine loss

parity alone. Cesarean delivery has a protective effect, similar to nulliparity, on the development of pelvic floor disorders when compared with vaginal delivery”. The Use of vacuum extraction or episiotomy during delivery was found to increase the risk of stress urinary incontinence [Vasavada et al., 2004, Ch. 3]. A possible explanation of the connection between pregnancy/parity and incontinence is pelvic floor denervation due to compression during pregnancy and delivery. The stretching or tearing of pelvic floor muscles (*levator ani* complex) and connective tissues is another relevant factor.

Menopause: It is known that in postmenopausal women the incidence of severe pelvic organ prolapse (POP) and urinary incontinence is significantly higher than in premenopausal women [Swift et al., 2001]. This may be related with alterations of the “...metabolism of connective tissue in the periurethral region with a significant decrease of collagen and vitronectin expression...” reported by Goepel et al. [2003].

Tobacco: The risk factor of developing genuine stress incontinence increases 2.5-fold in smokers [Bump and McClish, 1992]. The same authors concluded that although smokers have stronger urethral sphincters [Bump and McClish, 1994] due to a “cough training”, their violent coughing overcomes any protective advantage of a stronger urethral sphincter.

Race: According to Swift et al. [2001] white females have shown a significant increase in the percentage of severe pelvic organ prolapse (POP) over non-white females. However in subsequent studies [Moalli et al., 2003; Swift et al., 2005] this apparent relation between race and prolapse loses much of its importance.

According to Petros [2007] stress incontinence is the only type of incontinence that can be surgically cured. For other incontinence forms such as urge incontinence (UI) or mixed incontinence (UI+SUI), drugs¹⁰ and bladder training are the therapeutics commonly available.

2.3.2 Pelvic organ prolapse

Pelvic organ prolapse (POP) is an important issue in women’s health care, occurring in up to 50% of parous women [Maher et al., 2008; Olsen et al., 1997]. It cannot be

¹⁰only in short term treatments due to side effects

defined as a single condition but rather as a group of clinical conditions affecting women [DeCherney, 2007, Ch. 44]. POP impacts negatively in health-related quality of life

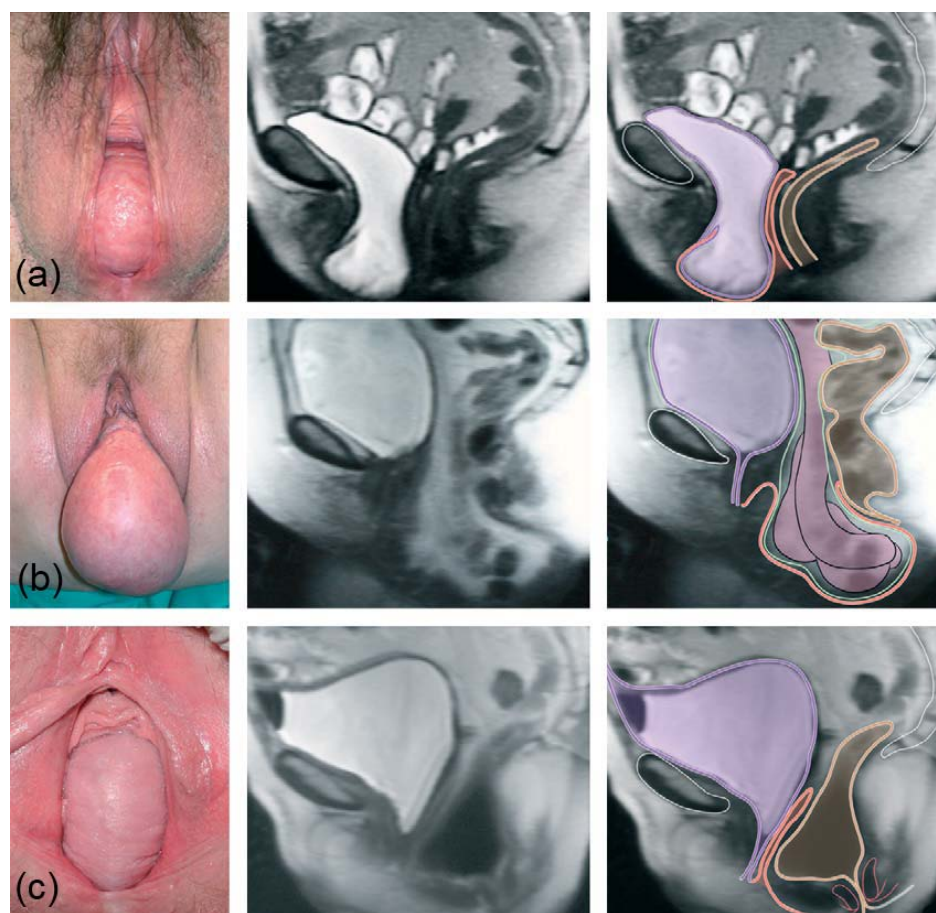


Figure 2.8: Photographs in lithotomy position and sagittal MRI showing vaginal-wall prolapse. Prolapse might include: (a) bladder (cystocele), (b) small bowel (enterocele), or (c) rectum (rectocele). Colour codes include purple (bladder), orange (vagina), brown (colon and rectum), and green (peritoneum). Adapted from [Jelovsek et al., 2007]

by affecting daily activities, body image, and sexual function [Lowenstein et al., 2009]. In a similar way to urinary incontinence the spread of POP in developed countries, where statistical data is both reliable and available, has epidemic proportions. The scale of the problem can be perceived if we acknowledge that in the United States, POP is responsible for more than 200,000 surgeries per year [DeCherney, 2007, Ch. 44]. For women, the lifetime risk of undergoing prolapse or urinary incontinence surgery is 11%, and the risk of reoperation after a previous POP surgery is close to 1/3 (29%). The high prevalence and high re-operation rates indicate the need of a different approach to POP [DeLancey, 2005]. This approach should extrapolate the anatomic relationships between the pelvic organs.

The downward descent of female pelvic organs may result in disruption on the normal anatomic relationships in the pelvis [Jelovsek et al., 2007]. The pathophysiology of POP is multifactorial and complex. Collagen levels, vaginal child birth, advancing age, and increasing body-mass index are the most consistent risk factors [Jelovsek et al., 2007; Swift et al., 2005]. As a matter of fact, most of the risk factors enumerated for urinary incontinence are common to POP. It is believed that POP is closely related to the integrity of pelvic floor muscles and perivaginal connective tissue [Ghoniem and Davila, 2006, Ch. II]. Some of the effects of pelvic organ descent may be observed in Figure 2.8: **(a)** Cystocele is a herniation¹¹ of the anterior vaginal wall and bladder caused by tearing, stretching, or a combination of the two, of the anterior wall endopelvic fascia. **(b)** An enterocele is a herniation of the superior portion of the posterior vaginal wall caused by tearing, stretching, or a combination of the two, of the posterior vaginal wall endopelvic fascia. **(c)** A rectocele is a herniation of the inferior portion of the posterior vaginal wall and rectum caused by tearing, stretching, or a combination of the two, of the posterior vaginal wall endopelvic fascia. Vaginal vault prolapse is a herniation of the vaginal vault caused by loss of support or weakening of the uterosacral ligaments, cardinal ligaments, and loss of attachment of the endopelvic fascia to the white line at the level of the sacrospinous ligament, or a combination of the above mentioned. Uterine prolapse is a herniation of the uterus caused by the loss of support from the uterosacral and/or cardinal ligaments.

Some of the principal symptoms of patients suffering from POP are:

- Sensation of vaginal fullness, pressure.
- Discomfort in the vaginal area.
- Presence of a soft, mass into the vagina, distending through vaginal *introitus*¹².
- With straining or coughing, increased bulging and descent of the vaginal wall.

¹¹abnormal protrusion of an organ or other body structure through a defect or natural opening in a covering membrane, muscle, or bone.

Dorland's Medical Dictionary for Healthcare Consumers. Elsevier. 2007. Retrieved 2010-04-30.

¹²Latin word meaning an entrance; a term used in anatomy.

Dorland's Medical Dictionary for Healthcare Consumers. Elsevier. 2007. Retrieved 2010-04-30.

- Back pain and pelvic pain
- Stress urinary incontinence (SUI) in association with severe POP.

To understand the fundamental causes for POP, some researchers have directed their research focus on the vaginal morphometric properties. Boreham et al. [2002b] found evidence of a decreased smooth muscle content in the vaginal *muscularis* of the anterior vaginal wall in women with prolapse, when compared with vaginal tissues from normal control subjects. The authors also found abnormal histologic features (disorganized smooth muscle bundles and decreases in the fractional area of smooth muscle in the *muscularis* layer) on the posterior vagina [Boreham et al., 2002a]. They concluded that morphologic features of the posterior vaginal wall are significantly altered in women with posterior wall prolapse compared with asymptomatic control subjects (Figure 2.9). These results were reinforced in respect to the anterior vaginal wall, from

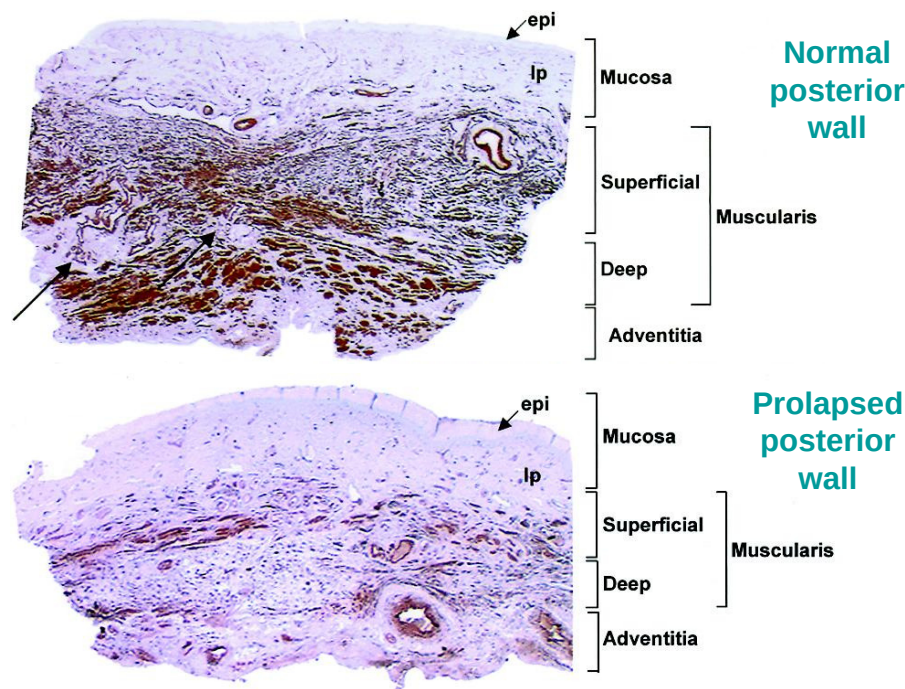


Figure 2.9: Posterior vaginal wall obtained at hysterectomy and immunostained with smooth muscle α -actin. The mucosa layer includes squamous epithelium (*epi*) and lamina propria (*lp*). Staining for smooth muscle α -actin was observed in small arterioles and venules perforating lamina propria and throughout muscularis in smooth muscle bundles. Arrow, Vascular plexus perforating junction of superficial and deep layers of vaginal muscularis. Adapted from Boreham et al. [2002a]

a morphometric analysis performed by Badiou et al. [2008]. The authors found that

the fractional area of nonvascular vaginal smooth muscle of women with POP was significantly decreased in comparison to control subjects, the fractional area of connective tissue was significantly increased. The fractional area of blood vessels was similar for both groups.

Changes on the collagen content of pelvic tissues from women with POP were observed as well. Takano et al. [2002] reported a lower amount of collagen in the parametrium of women with uterine prolapse, both in menacme¹³ and in postmenopause. Söderberg et al. [2004] concluded that young women with genital prolapse have a decreased collagen concentration. The authors interpreted this findings as evidence of a different organization of the endopelvic connective tissue extracellular matrix.

2.3.3 Vaginal atrophy - relation with menopause

During menopause women suffer significant physical changes, mainly due to hormonal changes [Schorge et al., 2008, Ch. 22], as the levels of estrogen in the female body are gravely reduced. One important changes is vaginal atrophy [Bride et al., 2010],

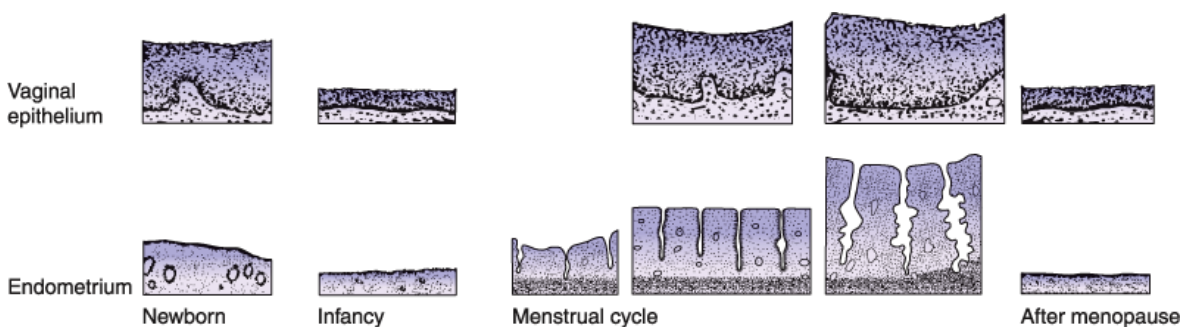


Figure 2.10: During the entire life of a woman, the structure and functions of the vaginal epithelium and of the endometrium depend on ovarian hormones. Adapted from [Junqueira and Carneiro, 2007, Ch. 22]

identified by the loss of *rugae*¹⁴ and a pale, dry vaginal mucosa (thin and inflamed). Some markers of vaginal atrophy are, a vaginal pH greater than 5.0 and a change in the vaginal wall's maturation index toward basal cell predominance (Figure 2.10). The growth of new cells on the outer most layers of the vaginal wall (and urethral

¹³the period of a woman's life during which there is menstruation.

Dorland's Medical Dictionary for Healthcare Consumers. Elsevier. 2007. Retrieved 2010-04-30.

¹⁴the irregular ridges in the mucous membrane

tract) are estrogen dependent and decrease in an estrogen-deprived environment. This effect is also seen in the *endometrium* (*epithelium* + *lamina propria*), Figure 2.10. Dyspareunia¹⁵, often accompanied with insufficient vaginal lubrication and bleeding, are common symptoms of vaginal atrophy [da Silva Lara et al., 2009].

2.4 Conclusions

The pelvic cavity in general and the pelvic floor in particular, brings an important set of scientific and technical problems that can be addressed either by experiments or models. In particular the nonlinear behavior of the soft tissues from the pelvic cavity will be considered later in this thesis.

To contextualize the next chapters, the internal anatomy of the female pelvic cavity was presented. This subject may be a basis for understanding the function and support of the organs in the pelvic cavity.

Pelvic pathologies were considered as well, since they are of fundamental importance due to the suffering they inflict to women and as a major driving force for research.

From the bibliography reviewed, it was possible to establish a connection (for pelvic tissues) between the morphology, the histologic features and pathologies like urinary incontinence or POP. In engineering terms, it means that some pelvic diseases are accompanied by micro-structural changes of the pelvic tissues. These “material” changes may alter the macro-scale mechanical properties measured via mechanical testing.

¹⁵difficult or painful sexual intercourse.

Dorland’s Medical Dictionary for Healthcare Consumers. Elsevier. 2007. Retrieved 2010-04-30.

Chapter 3

Soft tissue structure of the pelvic cavity

3.1 Introduction

This chapter looks into the structure of the tissues considered later in the thesis. The term structure appears on an engineering context. The purpose is to introduce the reader to the morphology of the pelvic soft tissues in terms of their structural constituents (epithelial tissue, connective tissue, muscle tissue, etc.) and the geometrical arrangements (micro-structure) of those constituents. After a review of the basic tissue types, understood as building blocks, the more complex pelvic tissues and organs will be considered.

3.2 The four basic types of tissue

There are four basic types of tissues [Junqueira and Carneiro, 2007], acting as building blocks for the complex organs and systems in the Human body. The combinations achieved either by the proportion of a given type and the number of types involved

allows all the diversity (and complexity) observed in the Human body and other mammals as well.

3.2.1 Nervous tissue

Nervous tissue spread throughout the body forming a complex system specialized on transmitting and processing information, the nervous system. It is composed by nerve cells called neurons which are connected into circuits i.e., networks of neurons that have some specific purpose. The nervous system has two major components, the central nervous system and the peripheral nervous system. The central nervous system (stimulus emission and information processing), composed by the cerebrum, cerebellum, and spinal cord, with only a small amount of connective tissue has the consistency of a gel. The peripheral nervous system (connects the body, i.e. muscles and organs to the central nervous system), is made of nerve fiber bundles held together by connective tissue sheaths. There are two types of fiber bundles, the nerves and the ganglia. Nerves transmit information between brain and spinal cord centers and the sense organs, muscles, etc. The ganglia relays nervous impulses between different neurological systems.

3.2.2 Epithelial tissue

The epithelial tissue (Figure 3.1) protects the human body by covering all its surface, both internal and externally (e.g. lining of intestine, dermis). It also plays a major role on the production of secretions (e.g. sweat glands, vagina). By the processes of secretion (extrusion of substances) and resorption (uptake of substances) epithelial tissue connects the body to the environment [Faller and Schuenke, 2004, Ch. 3]. Therefore epithelia are classified as *covering* or *glandular*.

There are also epithelial layers responsible for absorption (e.g., intestines), sensation (e.g., gustative and olfactory neuroepithelium), and contractility (e.g., myoepithelial cells). The cells forming the epithelial layers have a polyhedral form. These cells

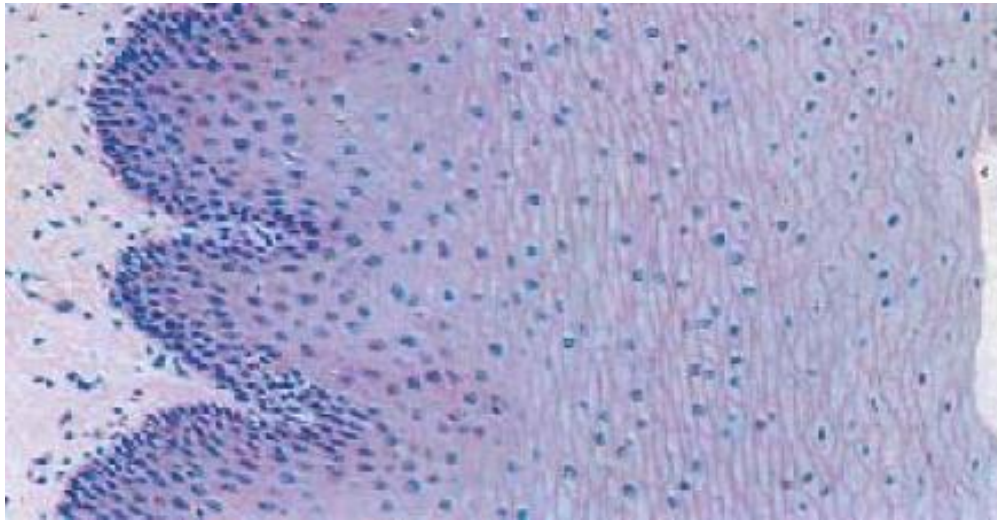


Figure 3.1: Multilayered Stratified Nonkeratinizing Squamous Epithelium -Esophagus. Adapted from [Kuehnelt, 2003, 116, pg. 87]

lay on a sheath of connective tissue (called *lamina propria* for vaginal tissue) which provides support, nutrition and a bound between the tissue and its neighboring structures. There are epithelia formed by a single layer of cells (over the *lamina* providing the basis), called simple epithelium and by multiple layers of cells, called stratified epithelium.

3.2.3 Connective tissue

Connective tissue gives support and protection to the body. It is responsible for the shape taken by tissues in the body. Connective tissue is mainly composed of extracellular matrix, a combination of protein fibers and ground substance which is viscous and hydrophilic. Ground substance is responsible for the strength and rigidity of the extracellular matrix. The other components of connective tissue are cells (in small numbers when compared to other tissue types) and fibers, mainly collagen.

Among the several types of connective tissue that form the Human body, those related with structural characteristics of the tissue are specially relevant on the context of this thesis. Excluding the supportive connective tissues (bone and cartilage), connective tissues of type loose, dense and elastic fall in this category.

Loose connective tissue (Figure 3.2) forms the *stroma* that connects tissues of organs. It holds nerves and vessels in place, forming sheaths in which they traverse their sur-

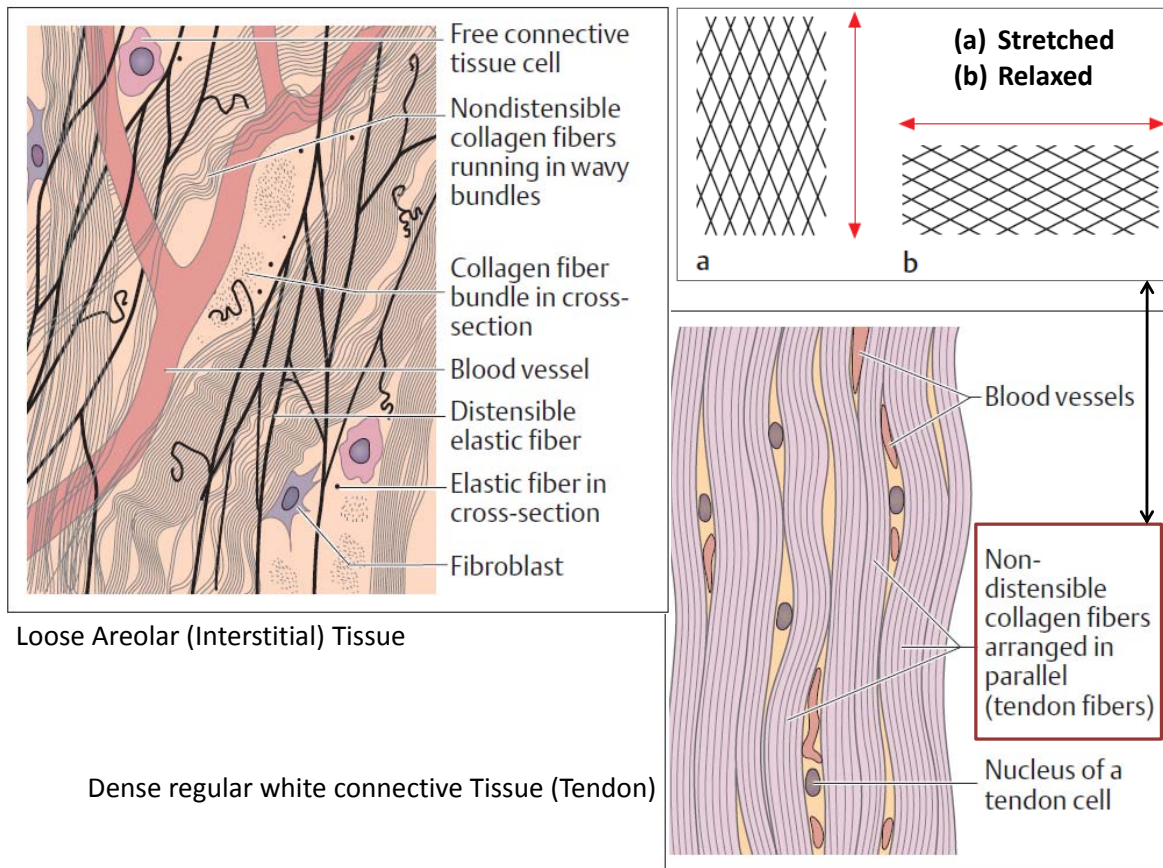


Figure 3.2: Loose and dense connective tissue. Behavior of distensible collagen fibers. Adapted from [Faller and Schuenke, 2004, Ch.3]

roundings. It can be found in mucous membranes lining hollow organs (e.g. the *lamina propria* layer in vaginal mucosa) as a support for epithelial cells. It is a protection for biological structures subjected to low friction and pressure as it stores water and allows other tissues room to shift. Dense (Figure 3.2) connective tissue (e.g. the *adventitia* layer in vaginal tissue) is made of the same elements of loose connective tissue but with a significant increase in collagen fibers which makes it stiffer and more resistant to stress.

In dense white connective tissue there only a few cells, with fibers being the dominant element. There are two arrangement of the tissue, as dense irregular and dense regular 3.2) white fibrous connective tissue. The irregular form as collagen fibers arranged in weave bundles (capsules of organs, dermis, sclera, *dura mater*¹). Regular connective tissue is molded by directional forces that induce the formation of parallel bundles visible to the naked eye (tendons and aponeuroses).

¹Membrane in contact with the bone and covering of the brain [Van de Graaff, 2001, Ch. 11].

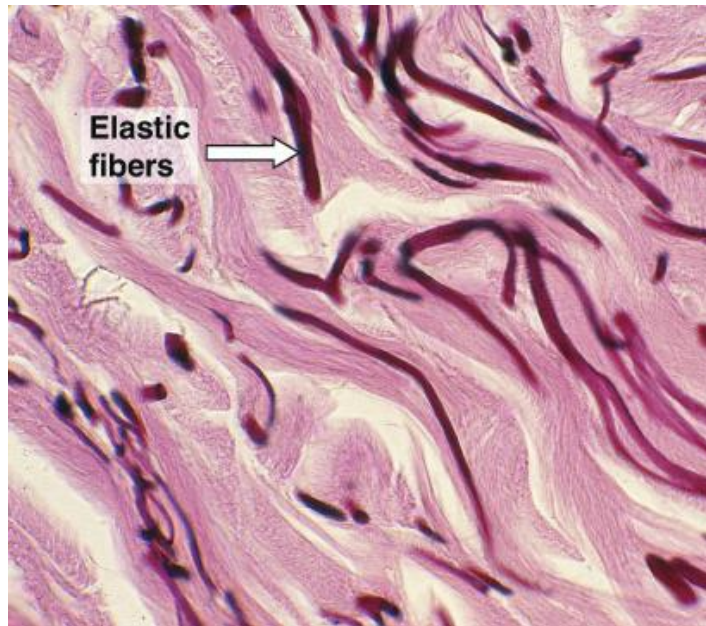


Figure 3.3: Elastic fibers of skin. Adapted from [Junqueira and Carneiro, 2007, Ch.5]

Elastic tissue is made of elastic fibers grouped in thick parallel bundles (Figure 3.3). There is not an abundance of elastic tissue in the Human body, but it is present in regions where high elasticity is required (e.g. the urethral wall [Edmonds, 2007, Ch.49, pg.507]).

3.2.4 Muscle tissue

Muscle tissue is capable of producing movement through cellular contraction. Muscle contractions are called isometric if muscles do not shorten or shorten very little. During such contractions, typically slow, the muscle is tensed without length change. This process allows the muscles to develop their maximum strength.

If the muscle shortens without changing its tension, typically in very fast movements, the contraction is called isotonic. Only little strength can be developed in this process. Muscle cells can be excited by chemical and electrical stimuli, similarly to nerve cells. These cells, with an elongated shape (Figure 3.4) have the ability to contract themselves through contractile proteins, *myofibrils*. The forces generated by contracting muscle cells are transmitted by the connective tissue involving the muscle fibers which are assembled in regular bundles. There are three types of muscle tissue: skeletal, smooth and cardiac.

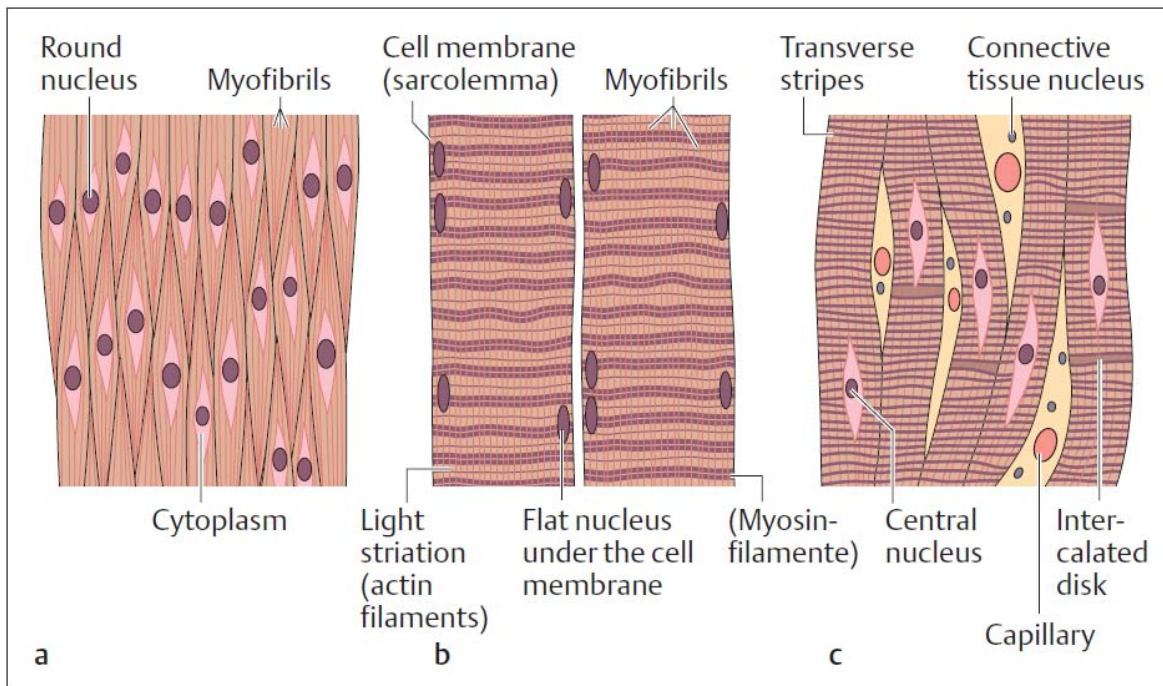


Figure 3.4: Muscle tissue in longitudinal section. **a** Smooth muscle. **b** Striated (skeletal) muscle. **c** Cardiac muscle. Adapted from [Faller and Schuenke, 2004, Ch.3]

Skeletal muscles are able to produce quick and voluntary contractions when the nerves give signal to the muscles to move. These muscles are responsible for 40-50% of the total body weight. They are connected to the bones by the tendons, being responsible for body movement. Skeletal muscles display a merge between muscular fibers and connective tissue (see Faller and Schuenke [2004, Ch. 3, pg. 89]). The muscle fascias are strong connective tissue sheaths (made of dense collagen) that involve, contain and allow the muscles to slide through neighbouring structures.

In general, smooth muscle forms large sheets such as those found in the walls of hollow viscera like the uterus or the intestines. The contractions produced by smooth muscles are slow and involuntary (slow-twitch fibers), the peristalsis in the intestine constitutes a good example of this type of movement. Smooth muscle can remain contracted for long periods, a relevant feature for the urinary continence mechanisms discussed in the previous chapter. The cardiac muscle is responsible for the contractions and relaxations that produce the heart beatings. The bundles of muscle tissue in the heart are knitted in a tight mesh, in such a manner that the contraction has a wave like pattern. Contractile proteins in cardiac cells have a structure and function similar (not completely equal) to those in skeletal muscle.

Pelvic Viscera and Perineum: Female - Median section (Sagittal)

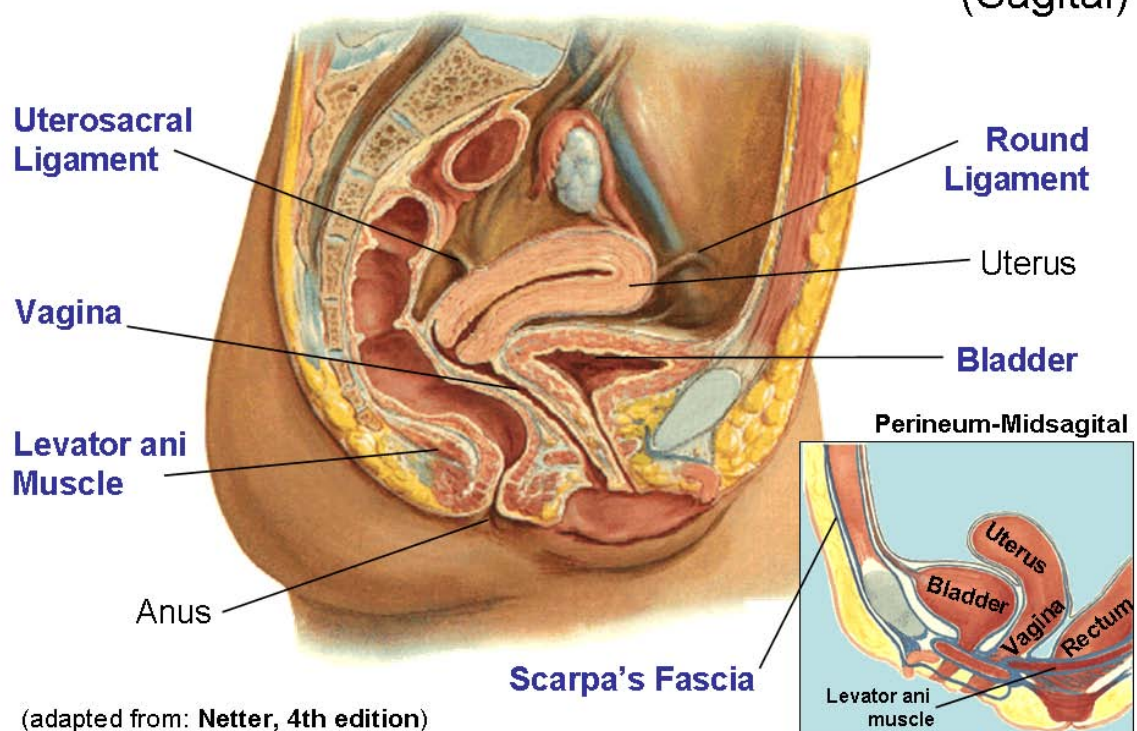


Figure 3.5: Anatomical detail of female pelvic anatomy. Adapted from Netter [2002]

3.3 Studied soft tissues

The experimental results presented in Chapter 5 concern different types of human soft tissues from the female pelvic cavity. The bulk of the experiments consisted in uniaxial tensile tests, also known as simple tension tests. The soft tissues tested, range from highly hydrated soft tissues with muscle layers (*levator ani* complex muscle), to internal organ's tissue (vaginal and bladder walls).

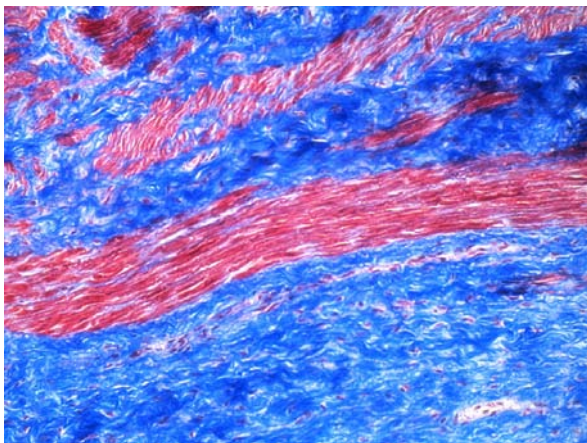
Ligaments (round and uterosacral) and fascias (*Scarpa's* fascia), i.e. tissues with higher collagen contents were considered as well (Figure 3.5). All these structures are a combination (with variable degrees of complexity) of the basic tissue types introduced in Section 3.2.

3.3.1 Vaginal wall tissue

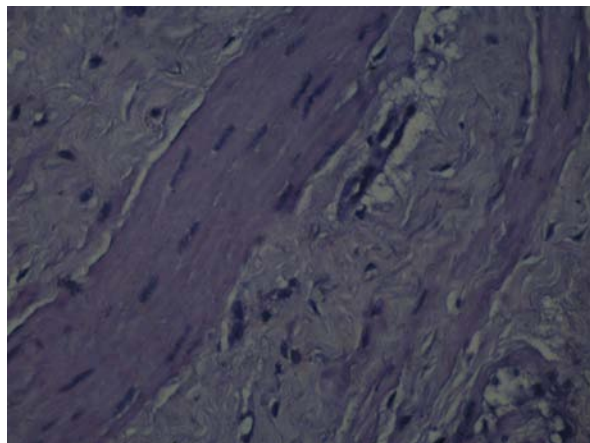
The vaginal wall includes three layers: a mucosa, the muscular layer, also known as *muscularis* and an *adventitia* [Junqueira and Carneiro, 2007, Ch. 22].

The vaginal mucosa has two layers. The first layer is the vaginal epithelium which is a stratified squamous nonkeratinized epithelium with thickness varying between 150-200 μm . The vaginal epithelium, under the estrogen stimulus synthesizes glycogen. Some bacteria in the vagina metabolize the accumulated glycogen producing an acidic ambient with a protective action against microorganisms.

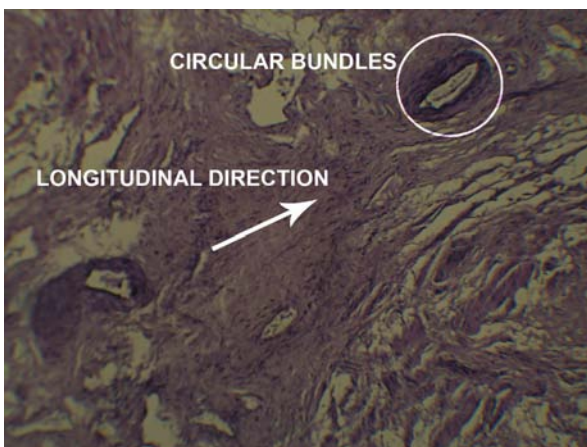
The second layer of the mucosa is the *lamina propria*. This layer contains loose connective tissue and is rich in elastic fibers, with a few smooth muscle cells [Badiou et al.,



(a) Masson's trichrome stain



(b) Hematoxylin-and-eosin stain



(c) Histology before uniaxial tensile test

(a) Masson's trichrome (MT) and (b) Hematoxylin and Eosin (HE) stains of a vaginal sample. MT-Connective tissue is stained blue, nuclei are stained dark red/purple. HE-Nuclei are stained blue; cytoplasm and extracellular matrix have varying degrees of pink staining.

(c) Details showing circular bundles of smooth muscle fibres.

Figure 3.6: Masson's trichrome and Hematoxylin and Eosin stains of vaginal samples from patients with vaginal prolapse. From results published in [Peña et al., 2010] and [Calvo et al., 2009]

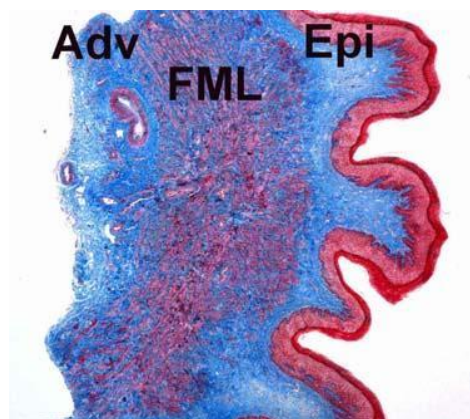


Figure 3.7: Anterior vaginal wall; Full thickness: **Adv** - *adventitia*; **FML** - fibromuscular layer; **Epi** - epithelium. Staining: Mason's trichrome, smooth muscle and epithelium stain red, connective tissue, blue. Adapted from [Clark, 2004].

2008]. The thickness of the *lamina propria* is dependent of the vascularization.

The *muscularis* layer is mostly² made of smooth muscle bundles oriented longitudinally (Figure 3.6.a-b). In the innermost part of this layer (close to the mucosa) some circular bundles of muscle fibres can be seen (Figure 3.6.c). According with Tulikangas et al. [2001], the thickness of the *muscularis* layer in women without vaginal prolapse is 3.2 ± 0.8 mm. The outer layer, the *adventitia*, is made of dense connective tissue with many elastic fibers of collagen and elastin, extensive nervous supply and venous capillaries. It provides a separation between the muscular wall of the vagina and the paravaginal tissues [Hsu et al., 2005]. A histological profile (full thickness) of the anterior vaginal wall can be observed in Figure 3.7.

During the experimental process developed on the scope of the current thesis, it was made an effort to characterize the microstructure of prolapsed vaginal tissue. For that purpose, some histological studies were carried out at the University of Zaragoza (Spain). In Figure 3.6 are shown several untested specimens of vaginal tissue, fixed in paraffin wax and sectioned at $4\mu\text{m}$ thickness slides in parallel directions to the test. Hematoxylin and Eosin and Mason's trichrome stains were applied to observe the tissue microstructure. The preferred orientation direction of the constituents is clearly observed.

Based on these histologic results and the nature of the tissue, the vaginal tissue was assumed to behave as a transversely isotropic hyperelastic material.

There are differences on the morphology of the anterior and posterior vaginal walls.

²The *muscularis* layer also contains collagen, and elastin.

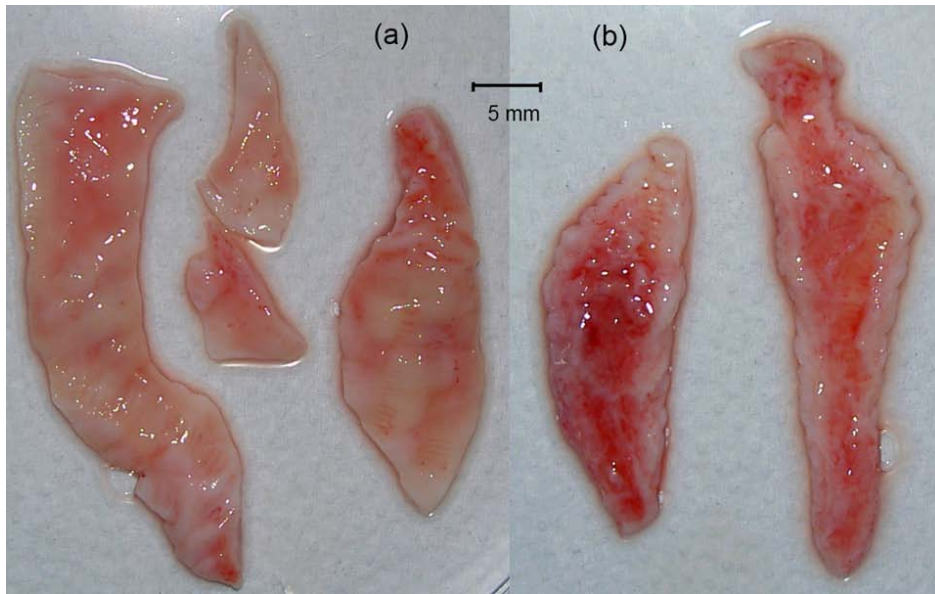


Figure 3.8: Prolapsed vaginal tissue from one patient. (a) Anterior vaginal wall, *adventitia* view. (b) Posterior vaginal wall, *epithelium* view.

In average, the length the anterior vaginal wall is 7 *cm* and the length of the posterior wall is 9 *cm*. The anterior wall is shorter due to the anterior position of the uterine cervix in most women [Schorge et al., 2008, Ch. 38].

Some of the experimental data presented in the next chapters is from the anterior and posterior vaginal walls, from both living patients (prolapsed vaginal wall) and cadavers. Most of the samples were from postmenopausal women (Tables 5.3 and 5.4), in particular on the group of patients with prolapse (POP-group). In a significant majority of those samples the effects of estrogen deprivation i.e., vaginal atrophy (consult Section 2.3.3) were observable. In Figure 3.8 the marks of vaginal atrophy can be observed on tissue excised from a 62 year old patient with severe vaginal prolapse (see Vv093 in Table 5.3).

3.4 Uterosacral and Round ligaments

The pelvis has a diversity of ligaments, with variable composition and function. These ligaments can range from dense connective tissue (those that support the bony pelvis and pelvic organs) with a significant support role, to smooth muscle, with small relevance for pelvic organ support [Schorge et al., 2008, Ch. 38]. The Uterosacral ligaments

(USL) and the Round Ligaments (RL) are two of these ligamentous support structures. Other ligamentous structures highly relevant for the understanding of the Uterus supporting mechanisms are the Cardinal ligaments. These bands of dense connective tissue attach the lateral margins of the cervix to the pelvic sidewalls. However, it proved difficult to dissect and isolate cardinal ligaments. To excise the USL and the RL, the Uterus was kept in tension. As soon as this tension was alleviated, the connective tissue bands forming the Cardinal ligaments were not able to be differentiated from the surrounding tissue.

3.4.1 Uterosacral ligaments

The Uterosacral ligaments (USL) (Figure 3.9) represent an important part of the pelvic support system (see Figure 2.5) and establish the level 1 support of the cervix and the upper vagina [Nichols and Randall, 1996; DeLancey, 1992]. They are condensations of visceral connective tissue consisting primarily of smooth muscle, connective tissue and some of the pelvic autonomic nerves [Schorge et al., 2008, Ch. 38]. They can be

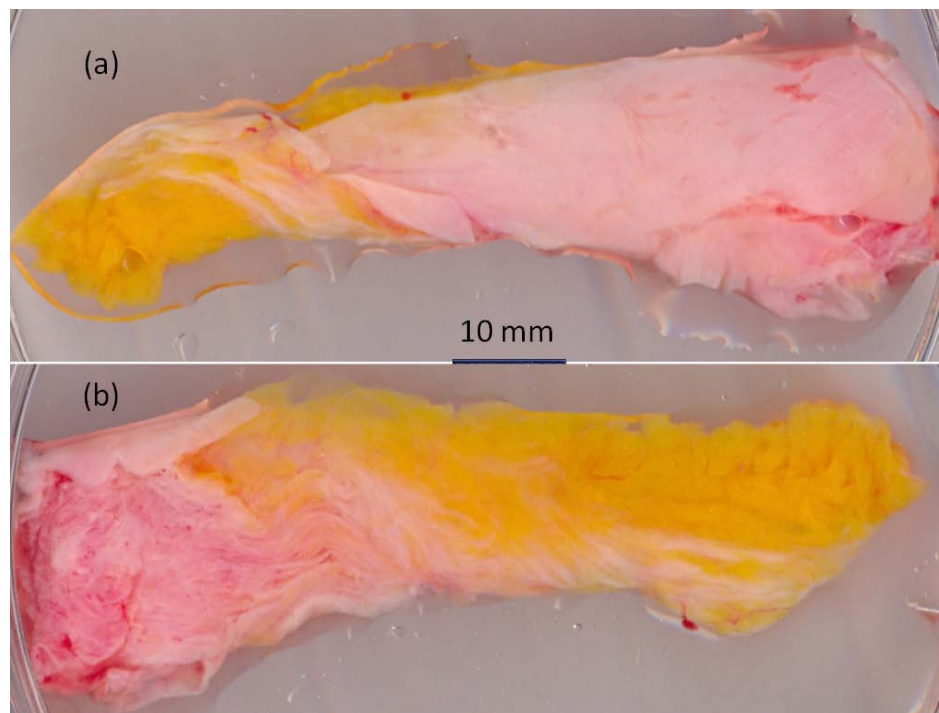


Figure 3.9: Uterosacral ligament. (a) View showing the outer layer of dense connective tissue. (b) View showing adipose tissue, smooth muscle and highly oriented white connective tissue fibers. Cadaver Cd11 (Table 5.4)

divided into a cervical, an intermediate, and a sacral portion [Ghoniem and Davila, 2006, Ch. II]. The cervical portion is mainly composed of smooth muscle and connective tissue. It also contains nerve fibers. The middle portion is made of a dense connective tissue band, the *stratum fibrosum* at its anterior margin and loose connective tissue posteriorly.

The direction of USL is vertical and provides a suspension of the upper vagina and cervix. USL are mainly composed of smooth muscle and connective tissue.

3.4.2 Round ligaments (of the Uterus)

The round ligaments of the uterus (RL) (Figure 2.6) are fibrous cords containing smooth muscle (longitudinal) from the outer layer of the uterine body [Schorge et al., 2008, Ch. 38]. Figure 3.10 shows a sample of RL from cadaver. The RL are actually

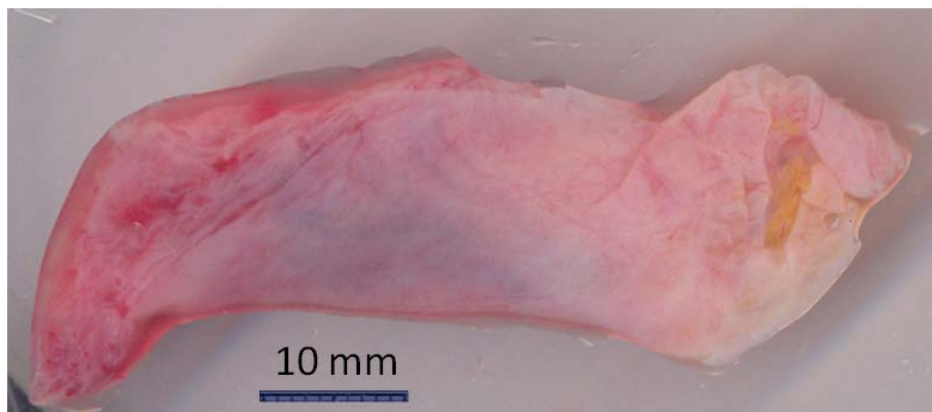


Figure 3.10: Round ligament. Cadaver Cd08 (Table 5.4).

continuations of the ovarian ligaments that support the ovaries. They emerge laterally from the uterine body and anterior to the origin of the fallopian tubes. RL extend laterally to the pelvic sidewall. It is considered that RL have a small contribution to uterine support [Davila et al., 2006], but may assist in maintaining the body of the uterus in its typical position over the bladder [DeCherney, 2007, Ch. 2]. However, it was found by Ozdegirmenci et al. [2005] that women with pelvic organ prolapse have a decrease on smooth muscle content of Round ligaments.

3.5 Bladder wall tissue

The bladder is composed of smooth muscle (the detrusor muscle), covered on its dome by a serosal layer and lined by a submucosa and transitional cell epithelium. The detrusor muscle is composed of three separate bundles of smooth muscle arranged in an internal and external longitudinal and an intermediate circular layer [Ghoniem and Davila, 2006, Ch. I]. Although separate layers of the detrusor are described, they are not as well defined as the layers of other viscous structures, such as the bowel or the ureter [Schorge et al., 2008, Ch. 38]. The muscle fibers of the bladder run in every direction (without distinct layers) until they approach the bladder neck [Junqueira and Carneiro, 2007, Ch. 19]. If the tissue specimens are prepared from bladder samples taken from the superior wall of the bladder, the biomechanical properties should be independent of the direction. This was experimentally verified (Table 5.9).

With full bladder (Figure 3.11 (b)), the epithelial cells slide upon one another which reduces the thickness of the epithelium. The interior surface of the bladder increases. Figure 3.11 (b) also shows thin strands of collagen fibers separating bundles of smooth muscle cells.

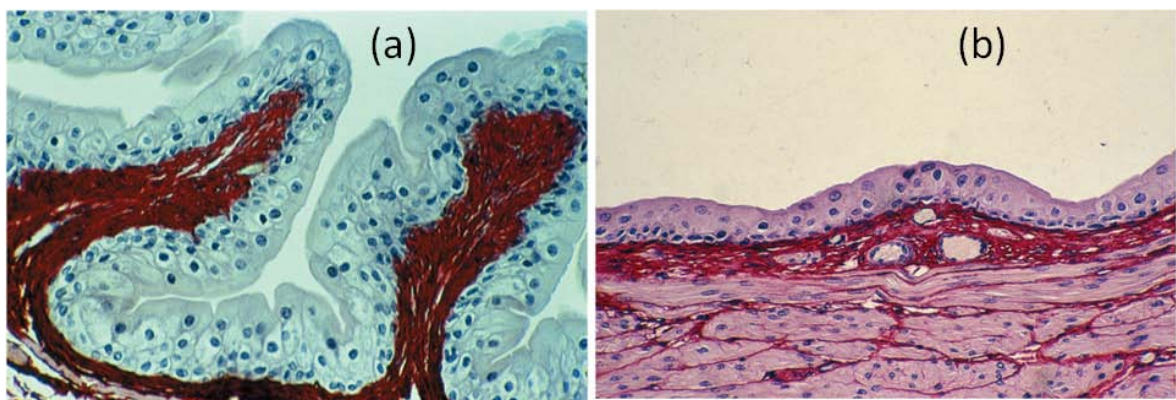


Figure 3.11: Transitional epithelium of the urinary bladder; (a) bladder empty, (b) full bladder .Adapted from [Junqueira and Carneiro, 2007, Ch. 19]

3.6 *Levator ani* muscle

The *levator ani* muscle complex is a thin sheet of muscle. Together with Uterosacral ligaments and Cardinal Ligaments (Figure 2.5) it supports the uterus and cervix. It is composed of slow-twitch fibers (Type I) able to sustain a contraction for a long period of time (constant tone) without fatigue [Fox, 2003, Ch. 12] (fundamental for the continence mechanisms). It also contains fast-twitch fibers (Type II) for a fast response to sudden rises in intra-abdominal pressure [Ghoniem and Davila, 2006, Ch. I].

The *levator ani* is subdivided into three muscles: *puborectalis*, *pubococcygeus* and *iliococcygeus* (see Section 2.2.2). The criteria for this division is based on the muscle fiber directions and their respective attachments to the surrounding structures (Figure 2.3). Although the studies included in Chapters 5 and 6 did not include the analysis of *levator ani* tissues, the experimental data can be found in Appendix A. Table A.1 contains the experimental results for the *Levator ani* muscle complex. The tensile strength (σ_{max}) and tangent modulus (E_t) were significantly smaller when compared to all the soft tissues tested. Directional differences (Longitudinal versus Transversal) are significant (Figure A.1). This result is consistent with tissue morphology, since muscle fibers are highly oriented (locally) in every region of the *levator ani*.

3.7 *Scarpa's* Fascia

There is a layer from the anterior abdominal wall called the subcutaneous Layer. It can be separated into a superficial, fatty layer known as Camper fascia and a deeper, more membranous layer known as *Scarpa's* fascia [Schorge et al., 2008, Ch. 38]. Camper fascia and *Scarpa's* fascia are a continuum of the subcutaneous tissue layer. *Scarpa's* Fascia is better developed in the lower abdomen and it is found just superficial to the *rectus abdominalis* fascia. The parallel fiber bundles observed in Figure 3.12 are molded by directional forces. The fiber bundles are visible to the naked eye (in fascias and tendons). These strong connective tissue sheaths are made of dense collagen. They enable the muscles to slide through neighboring structures.

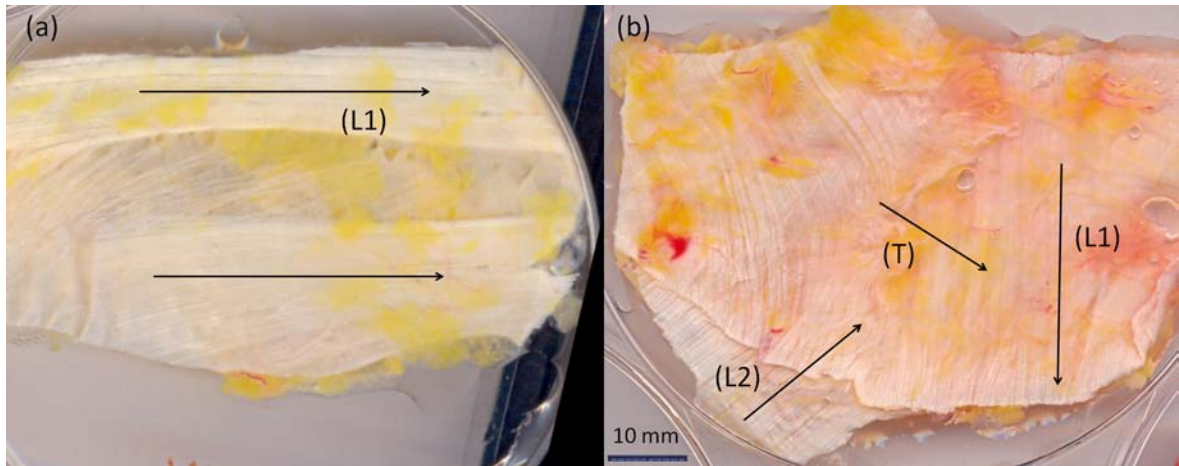


Figure 3.12: *Scarpa's fascia* samples. (a) Highly oriented sample from cadaver Cd15 (Table 5.4). (b) “Double layer” sample from cadaver Cd17 (Table 5.4); the directions of the specimens prepared are labeled as L1, L2 and T.

Although the studies included in Chapters 5 and 6 did not include the analysis of *Scarpa's Fascia*, the experimental data can be found on Table A.2, in Appendix A.2. The tensile strength (σ_{max}) and tangent modulus (E_t) were significantly higher when compared to all the soft tissues tested. The mean results of the mechanical behavior (stress-stretch diagram) for the fiber direction 1 (L1) are shown in Figure A.3, as well as the mechanical behavior for the different fiber directions identified, Figure A.4. These fiber directions were established according with the criteria explained in Section 5.1.

3.8 Conclusions

This chapter reviewed the structural constituents of biological tissues. These blocks are combined to form more complex structures, such as ligaments, muscles or organs. Structures such as fascias, or the *levator ani* muscle complex are critical to the global performance of the pelvic floor behavior. The pelvic tissues considered in more detail were:

- Bladder
- Vaginal wall; anterior and posterior

- Round an Uterosacral ligaments
- *Scarpa's* Fascia
- *Levator ani* complex muscles

These tissues were selected in the context of the experimental work and hyperelastic material modeling that will appear on the next chapters.

Any realistic model of the pelvic cavity should account for the nonlinearity of the soft tissues. Models, such as finite elements, also depend largely on the geometry and boundary conditions. The inherent nonlinearity of the tissues may be covered by the nonlinear theory of elasticity combined with structural information. Under such theory, tissues are treated as hyperelastic materials. The boundary conditions, geometry and the overall structural complexity are only attainable using anatomical and physiological knowledge combined with medical imaging techniques.

Chapter 4

Mechanical testing of Soft Biological Tissues

4.1 Introduction

The mechanical testing of biological soft tissues, a particular type of hyperelastic materials, is a core subject of this thesis. To investigate the mechanical properties of the tissues described in Chapter 3 a suitable experimental technique was required. One possible alternative is the simple tension (or uniaxial tensile) test, one of the most common mechanical stress-strain tests. The chapter is structured as follows:

The first part (Section 4.2) introduces materials science concepts and definitions required to understand the relation between force-displacement data experimentally observed and the stress-stretch (or stress-strain) mechanical behavior diagram.

Section 4.3 gives an overview of the uniaxial tensile test, and the experimental techniques for strain measurement in highly deformable materials (Section 4.3). A review of non-contact (imaging) strain measurement techniques and their technical shortcomings is presented.

Section 4.4 explains the experimental solutions used to produce the data required for the investigations carried out. The technical requirements for working with highly hydrated biological tissues in general, are discussed. Finally, the specific details to the

mechanical tests performed are presented.

The Chapter ends (Section 4.5) with some considerations on the micro-structural changes observed in tested specimens. Evidence of the alterations were observed in histologies of the specimens tested.

4.2 Materials Science concepts and definitions

The main objective of mechanical testing is the production of experimental data from which the mechanical properties of the tested materials can be directly calculated or inferred. For the particular case of tension tests (uniaxial or biaxial), the physical quantities directly measurable are the load, F and the elongation, Δl .

These quantities are normalized to account for the test specimen's geometry. The normalized engineering parameters are the engineering stress, σ and engineering strain, ε . Engineering stress is the ratio between the load F and the sample's cross section i.e., $\sigma = F/A$. Engineering strain is given by

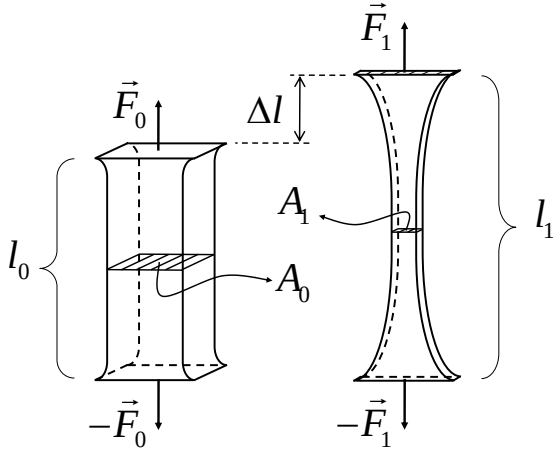


Figure 4.1: Volume preserving transformation during uniaxial tensile test.

$$\varepsilon := \frac{l_1 - l_0}{l_0} = \frac{\Delta l}{l_0} \quad (4.1)$$

where l_0 is the initial length and l_1 the deformed length as shown in Figure 4.1. The stretch in the testing direction can be obtained from the strain using the relation

$$\lambda := \frac{l_0 + \Delta l}{l_0} = 1 + \varepsilon \quad (4.2)$$

Throughout this thesis λ will be used instead of ε because the stress-stretch diagram i.e., the relation $\sigma = f(\lambda)$, is particularly suited for the study of nonlinear mechanical

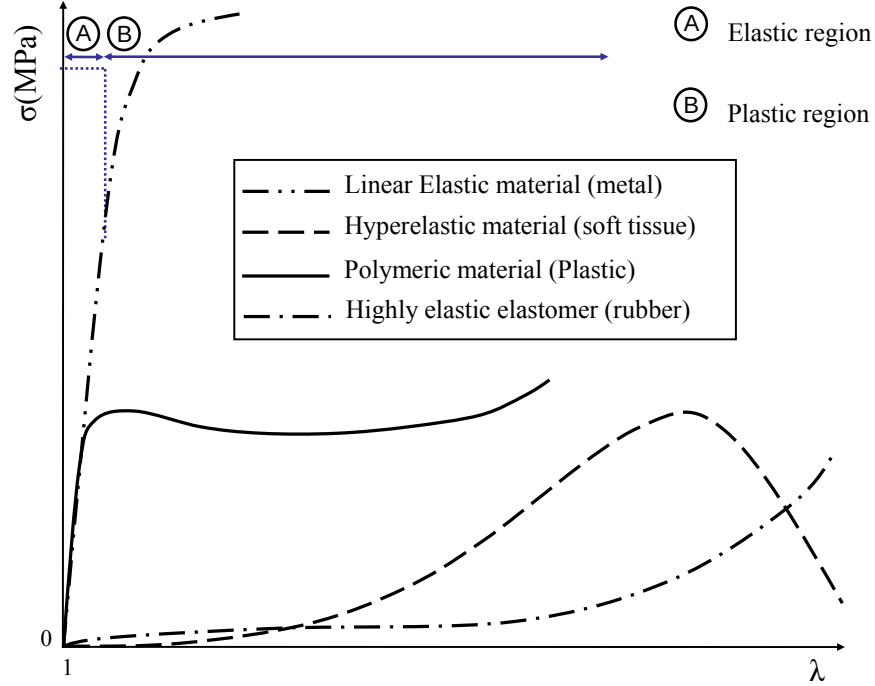


Figure 4.2: Mechanical behavior of linear elastic and hyperelastic materials.

behavior using the nonlinear theory of elasticity [Holzapfel, 2000].

The mechanical behavior of many classical engineering materials like steel or brass, has two main regions the elastic (Figure 4.2 (A)) and the plastic (Figure 4.2 (B)). On the elastic region there is a linear relation between the stress, σ and the strain ϵ , following equation 4.3.

$$\sigma := E\epsilon \quad (4.3)$$

Equation 4.3 is known as Hooke's law, and the constant of proportionality E is the modulus of elasticity, or Young's modulus i.e., it is the slope of the stress-stretch curve in the linear (elastic) region [Callister, 2001, Ch. 7].

This proportionality is observed at very small strain ranges for metals and polymeric materials (Figure 4.2). For rubber like materials Hooke's law is not valid, and alternate nonlinear mechanical behavior models must be used [Holzapfel, 2000; Ogden, 1984].

The deformation is permanent (or nonrecoverable) if strain (or stretch) exceeds the elastic limit. This deformation is called plastic and occurs in region (B), Figure 4.2. The stress at which plastic deformation starts is called Yield strength, σ_Y . Above this stress level, the stress-stretch curve is not linear. It is important to focus the discussion on the biological soft tissue case, stressing the mechanical behavior differences between

these materials and other types of materials (Figure 4.2).

4.2.1 The specificity of biological soft tissues

Biological soft tissues display a mechanical behavior described as nonlinear. In this particular context, the term nonlinear describes the mathematical function providing the relation between stress σ and stretch λ (or strain ε) i.e., $\sigma = f(\lambda)$.

Figure 4.3 shows the stress-stretch diagram for a typical biological soft tissue and the nonlinear equivalents of the *classical* mechanical properties. The term *classical* is

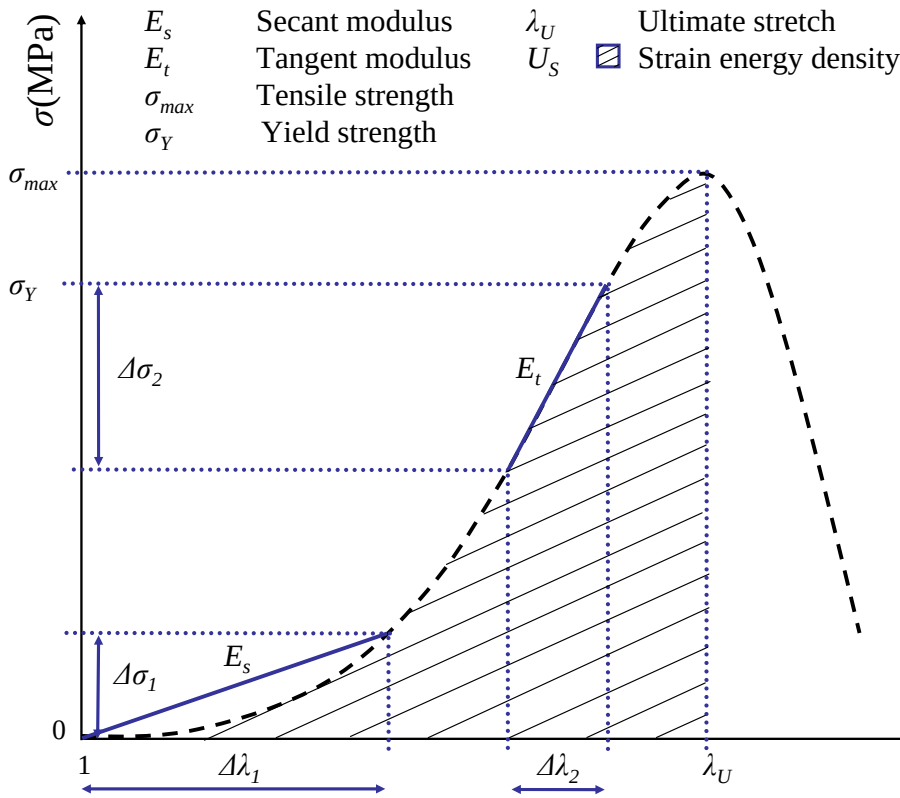


Figure 4.3: Typical mechanical behavior of biological soft tissues. *Classical* mechanical properties of soft tissues.

used in this context to enforce the differences between the service characteristics of conventional engineering materials and those of biological soft tissues. Their complex mechanical behavior can only be described using different mechanical models with different mechanical parameters (Chapter 6).

Secant (Eq. 4.4) and tangent (Eq. 4.5) moduli are *regional* linear modulus (Figure 4.3).

In general they are used when the stress-strain curve is not linear. For soft biological tissues however, tangent modulus (E_t) is used in the linear region of the stress-stretch diagram since Young modulus is only adequate for materials where Hooke's law is valid at the beginning of the stress-stretch diagram.

$$E_s = \frac{\Delta\sigma_1}{\Delta\lambda_1} \quad (4.4)$$

$$E_t = \frac{\Delta\sigma_2}{\Delta\lambda_2} \quad (4.5)$$

Secant modulus (E_s) is used in the conventional way to have a basic notion of the mechanical behavior in a given region [Afonso et al., 2008]. Yield strength σ_Y , also defines the upper limit for the calculation of E_t , and coincides with the beginning of the yielding region i.e., the stretch-stress curve re-enters the nonlinear regime.

The tensile strength (σ_{max}) is the maximum value of tension in the stress-stretch curve, the corresponding stretch, λ_U is called the ultimate stretch. These quantities define the limit for the calculation of the strain (or stretch) energy per unit volume or strain energy density U_S [Woo et al., 2005]. Strain energy density is defined by equation 4.6. This energy is absorbed by the material while being deformed [Abramowitch et al., 2009] and corresponds to the area below the stretch-strain curve (Figure 4.3).

$$U_S := \int_0^{\varepsilon_U} \sigma d\varepsilon = \int_1^{\lambda_U} \sigma d\lambda \quad (4.6)$$

In the study of the mechanical deformation of metals, it is often used the modulus of resilience $U_r := \int_0^{\varepsilon_Y} \sigma d\varepsilon$, which is a measure of the capacity of a material to absorb energy while being deformed elastically and upon unloading, to have this energy recovered. ε_Y is the strain (or $\lambda_Y = 1 + \varepsilon_Y$ is the stretch) corresponding to the yield strength (σ_Y). However, a complete recovery of the stored energy does not occur (in general) with biological tissues. The loading-unloading cycle commonly displays an hysteresis effect, which can be related with viscoelastic phenomenon easily identified in the load-deformation (or stress-strain/stretch) diagram by the path variation (Figure 4.4) of the curve [Fung, 1993, Ch. 7]. The modulus of resilience U_r can (hypothetically) be used when studying biological soft tissues if the experimental protocol includes the

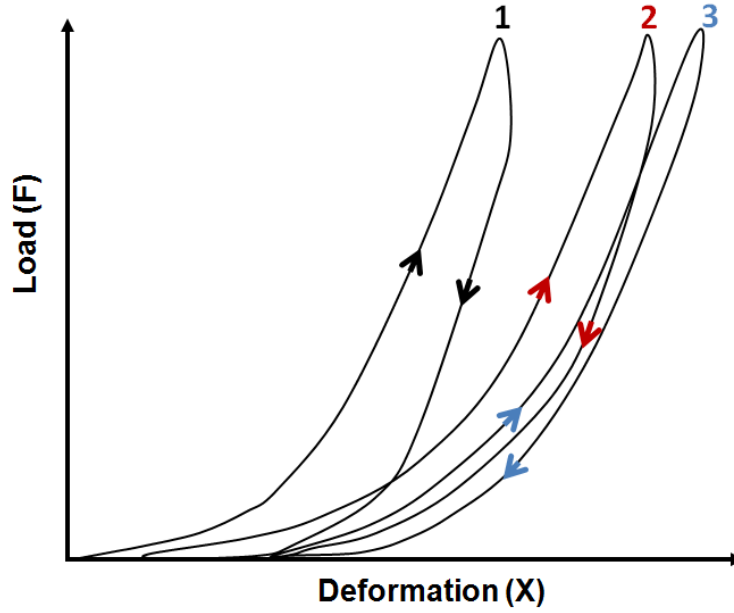


Figure 4.4: Preconditioning of an anterior cruciate ligament. Each cycle shows evidence of hysteresis. Adapted from [Fung, 1993, Ch. 7-Figure 7.3:6]

consecutive repetition of identical loading-unloading cycles. A recovery period where the material is completely unloaded should be maintained between the cycles. This procedure is called preconditioning and it (eventually) eliminates the hysteresis effect [Fung, 1993, Ch. 7].

The samples were not preconditioned because some of the tissues tested were very fragile, therefore excessive tissue manipulations had to be avoided. Also because, the testing machine was purposely built for the testing of biological soft tissues [Martins et al., 2006b]; being an *in-house* solution, the automation features available lack the flexibility and ease of use to perform preprogrammed loading-unloading cycles.

4.2.2 True stress and volume preserving deformations

As seen in Section 4.2 the uniaxial tensile test can be used to evaluate several mechanical properties of materials [Callister, 2001, Ch. 7]. A specimen is usually deformed, until complete rupture, with a gradually increasing tensile load that is applied uniaxially along the long axis of a specimen and producing a deformation, that for the materials analyzed throughout this thesis, is approximately volume preserving. This approximation is more accurate if specimen's deformation is measured on the homoge-

neous deformation zone where the cross section is uniform along the sample's length (Figure 4.5-Ⓐ). The diagram Ⓑ of Figure 4.5 shows the uniaxial tensile test of a

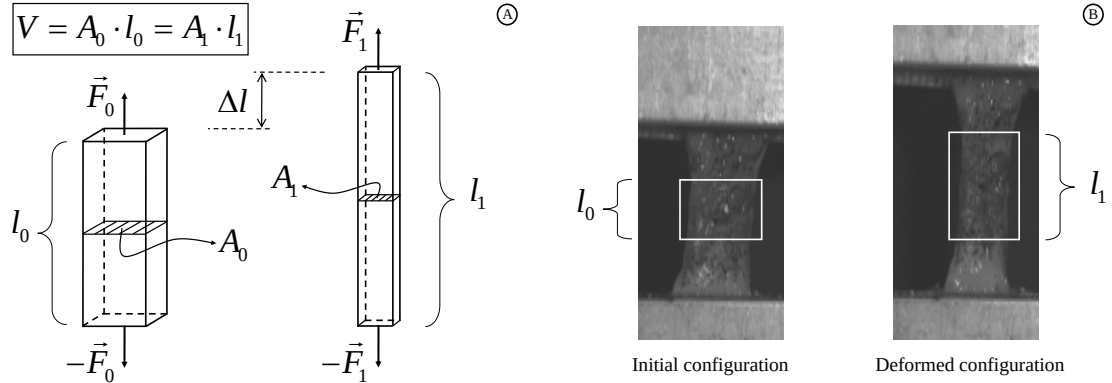


Figure 4.5: The volume preserving deformation occurs in the central homogeneous region of the specimen. Diagram Ⓑ illustrates this phenomenon as seen in a prolapsed vaginal tissue specimen. In the volume preserving regions (white squares of Ⓑ), areas A_0 and A_1 remain constant as shown in diagram Ⓐ.

prolapsed vaginal tissue specimen. The white boxes highlight the homogeneous deformation region as it changes from the central region, at the beginning of the test, to the longer, thinner region during the test.

This effect, observed in most of the tissues studied (Chapter 3), is known as necking [Callister, 2001, Ch. 9], and is a known phenomenon in metals and other engineering materials. Figure 4.6 illustrates the effect with four different types of soft tissue (a)↔(d), and one surgical mesh (e). The necking effect differs for different materials. For biological tissues there is even more variability because the same type of tissue, harvested from different subjects (cadavers or living patients), can have a significantly different mechanical behavior. An appreciation of the experimental data in Chapter 5 supports this consideration.

As shown in diagram Ⓐ of Figure 4.5, incompressibility relates the cross-sectional area (A_i) with length (l_i) on two given deformation instances (ex. 0 and 1) by relation, $V = A_0 \cdot l_0 = A_1 \cdot l_1$. Taking the initial (l_0 , A_0) and deformed (l_1 , A_1) configurations (Figure 4.5-Ⓐ), using Eq. 4.2 the stretch that corresponds to the deformed state is $\lambda_1 = \frac{l_1}{l_0}$. Assuming incompressibility ($l_1 A_1 = l_0 A_0$), the updated cross-section is

$$A_1 = \frac{A_0}{\lambda_1} \quad (4.7)$$

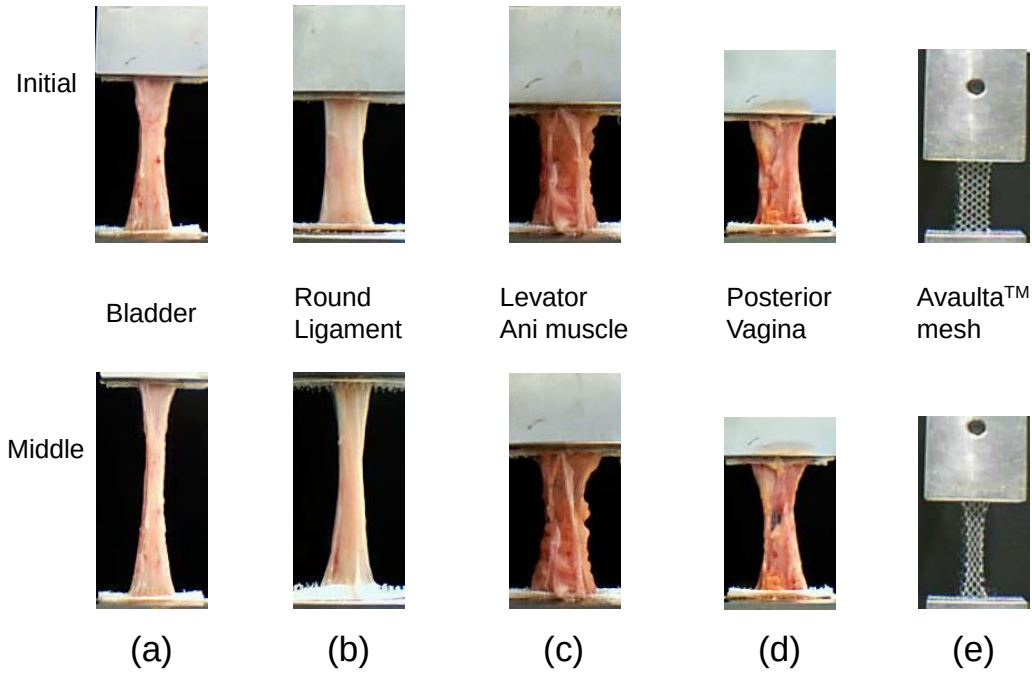


Figure 4.6: Necking effect during uniaxial test at **Initial** and **Middle** stages. The effect gets more pronounced as the test advances. **(a)↔(d)** Different tissues from the pelvic region (female cadaver); **(e)** Mesh used in urogynecology surgeries.

The *true* stress corresponding to the deformed configuration must account for the cross-section variation. Therefore on the stress-stretch diagram (Figure 4.3) stress is obtained from the initial area A_0 and the stretch λ_1 by,

$$\sigma(\lambda_1) = \frac{f}{A_1} = \lambda_1 \frac{f}{A_0} \quad (4.8)$$

In a recent paper, Abramowitch et al. [2009] reviewed some of the experimental techniques used for the study of pelvic organ prolapse. The issue of a correct stress determination is raised, because it is particularly sensitive to the cross sectional area estimation. One of the techniques mentioned, consists on a laser reflectance system that can measure the initial cross-sectional area and shape of concave surfaces [Moon et al., 2006]. However, this approach is only fit to measure the initial area A_0 , because the laser has to rotate 180 degrees about the specimen (the remaining 180 degrees profile is extrapolated).

To the author's knowledge, a technique able to follow the real time 3D deformation of a specimen during a uniaxial tensile test was not yet proposed.

In Section 4.4.3 the approach followed for the initial cross-sectional determination is explained.

4.3 Experimental techniques for strain measurement in highly deformable materials

In typical (hard) engineering materials like metals and composites, strain may be measured at any instance during the mechanical test, using different techniques [Czichos et al., 2006, Part C, 7.1]. Some techniques require a close contact with the samples during the test. The use of extensometers attached directly (glued with cyanoacrylate

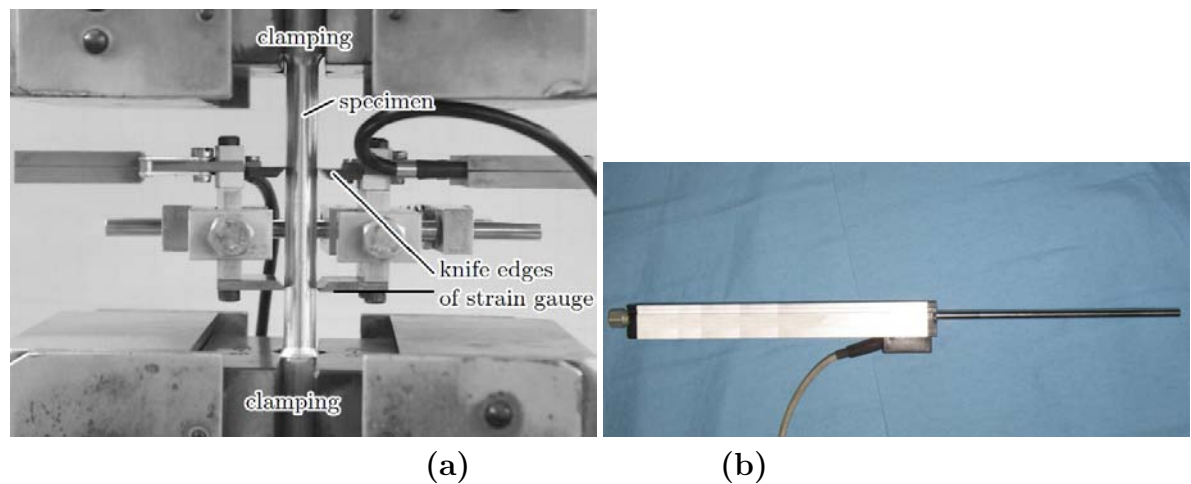


Figure 4.7: (a) Tensile test using a strain gage. Adapted from [Rösler et al., 2007, Ch. 3-Fig.3.9]. (b) LVDT used to measure displacement (specimen length).

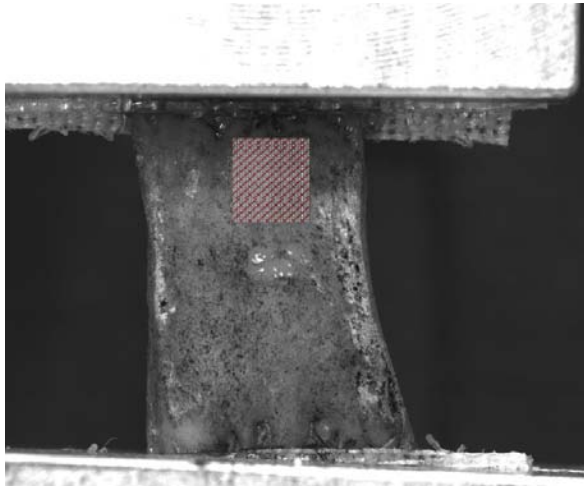
adhesives, for instance) to the gage section of the test specimen, and strain gages that are mounted directly to the specimen (Figure 4.7) are the most common solutions. For the strain measurement in *hard materials*, Clip-on extensometers are advised [Czichos et al., 2006, Part C, 7.1] since they are more accurate than other techniques.

The most common (and widely used) clip-on extensometers are the Linear variable differential transducers or LVDTs (Figure 4.7-(b)) and strain gages (Figure 4.7-(a)). It is not advisable (not feasible for the tissues studied in the current thesis) to apply an extensometer directly to the outer layer of a soft material. If correctly bound, the stress concentration on the boundary would break the specimen outer layer. Since

strain gages have knife edges to define the exact points of contact with the specimen (Figure 4.7-(a)), they are also incompatible with soft tissues. LVDT sensors require contact between the transducer's tip and the specimen's surface. Since the materials considered are highly deformable (hyperelastic) even a slight contact could disrupt the test results. These limitations affect the measurement of the effective cross-sectional area of the specimen i.e., ideally specimen's width and thickness should be determined by non-contact measurement techniques.

4.3.1 Digital image correlation - DIC

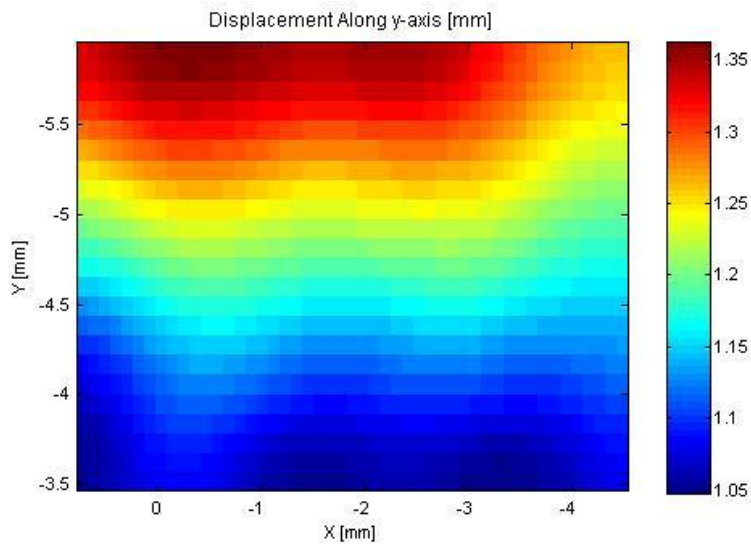
Imaging techniques such as video extensometry or digital image correlation [Eaton-Evans et al., 2009, pg.14-15,25], may be an alternative to the contact methods. Digital image correlation (DIC) is based on a process called image matching which is common to other imaging techniques [Sutton, 2009, Ch. 5]. DIC is a full field, non-contact technique that may be used in a variety of mechanical tests [Eaton-Evans et al., 2009, pg.14-15]. It is possible to use this technique to measure surface displacement and build up full field 2D and 3D deformation vector fields and in-plane strain maps. It requires a set of markings, known as speckle, on the analyzed surface(s). For some biological materials like human skin [Marcellier et al., 2001], the natural random markings and patterns (pores, wrinkles, scars, etc.) may be used as speckle for image matching. In highly hydrated soft tissues like vaginal tissues (Figure 4.6-(d)), obtaining a proper random pattern is a difficult task. Ribeiro et al. [2009] tested a variety of techniques used for random speckle creation in *conventional* materials and verified that it was difficult to produce satisfactory results with vaginal tissues. Eventually, some acceptable results were obtained by the application of laser printer toner powder to the specimen's surface. The DIC system Aramis from GOM [2010] was efficient in capturing the displacement field (Figure 4.8-(c)) in different regions of the specimen. It was seen that the displacement was not uniform through the specimen's surface. Central region had higher displacements than border regions. The distribution of the elastic fibers (mainly elastin and collagen) on the different tissue layers, although presents some degree of orientation (Figure 3.6) is not uniform, fact that may explain to some extent



(a) Beginning of tension test. Measured region highlighted.



(b) Specimen during the mechanical the test.



(c) Displacement field measured in the vertical direction for the highlighted region in (a).

Figure 4.8: Measurement of displacement field in a prolapsed vaginal tissue specimen using DIC (Digital Image Correlation). From results published by Ribeiro et al. [2009].

the local variations detected.

The speckle creation was the most important technical limitation for an intensive use of DIC with highly hydrated soft tissues. Figure 4.8-(b) shows the specimen's surface affected by a washout effect. The toner powder used to provide the speckle, was dislocated by the pouring fluids, released while the specimen was stretched. These fluids contain some of the preserving saline solution, that was absorbed prior to the test, and also some remaining blood. This cumulative speckle pattern destruction makes DIC an inadequate technique to evaluate the displacement field in later stages of the test. Therefore, phenomena such as damage cannot be studied this way.

4.3.2 Video extensometry

Video extensometers performing principle is based on tracking i.e., on following the position of a given number of markings (Figure 4.9-(a)) or regions of interest (ROI) selected by the user. As with DIC, the video extensometry based analyses may be carried out after the experiment as many times as required, since video data is recorded. In plane marking displacements are tracked and the strains (between different marks) can be computed, allowing material properties like the Modulus (E) or Poisson's ration ν to be computed. This method has been successfully applied to study the anisotropic hyperelastic response of soft tissues by Peña et al. [2010]. The markings were made using a viscous resin without mixing the accelerator. This technique resists well to the washout effect described earlier. It is possible to measure the strain until the end of the test (complete failure of the sample), and have information on the damage zone. Though this is true in principle, the laser video extensometer Instron 2663 – 281 frequently lost track of the markings. Another problem was the size of the markings, a critical factor in small samples, as can be observed in Figure 4.9-(a). The lack of full-field strain information seems a shortcoming of video extensometry if compared with DIC. However, there are nowadays highly robust tracking systems, able to follow a large number of markings thus providing fairly complex strain maps.

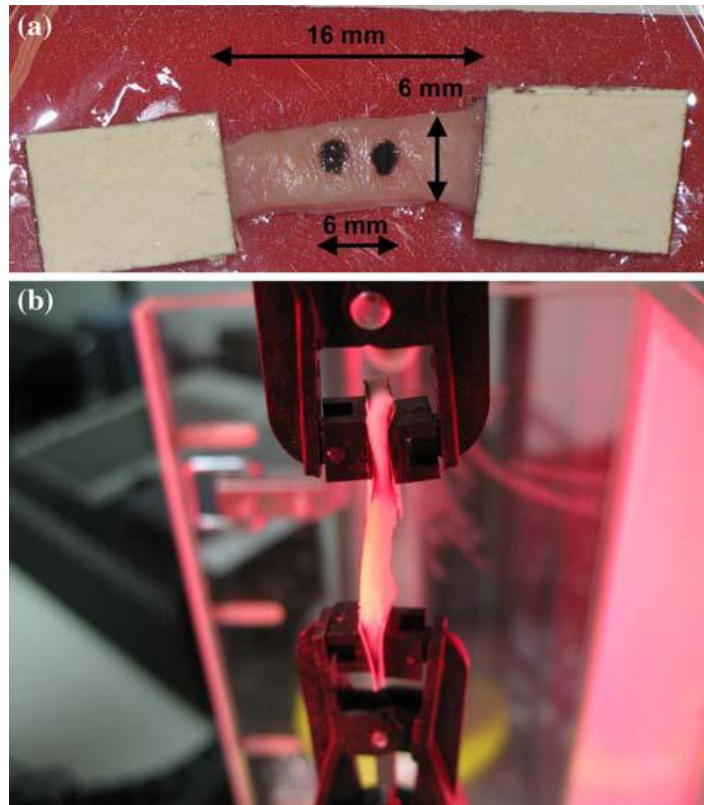


Figure 4.9: Tensile test using a video extensometer. (a) details of the sample marked using black resin. (b) Experimental setup. Strain measured using the laser video extensometer Instron 2663 – 281, with a megapixel sensor ($0.5 \pm 0.5\%$) high performance digital camera [Peña et al., 2010].

4.4 Mechanical test solutions

The large majority of the materials tested consisted on Human soft tissues (see Chapter 5). There are some technical problems arising from the manipulation and experimentation on such materials. A number of hygiene measures and protocol procedures adequate to biohazards and infectious biological agents were followed. The details of such procedures can be found in laboratory techniques literature [Gallagher and Wiley, 2008, App. 1A]. Prior to the mechanical test, the individual specimens were prepared from the original sample (Figure 4.10). The samples (either from cadaver or excised during surgery) presented a diversity of shapes and sizes (see Chapter 3). In addition, it was also established (for most tests) a preferential cutting direction in reference to the dominant fiber direction (see Figure 5.1) and a tissue defect exclusion policy. Tissue defects (like scars or deep forceps marks) were excluded because they could affect the local stiffness (ex. stress concentration effect) altering the mechanical test outcome.

These factors were accounted while cutting the specimens in strips (approximately rectangular) using scalpel and tweezers.

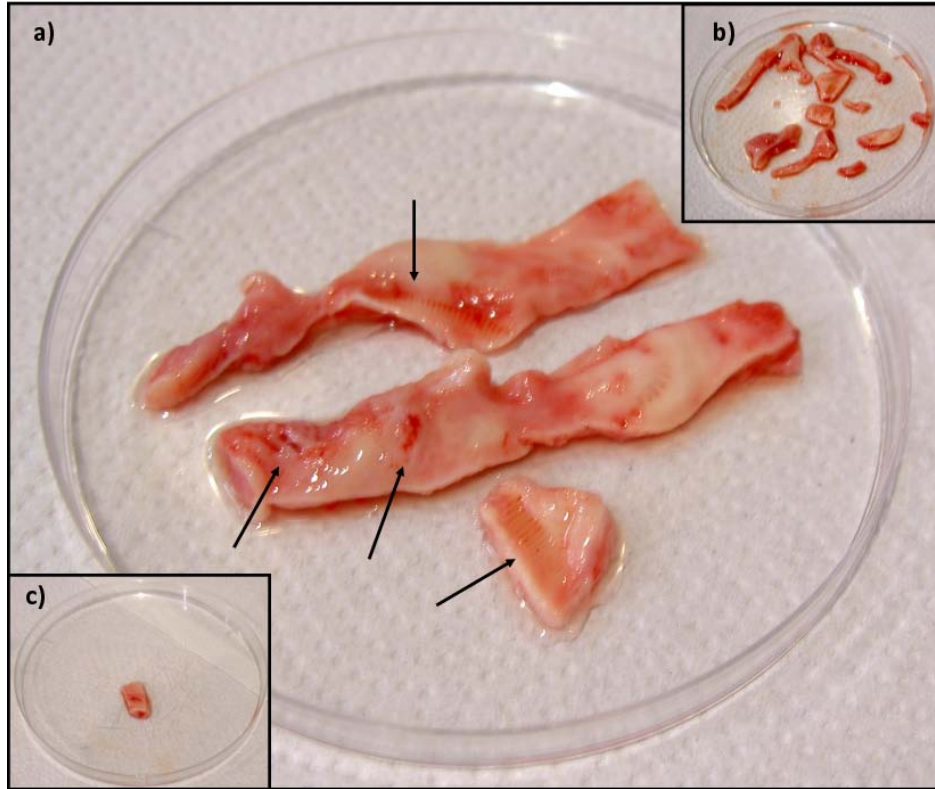


Figure 4.10: Specimen preparation details. The arrows point out tissue defects, both natural and made by the surgeon's forceps.

(a) Original tissue sample of prolapsed vaginal tissue. (b) Intermediary process of specimen cutting. (c) Final specimen.

4.4.1 Specimen preparation

The conventional approach to prepare the specimens for uniaxial tensile testing, involves the control of geometry by cutting *dog-bone* shaped specimens from the original sample, using casts, machining or other techniques to obtain a standard geometry. This effort towards test reproducibility was abandoned due to the reasons mentioned above, despite its potential to be a source of error.

4.4.2 The uniaxial tensile test

The uniaxial tensile test, also called simple tension test requires an experimental setup. There are commercial solutions available from companies like Bose® (ElectroForce® Systems Group), INSTRON® or MTS® however, the budget constraints of the research project led to an alternative solution. The scheme in Figure 4.11 represents

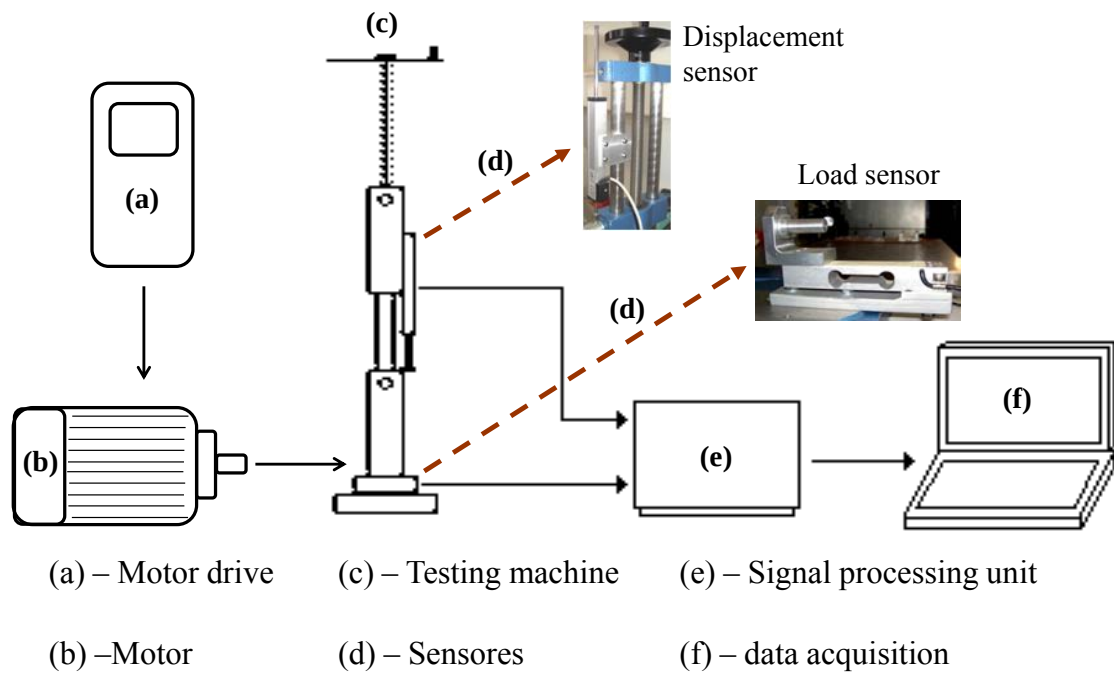


Figure 4.11: Diagram of the experimental setup.

of the scheme because it is an add-on to the basic system, despite its importance to the testing solution. The major technical details for each component are summarized in Table 4.1. The experimental approach used was validated for hyperelastic, rubber-like materials (silicon rubber) [Martins et al., 2006b], and later successfully applied to the testing of biomaterials (polypropylene meshes) [Afonso et al., 2008] and human soft tissues [Calvo et al., 2009; Martins et al., 2010b]. The mechanical testing of soft tissues requires particular care in every step of the process. The sample was mounted on the testing machine, using a set of aluminum grips (Figure 4.12-(b)). Due to the fragile nature of the tissues, it was required a grip support system (Figure 4.12-(a)) to mount the samples on the testing machine. This procedure minimizes sample damage prior to

Table 4.1: Components of the experimental setup.

Component	Fig. 4.11	Comm. Ref.	Main Characteristics
Motor drive	(a)	COMMANDER SK SKA1200025	AC variable speed drive 0.25 to 132 <i>kW</i>
AC Motor	(b)	AIS56B4 T2	Asynchronous, Three-Phase 0,09 <i>kW</i> ; 1340 revs/min 50 <i>Hz</i>
Gear Box	(b)	NMRV-025	Gear Box (speed reducer) Output torque: 13 <i>N.m</i> -1050 <i>N.m</i>
Testing support	(c)	-	-
Displ. sensor	(d)	-	LVDT
Load sensor	(d)	Huntleigh Model 1022	Low capacity (20 Kg) Single point load cell
Sig. proc. unit	(e)	NI CB-68LP	Unshielded 68-Pin I/O Connector Block
Data acquisition	(f)	NI DAQCard-6024E (PCMCIA)	Data acq. card; 200 <i>kS/s</i> , 12-Bit 16 Analog Input DAQ
		Laptop/desktop PC SO: Windows XP	PIV, 2.0 <i>GHz</i> , 512 <i>MB</i> Ram NI LabVIEW application
Video acquisition	-	2×CMOS-300k pix 320x240 QVGA	RGB 30 <i>fps</i> ; Lens:f=4.3 <i>mm</i> , F1.8 Focus: 0,2 <i>m</i> ↔ ∞

the test beginning. It also provides for an easier adjustment of the sample in the test rig (Figure 4.12-(d)). The black screws (Figure 4.12) in the support system maintain the sample with minimum stress and in correct position during the initial adjustments. A strip of velcro glued to sandpaper (Figure 4.12-(c)) placed between the sample and the grip surface minimizes slippage (sandpaper side), providing at the same time better contact and stress distribution on the grip-tissue contact region (velcro side). The assembly support shown in Figure 4.12 is taken to the testing machine, as seen in Figure 4.12-(d). The whole system sample-grips-support lays in suspension on the upper pin of the testing machine. Some initial span¹ adjustment may be required in order to place the system in place. It may be done manually (using the wheel on the top of the test rig) or with the motor drive controls.

When the system is properly placed, the assembly support is removed. Since the grips are free to rotate (Figure 4.12-(d)) on the pin supports, some minor misalignments of the specimen may be corrected.

The testing process did not involve the preconditioning of the tissues, however in prac-

¹Span in this context means the distance between the upper and lower support pins.

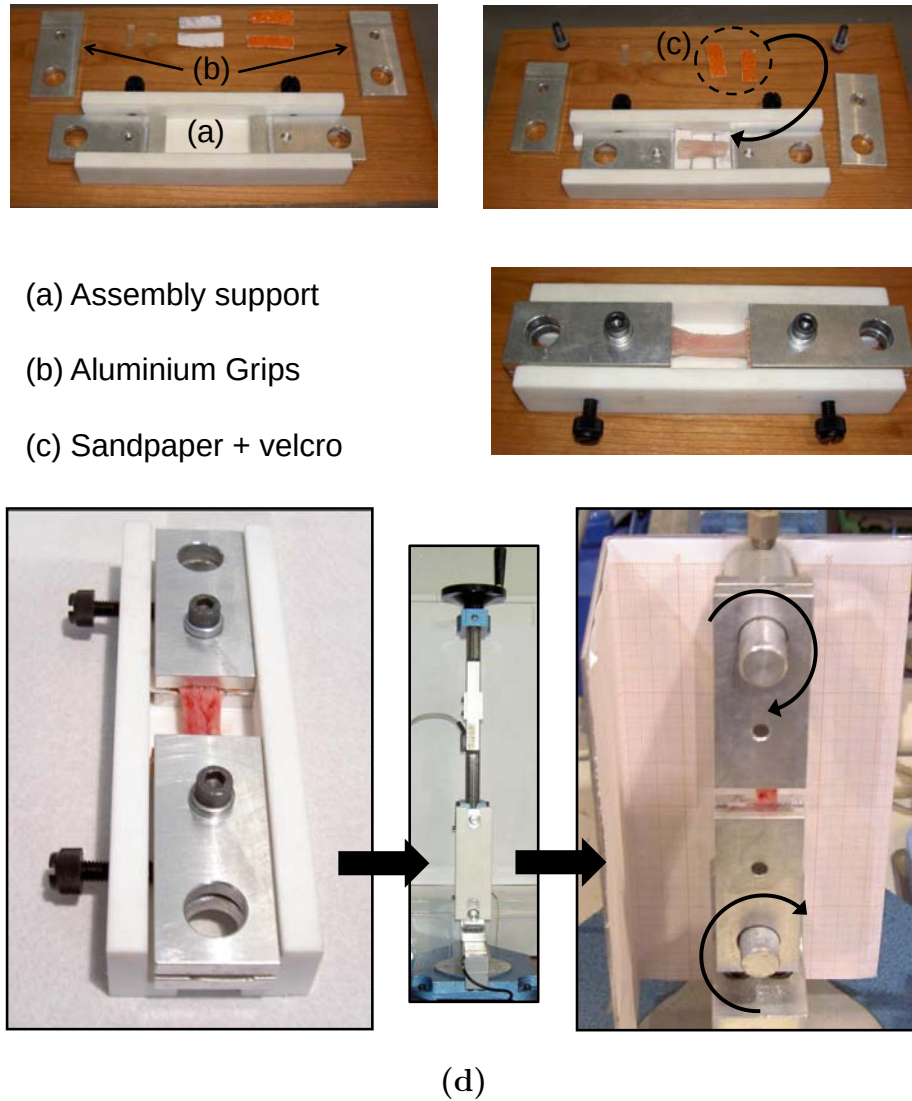


Figure 4.12: Sample preparation. (d) Mounting the sample in the test rig.

tice the weight of the inferior grips (330 g) act as a preload. This ensures that some residual stresses making the samples wrinkle or twist are compensated. Using image analysis, the initial length, width and thickness may be measured with better accuracy. It is known [Weiss et al., 2002] that using the distance between the grips to determine the initial length of the specimen (l_0) for calculations of stretch or strain, may be an error source in strain measurements (due to local damage and stress concentration on the grip-tissue boundary). These measurements should have been taken inside the homogeneous zone defined in Figure 4.5-B. However, the non-contact techniques available (discussed in Section 4.3) led to use the distance between grips for the estimation of l_0 (Figure 4.13).

All the tests were performed at room temperature ($\approx 20^\circ\text{C}$). No significant dehydra-

tion effects were observed along the duration of the tests ($5 \leftrightarrow 7 \text{ min}$).

4.4.3 Geometrical analysis - sample dimensions

The geometrical properties were acquired through digital image analysis. Using two USB webcams connected to the data acquisition PC, front and side view photographs were taken. The specimen dimensions were measured using the software ImageJ [Open-Source, 2007]. Figure 4.13 shows the image acquisition process. The grip di-

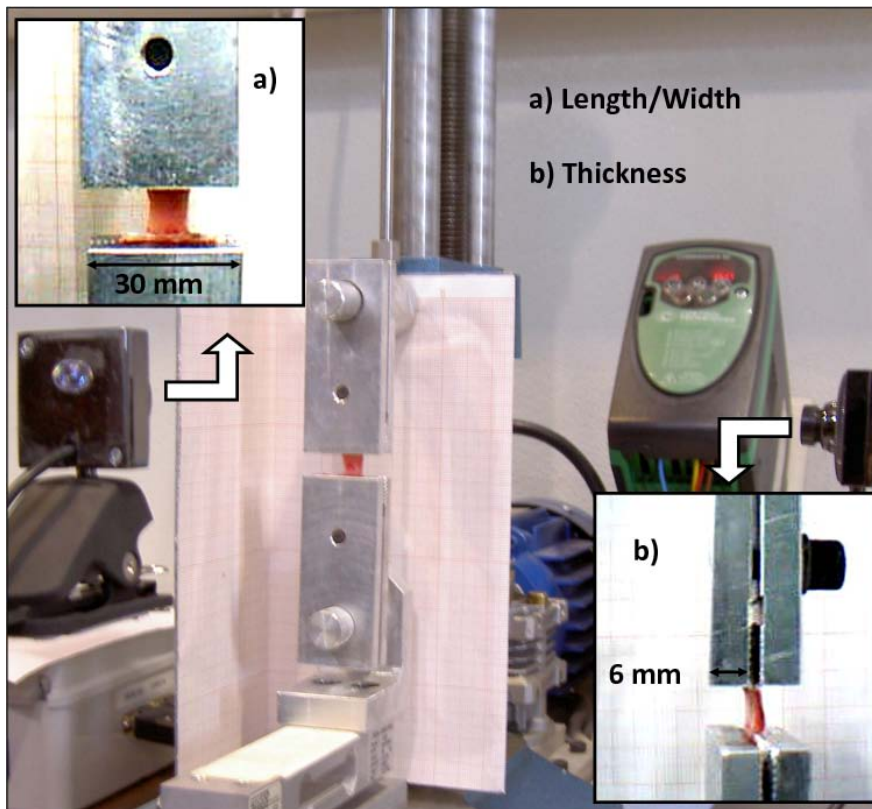


Figure 4.13: Image acquisition setup.

mensions act as references for the measurements taken. Although the tissue samples displayed significant thickness variations on different sites, the specimens were cut from *homogeneous* regions. The criteria followed on the present work was to take the initial thickness at half length if the thickness variations along the specimen's profile were small. The initial area A_0 , is estimated by,

$$A_0 = \text{Width} \times \text{Thickness} \quad (4.9)$$

The front-view camera record each mechanical test. The video was used to validate the test, since anomalous occurrences such as slippage or significant misalignment are easily detected.

4.5 Effects of uniaxial testing in soft tissues

The current chapter started by stressing the differences on the mechanical behavior of different types of materials. It was seen that for hyperelastic materials (like most soft tissues), small loads produced big stretches. In fact a direct comparison between the mechanical behavior of bladder wall tissue (Figure 4.14-(a)) and a ferritic steel (Figure 4.14-(b)), shows significant differences on the stress and strain ranges between the materials. These differences in mechanical behavior observed at a macroscopic scale, certainly have their microscopic counterparts. A detailed micro-structural analysis falls outside the current thesis's scope. However it is possible to draw some conclusions from the histologies presented in Chapter 3. Several untested specimens were fixed in paraffin wax and sectioned at 4 μm thickness slides in parallel directions to the test. Hematoxylin-Eosin and Mason's trichromic stains were applied to observe the tissue microstructure, Figure 3.6. It was seen that vaginal muscle is mostly smooth muscle oriented longitudinally (see Figure 3.6), with some circular bundles. The circular cell nuclei shape points out that the bundles run parallel to the test direction, being these the directions of anisotropy. For this reason, specimens were included in the study only if the resected tissue was in the longitudinal axis of the vagina.

Based on histologic results and the nature of the tissue (Figure 3.6), the vaginal tissue was assumed to behave as a transversely isotropic hyperelastic material.

Some of the micro-structural effects of the uniaxial tensile test can be seen in Figure 4.15. Comparing this histology with Figure 3.6-(c) it is seen that the dominant fiber direction is aligned with the test direction as expected. The circular fiber bundles (center of Figure 4.15) remain visible and *seem* identical to those in the tested samples. There are several ruptures in the tissue's matrix, transversally to the test (and fiber) direction. These microcracks [Lemaitre and Desmorat, 2005, Ch. 1] are a proof of material damage induced by the continuous stretch of the sample until rupture.

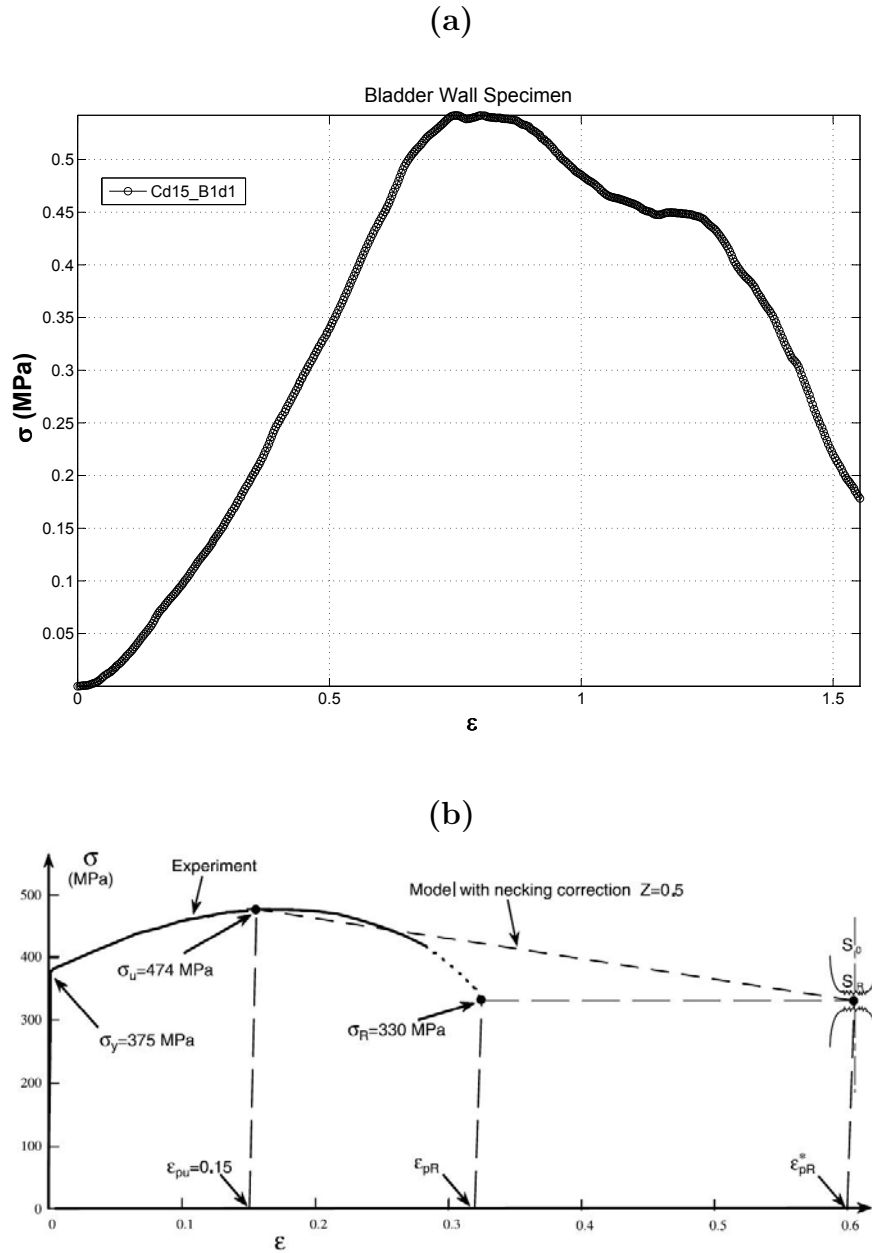


Figure 4.14: (a) Tension test on a cadaver bladder wall sample. (b) Tension test on ferritic steel at room temperature, from Lemaitre and Desmorat [2005, pg. 36].

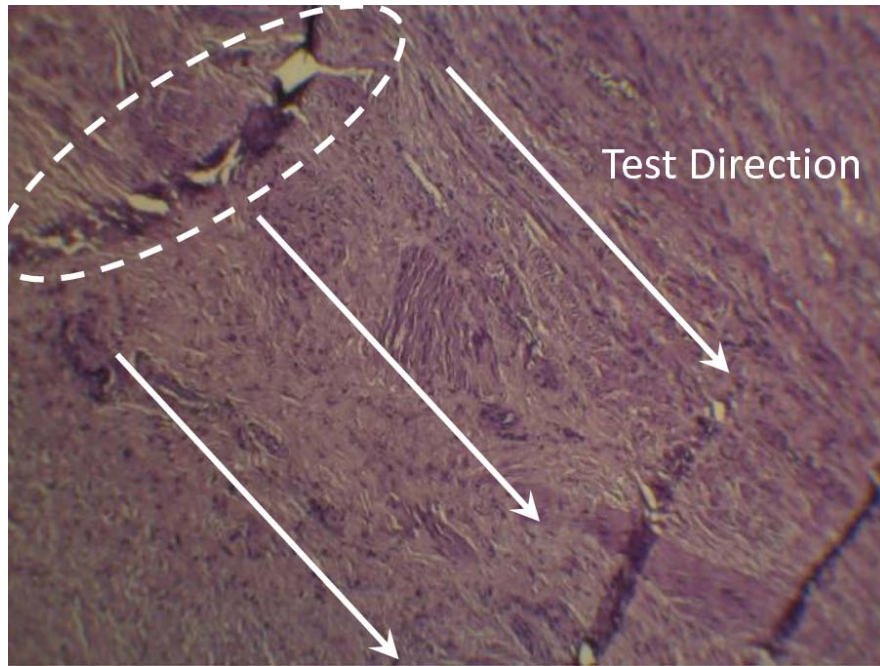


Figure 4.15: Effects of the uniaxial tensile test to a prolapsed vaginal tissue sample. Histology of a tested sample. Adapted from Calvo et al. [2009].

4.6 Conclusions

This chapter introduces the experimental set-up, as well as the experimental process used on the production of the results used in later chapters. The experimental set-up for uniaxial tensile tests, was designed and built by our group, being an important contribution to/from this thesis.

The typical sample geometry, fittings and grips were presented, as well as the typical curves obtained in uniaxial tests.

The present experimental approach was compared with other techniques by Feup or University of Zaragoza and discussed. The competitive methods showed limitations to analyze the samples considered in the present work. The technique proposed shows a more flexible set-up to analyze a broader set of tissues. Perhaps this flexibility produces a less accurate environment. However, this new approach may enable the study of more types of tissues and therefore fulfill the aspect of building a more complete database of results.

The results of the experimental approach followed are includes in Chapters 5, 6 and in Appendix A.

Chapter 5

Experimental Results

5.1 Introduction

The experimental component is the principal element of the research here presented. All the theoretical interpretations and in particular the hyperelastic models proposed are based in experimental data produced by the author of this thesis and his colleagues. There was a synthesis effort on the preparation and presentation of the experimental results that follow. A significant amount of experimental data, although being treated and analyzed, had to be discarded from the studies here presented mainly due to lack of proper information on the patients. The data shown in Tables 5.9 to 5.15 was collected according to the techniques discussed in Chapter 4. The physical quantities presented (Table 5.1) were extracted directly from the experimental data (see Figures 4.3 and 5.15 and Section 4.2.1 for details).

To ease the consultation of the research data, and in particular sample, specimen or group identification, all the samples were named following the nomenclature conventions detailed in Table 5.2. The diagrams in Figure 5.1 show the fiber direction in the samples according to the designation longitudinal (L) and transversal (T). The fiber direction is determined by visual inspection in highly oriented fibered materials.

Table 5.1: Physical quantities measured from:

Stress-stretch curve Soft tissues
Load-displacement curve Polypropylene meshes

Physical quantity	Symbol	Unit
Stress-stretch curve		
Tensile strength	σ_{max}	MPa
Ultimate stretch	λ_U	Nondimensional
Strain energy density	U_S	$nJ.m^{-3}$
Tangent modulus	E_t	MPa
Secant modulus	E_s	MPa
Yield strength	σ_Y	MPa
Load-displacement curve		
Ultimate displacement	d_U	m
Ultimate load	L_U	N
Secant modulus [0 $\leftrightarrow$ 500] g	E_I	N/m
Tangent modulus [500 $\leftrightarrow$ 2000] g	E_{II}	N/m

Table 5.2: Nomenclature conventions for the experimental data presented.

General Rule:		(Sample ID)_(Tissue type)(Specimen nr.)(Orientation)			
Example:		Sample of posterior vagina of cadaver nr. 6 cut along longitudinal direction. The 2 nd specimen tested in the session.			
Cd06_PV2L					
Sample				Specimen	
Type	Abbrev	nr.	ID	nr.	Orientation
Cadaver	Cd	(##)	Cd(##)	(#)	L/T/L1/L2/d1/d2
Vivo	Vv	(###)	Vv(###)	(#)	L/T
Mesh	Mh	—	Mh_(<i>type</i>)	(#)	—
Tissue type				Fibers	
Abbrev		Abbrev			
B	Bladder	L	Longitudinal (to fiber direction)		
F	Abdominal fascia	T	Transversal (to fiber direction)		
RL	Round ligaments	L1	Direction of the 1 st fiber family		
USL	Uterosacral ligaments	L2	Direction of the 2 nd fiber family		
LA	Levator ani muscle		(no dominant fiber direction)		
AV	Anterior vaginal wall	d1	Arbitrary direction 1		
PV	Posterior vaginal wall	d2	Arbitrary direction 2 ($\perp$ to d1)		
Mesh type					
Abbrev					
Aris	Aris TM				
Avlt	Avaulta TM				
TVTO	TVTO TM				
AuSt	Auto Suture TM				
Urtx	Uretex TM				

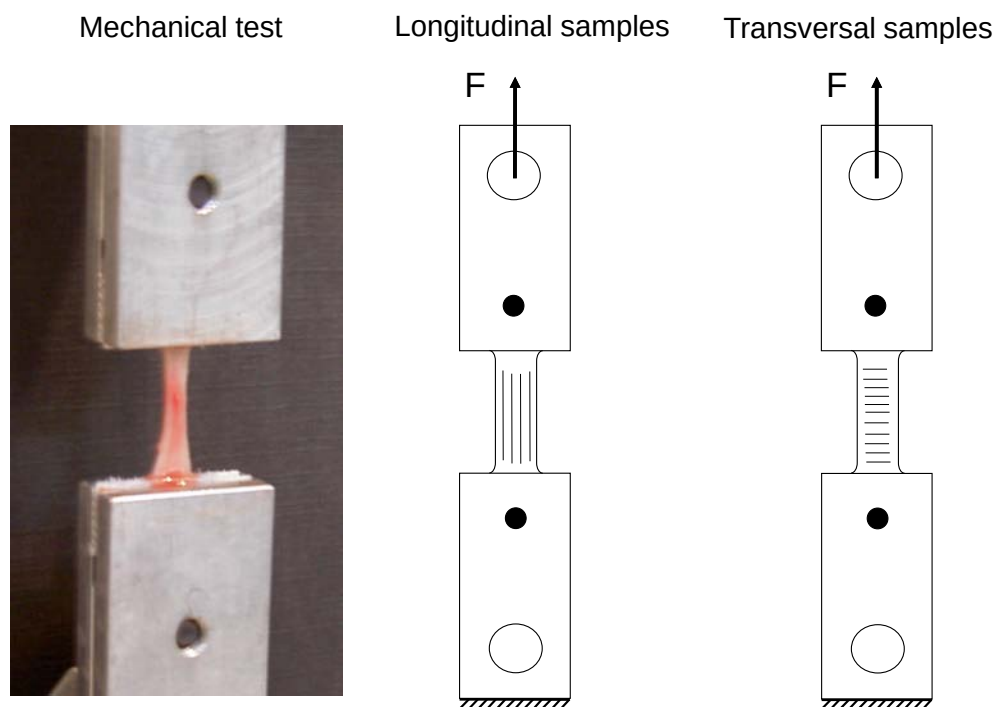


Figure 5.1: Dominant direction of the fibers, relative to the mechanical testing. Longitudinal (L) and transversal (T). $\mathbf{F}$ is the load applied during the test.

5.1.1 Human tissue samples

The majority of the experimental data presented in this chapter, was produced during the mechanical testing of human tissue samples. The tissues were from both living patients and cadavers.

Living patients were submitted to pelvic surgeries for prolapse correction. During surgery, as a consequence of the anatomical reconstructive procedures, there were portions of vaginal tissue excised. These tissues, normally incinerated, in compliance with standard waste disposable procedures of Health institutions, constitute the samples of the POP patient group. The Ethical Research Ethics Committee guidelines of *Hospital de São João* (Porto, Portugal) were followed throughout the study. There was also the informed consent provided by all patients involved in the investigation. The patients were preoperatively evaluated in terms of history, physical examination and a urodynamic study was performed when indicated. The Pelvic Organ Prolapse Quantification (POP-Q) system [Bump et al., 1996] was used to quantify the pelvic organ prolapse severity on all the patients. Table 5.3 presents the list of patients (valid data) and

information available. Patients were excluded when: the tissue sample was too small or too damaged to be tested; there was not enough data (on the patient) to support its inclusion in the study. Patient age ranged from 38 to 84 years, parity ranged from 0 to 10.

The mechanical tests performed in this thesis belong to the category of destructive tests. This means that the specimens tested are completely (at least irreversibly) damaged. Although, there are other techniques to infer/estimate the biomechanical properties of soft biological tissues without damage [Kauer, 2001; Kauer et al., 2002; Epstein et al., 2007; Cox et al., 2008], the information acquired is not adequate for an accurate estimation of the Biomechanical properties. Destructive testing procedures such as uniaxial, biaxial or compression tests, cannot be carried out in (or with samples taken from) living patients. This situation led to the establishment of a research protocol with the Forensic Pathology Service of the *North Branch of INML, I.P.*. Female cadavers with ages ranging from 18 to 65 years, without clinical noticed pelvic floor dysfunctions or POP were included in the study. The tissue samples collected were from the bladder wall, the *Levator ani* muscle, Round Ligaments (RL), Uterosacral ligaments (USL), anterior and posterior vaginal wall and abdominal fascia. The sample collection was carried out at the end of the normal forensic autopsy procedures, in agreement with a procedure approved by the direction board of the Forensic Pathology Service of the *North Branch of INML, I.P.*. The cadavers were not formolised but refrigerated for preservation. Table 5.4 presents the list of patients and the information available/considered for each patient.

In Tables 5.3 and 5.4, gravidity (G) is the total number of pregnancies and parity (P) is the outcome of completed previous pregnancies as defined by Hamilton-Fairley [2004, Ch.9, pg. 107].

In Table 5.4, the calculation of the body mass index (BMI) [Mommensen and Foldspang, 1994] for the cadaver population was made using (5.1)

$$BMI = \frac{Weight (Kg)}{Height (m)^2} \quad (5.1)$$

Table 5.3: Patients with pelvic organ prolapse: 70 cases studied; 40 cases considered.

Tissues studied: Vagina - anterior and posterior wall									
Information available: Yes=1 No=0									
G gravidity									
P parity									
Mnp menopause status									
ΔT Mnp duration (approximate) of menopausal state									
HT hormonal therapies									
POP Ant pelvic organ prolapse of the anterior vaginal wall									
POP Post pelvic organ prolapse of the posterior vaginal wall									
SUI stress urinary incontinence									
Patient	Age	G	P	Mnp	ΔT Mnp	HT	POP	POP	SUI
	(Years)			(Y/N)	(Years)	(Y/N)	Ant	Post	(Y/N)
Vv017	79	1	1	1	34	0	3	3	0
Vv018	64	3	3	1	24	0	3	1	0
Vv019	72	4	4	1	22	0	1	3	0
Vv020	49	3	3	1	2	0	3	0	1
Vv023	74	2	2	1	29	0	2	3	0
Vv024	59	5	5	0	—	—	2	0	1
Vv025	78	6	6	1	33	0	3	2	0
Vv026	84	4	4	1	32	0	2	3	0
Vv027	67	2	2	1	13	0	3	2	0
Vv028	38	4	4	0	—	—	2	0	1
Vv029	66	2	2	1	11	1	3	0	0
Vv030	68	2	2	1	23	0	0	3	1
Vv031	57	4	2	1	7	0	2	0	1
Vv032	60	4	4	1	13	1	2	0	1
Vv033	68	4	4	1	25	0	3	2	1
Vv038	56	4	3	1	6	1	2	0	1
Vv048	70	—	—	1	—	0	—	—	—
Vv049	66	3	3	1	12	1	3	0	1

(Continued on Next Page...)

(Table 5.3, cont.)

Patient	Age (Years)	G	P	Mnp (Y/N)	ΔT Mnp (Years)	HT (Y/N)	POP Ant	POP Post	SUI (Y/N)
Vv050	44	4	4	0	—	—	1	2	1
Vv051	83	10	9	1	43	0	3	2	1
Vv052	65	0	0	1	17	1	3	0	0
Vv053	69	0	0	1	19	1	2	2	0
Vv054	45	2	0	0	—	—	1	2	1
Vv055	57	3	3	1	7	1	3	0	1
Vv057	56	2	2	1	6	0	1	0	0
Vv060	75	3	3	1	30	0	2	1	0
Vv062	68	3	3	1	—	0	2	1	0
Vv066	49	2	2	0	—	—	2	0	0
Vv070	61	4	4	1	19	0	0	3	1
Vv073	60	2	2	1	7	0	3	1	0
Vv074	68	2	2	1	—	0	2	1	0
Vv086	49	3	3	0	—	—	2	3	0
Vv090	67	2	2	1	14	0	2	2	0
Vv091	53	3	3	1	6	0	2	2	0
Vv092	56	2	2	1	—	—	3	3	1
Vv093	62	1	1	1	—	0	3	3	0
Vv094	68	7	7	1	—	0	3	3	0
Vv096	73	3	3	1	23	0	3	3	0
Vv102	60	2	2	1	8	1	2	2	0
Vv104	70	10	10	1	18	0	2	1	0

Table 5.4: Cadaver population: 18 cases studied; 15 cases considered.

Tissues studied:		Bladder wall Levator ani muscle Ligaments - round and uterosacral Vagina - anterior and posterior wall Abdominal fascia.				
Information available:		Yes=1 No=0				
	Mnp	menopause status				
	BMI	body mass index				
Patient	Age	Mnp (Y/N)	Parity	Height (<i>m</i>)	Weight (<i>Kg</i>)	BMI (<i>Kg/m</i> ²)
Cd01	51	—	1	1.58	62.4	24.996
Cd02	51	—	1	1.56	55.0	22.600
Cd03	51	—	0	1.63	78.0	29.358
Cd04	47	0	1	1.68	91.0	32.242
Cd05	43	—	2	1.59	80.0	31.644
Cd06	40	—	1	1.50	57.8	25.689
Cd07	33	1	0	1.67	66.0	23.665
Cd08	36	1	0	1.54	58.0	24.456
Cd09	18	1	0	1.79	104.0	32.521
Cd10	38	1	1	1.63	89.0	33.498
Cd11	59	0	—	1.65	67.5	24.793
Cd12	40	—	1	1.58	53.0	21.231
Cd13	51	—	2	1.60	66.0	25.781
Cd15	54	1	—	1.51	—	—
Cd17	65	0	—	1.59	65.0	25.711

5.1.2 Studies performed

The results presented in this chapter (Tables 5.9 to A.1), may be analyzed from different perspectives. Given the multidisciplinary scope of the current thesis, engineering and in some cases clinical analyses may be conducted. All the work performed pays unquestionable tribute to the physics and engineering background of the author and his supervisors. However the collaboration with several medical doctors gave the research a broader scope.

The first study here presented is “The influence of POP (Pelvic Organ Prolapse) on the biomechanical properties of vaginal tissue” (Section 5.2). The variations of the biomechanical properties (Section 4.2.1 and Table 5.1) in cadaver (“normal” vaginal tissue) and vivo (prolapsed vaginal tissue) populations were investigated. The characterization of the two patient groups considered is presented in Table 5.3 for patients

with prolapse (POP) and Table 5.4 for cadavers (non-POP). A clinical interpretation of the results was proposed.

The second study is the “Uniaxial mechanical behavior of Human female bladder” (Section 5.3). It was not possible to obtain bladder samples from living patients. Therefore, thirteen bladder tissue samples from female cadavers without known pelvic disorders were tested. The mechanical tests were analyzed and a clinical interpretation of the results was produced.

The third study “Round and Uterosacral Ligaments Strength” investigates the tensile biomechanical properties of Round and Uterosacral ligaments. The ligament samples were obtained from fifteen female cadavers without known pelvic disorders. The mechanical tests were analyzed and a clinical interpretation of the results was produced. The fourth study considered [Afonso et al., 2008], compares the biomechanical properties (tensile stiffness and flexural stiffness) of different meshes used in urogynecological surgery, and one standard mesh. Two mechanical tests were carried out. Five different commercial meshes were considered: Aris™, Auto Suture™, Avaulta™, TVTO™ and Uretex™. The methods used consisted on uniaxial tensile test (tensile stiffness) and tape ring tests i.e., experimental continuous compression of the mesh loops (flexural stiffness). This study shows that there are significant differences on the mechanical properties between urogynecological surgery meshes.

5.2 The influence of POP on the biomechanical properties of vaginal tissue

5.2.1 Introduction

Studies have been addressed to evaluate biomechanical properties of pelvic ligaments and vaginal tissue [Cosson et al., 2003a, 2004; Goh, 2002; Lei et al., 2007; Rubod et al., 2008]. These studies are also important for the development of prostheses utilized in vaginal surgery [Goh, 2003]. However, the biomechanical repercussions of the overloaded vaginal tissues caused by POP remain unclear. Therefore, the aim of this study is to compare the biomechanical properties of vaginal tissue in POP and non-POP women. For this purpose, based on a uniaxial tensile test, following the techniques and processes outlined in Chapter 4, the factors influencing the tangent modulus and tensile strength of vaginal tissue were investigated.

5.2.2 Materials and methods

Forty (40) patients submitted to surgery for POP were prospectively evaluated (Table 5.3). The mean age of the patients was 63.3 ± 1.7 years (ranging from 38 to 84 years) and parity of 3.3 ± 0.3 (ranging from 0 to 10). The study was conducted following the Ethical Research Ethics Committee guidelines of *Hospital de São João* (Porto, Portugal) including an informed consent provided by all patients involved in the investigation. All patients were preoperatively evaluated by history, physical examination and an urodynamic study when indicated. The severity of pelvic organ prolapse was assessed in all patients, preoperatively, by the Pelvic Organ Prolapse Quantification (POP-Q) system [Bump et al., 1996].

The control group was composed of 15 female cadavers without clinical noticed pelvic floor dysfunctions or POP (Table 5.4). The samples were not formalised but refrigerated for preservation. The mean age of the cadavers was 45.1 ± 3.0 years (ranging

from 18 to 65 years) and parity of 0.8 ± 0.2 (ranging from 0 to 2). The vaginal tissue was collected in agreement with a procedure approved by the direction board of the Forensic Pathology Service of the *North Branch of INML, I.P.*.

Vaginal tissue samples

The tissue was excised in the longitudinal axis of the middle third vagina in the anterior and posterior vaginal walls. In the POP group the vaginal tissue was resected during surgery for anatomic restoration. In the cadavers (non-POP group), the dissection of the samples was made at the end of the normal forensic autopsy procedures. The specimens, of rectangular shape and approximately regular, were prepared according with the procedures detailed in Section 4.3. The samples were preserved in a saline bath and kept at 5° Celsius until 15 minutes before the mechanical tests were performed. The storage time of the soft tissues (until testing) did not exceed 6 hours, and for most of the samples it was inferior to 4 hours.

Geometrical properties were acquired through digital image analysis with the techniques presented in Section 4.4.3.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software program version 16.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of the data was verified using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The variables were described as means with their respective ranges and standard error of the mean. The statistical differences between the groups were tested using chi-square, ANOVA, Student's paired and unpaired t-test as required. Subsequently, using linear regression, it was possible to create a model to predict the factors influencing the biomechanical measurements in vaginal tissue. Correlations were calculated using the Pearson correlation coefficient. Significance level was established as $p < 0.05$.

Mechanical properties assessment

The biomechanical properties were evaluated by means of uniaxial tensile tests. To perform the tests, the tissue specimens were assembled on the testing machine using the tools and techniques described in Section 4.3. The mechanical testing process has been validated for different hyperelastic materials on a previous work [Martins

et al., 2006b]. The load (N) and displacement (mm) data were acquired using a load cell; ($F_{max} = 200 N$) and a displacement sensor. A constant displacement rate of $5 mm.min^{-1}$ was maintained throughout the test and for all tests. Both load and displacement data were recorded till complete specimen rupture. One of the webcams previously employed for geometry measurement was used to record each mechanical test on video. This procedure enabled the quality control of each test, by a posteriori elimination of tests where an inadequate alignment or slippage was detected.

The data available for prolapsed vaginal tissue (POP group) is from samples in the longitudinal (L) direction (Table 5.5). The experimental results for the cadaver samples, non-POP group are presented in Table 5.6. These results are from longitudinal (L) and transversal (T) samples, however in this work only the longitudinal data was used, since it compares directly with the POP group data.

The biomechanical properties extracted from the experimental results, are summarized in Table 5.1 and explained in detail in Figure 4.3. The properties considered on the scope of the present study are the tangent modulus (E_t) and tensile strength (σ_{max}).

Table 5.5: Experimental data extracted from prolapsed vaginal tissue. Anterior vaginal wall and posterior vaginal wall. Specimens taken from the original samples in the longitudinal (L) direction (see Table 5.2 and Figure 5.1).

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Vv017_AV5L	4.087	1.464	0.668	12.200	3.735
Vv017_AV7L	4.012	1.400	0.590	16.149	3.545
Vv018_AV2L	7.314	1.824	1.995	18.618	6.181
Vv018_AV3L	5.490	1.780	1.711	14.014	4.080
Vv018_AV4L	5.546	1.810	1.695	12.202	4.305
Vv019_PV2L	7.715	1.555	1.556	21.737	6.982
Vv019_PV4L	6.355	1.644	1.748	16.938	5.368
Vv019_PV6L	4.518	1.561	0.992	15.296	3.657
Vv020_AV1L	5.910	1.874	1.700	13.521	5.538
Vv020_AV3L	5.267	1.893	2.040	11.939	4.055
Vv020_AV6L	4.194	1.658	1.052	11.379	3.608
Vv023_PV1L	5.831	1.826	2.192	12.012	4.526
Vv024_AV1L	3.758	1.612	0.954	9.310	2.865

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(Table 5.5, cont.)

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Vv024_AV2L	3.946	1.580	0.879	11.062	3.719
Vv025_AV3L	4.874	1.675	1.482	11.905	4.163
Vv025_AV4L	3.770	1.525	0.858	12.535	2.815
Vv026_PV1L	5.272	1.741	1.545	14.140	4.441
Vv026_PV2L	4.024	1.510	0.846	16.755	3.413
Vv027_AV1L	2.957	1.635	0.713	10.453	2.414
Vv027_AV2L	2.826	1.735	0.858	6.615	2.454
Vv028_AV1L	2.242	2.015	1.082	4.498	1.598
Vv028_AV2L	2.336	1.458	0.486	8.504	1.681
Vv029_AV1L	2.468	1.647	0.656	7.154	2.067
Vv030_PV1L	4.878	1.526	1.078	17.820	3.770
Vv030_PV2L	5.562	1.699	1.786	13.424	4.341
Vv030_PV3L	4.661	1.653	1.181	14.360	3.936
Vv030_PV4L	4.714	1.965	1.715	11.911	3.477
Vv031_AV3L	6.860	1.634	1.329	22.056	6.559
Vv031_AV6L	9.207	2.064	3.531	16.103	8.381
Vv032_AV1L	8.869	1.713	2.210	23.344	8.232
Vv032_AV2L	7.686	2.332	3.930	9.191	7.035
Vv033_AV1L	8.314	2.183	3.889	12.273	6.625
Vv033_AV2L	8.299	1.808	2.717	17.794	6.941
Vv038_AV1L	8.756	1.810	2.678	17.359	7.706
Vv038_AV2L	9.446	1.949	3.490	17.679	8.750
Vv038_AV3L	7.454	1.657	1.744	20.278	7.082
Vv038_AV4L	10.617	1.850	3.279	21.304	9.073
Vv048_PV1L	10.223	2.204	4.854	14.214	8.211
Vv049_AV2L	4.105	1.514	0.759	12.913	3.866
Vv050_PV2L	2.112	1.424	0.354	10.180	1.897
Vv051_AV1L	4.063	1.673	0.903	10.926	3.925
Vv051_AV3L	4.180	1.929	0.858	11.815	3.996

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(Table 5.5, cont.)

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Vv052_AV1L	3.526	1.565	0.900	9.583	2.860
Vv053_AV1L	1.946	1.661	0.568	4.885	1.540
Vv053_AV2L	3.302	2.003	1.346	6.420	2.593
Vv054_PV2L	3.846	2.064	1.767	5.922	3.083
Vv054_PV3L	4.195	1.935	1.730	7.745	3.533
Vv055_AV1L	5.747	1.837	1.745	13.451	4.887
Vv055_AV2L	3.241	1.977	1.450	5.490	2.051
Vv055_AV3L	4.270	1.983	1.896	7.353	3.031
Vv057_AV1L	5.223	1.892	1.567	12.960	4.632
Vv057_AV3L	3.677	1.818	1.204	7.452	2.505
Vv060_AV1L	1.339	1.974	0.711	5.807	0.727
Vv060_AV3L	2.087	1.439	0.352	8.698	1.980
Vv060_AV4L	1.991	1.644	0.595	5.696	1.693
Vv062_AV1L	5.569	1.525	1.226	16.358	4.664
Vv062_AV2L	4.368	1.544	0.817	14.792	4.235
Vv066_AV1L	4.593	1.602	1.095	11.954	4.035
Vv066_AV2L	2.934	1.721	0.862	6.283	2.517
Vv070_PV3L	7.152	1.691	1.712	19.172	5.898
Vv070_PV4L	7.577	1.714	2.491	17.629	6.985
Vv070_PV5L	7.153	1.824	2.341	16.305	5.913
Vv070_PV6L	6.270	1.690	1.438	17.629	5.488
Vv073_AV2L	8.006	1.753	2.583	17.301	6.391
Vv073_AV3L	8.211	1.695	1.981	18.838	8.025
Vv073_AV4L	9.775	1.902	3.126	16.854	8.991
Vv073_PV1L	2.303	1.575	0.597	6.440	1.927
Vv073_PV5L	3.540	1.754	0.790	9.865	3.332
Vv073_PV6L	3.531	1.443	0.610	12.975	3.328
Vv074_AV1L	2.580	1.315	0.360	15.415	2.056
Vv074_AV2L	2.486	1.328	0.381	15.106	1.868

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(Table 5.5, cont.)

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Vv074_AV3L	6.294	1.485	1.156	23.501	5.647
Vv074_PV5L	4.620	1.436	0.877	21.224	3.494
Vv086_AV1L	4.340	1.787	1.661	13.888	2.970
Vv086_AV2L	3.391	1.563	0.917	13.586	2.818
Vv086_PV4L	2.292	1.542	0.553	7.993	1.782
Vv086_PV5L	3.206	1.387	0.450	14.797	2.879
Vv090_AV1L	1.942	1.456	0.286	8.254	1.850
Vv090_PV3L	1.118	1.555	0.257	3.815	0.941
Vv090_PV4L	2.181	1.546	0.506	8.130	1.843
Vv091_AV1L	6.665	1.511	1.237	21.673	6.248
Vv091_AV2L	10.964	1.666	2.410	30.115	9.697
Vv091_PV3L	4.717	1.640	1.129	13.517	4.059
Vv092_AV2L	3.849	1.551	0.762	13.671	3.357
Vv092_AV3L	7.363	1.529	1.324	27.869	6.956
Vv092_PV5L	2.065	1.356	0.307	9.608	1.849
Vv092_PV6L	2.435	1.525	0.465	8.601	2.186
Vv093_AV1L	16.449	2.407	7.674	22.955	15.352
Vv093_AV2L	17.702	1.799	5.372	34.006	15.259
Vv093_PV3L	2.487	1.466	0.534	8.394	1.876
Vv093_PV4L	3.014	1.862	1.076	5.354	2.571
Vv094_AV1L	3.849	1.785	1.171	7.677	3.557
Vv094_PV3L	3.252	1.615	0.776	10.469	3.054
Vv096_AV1L	2.501	1.381	0.316	12.432	2.282
Vv096_AV2L	2.769	1.429	0.401	12.906	2.599
Vv096_PV4L	2.464	1.616	0.630	7.844	2.015
Vv102_AV1L	5.742	1.917	1.887	11.108	5.346
Vv102_AV2L	6.022	2.436	3.027	9.904	5.177
Vv102_PV4L	4.709	1.685	1.290	11.485	4.067
Vv104_AV2L	4.944	1.449	0.892	14.256	4.870

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(Table 5.5, cont.)

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Vv104_PV3L	3.571	1.725	0.955	8.057	3.040
Vv104_PV4L	3.669	1.625	0.981	9.791	3.164

Table 5.6: Experimental data extracted from cadaveric tissue. Anterior (AV) and posterior (PV) vaginal wall. Specimens taken from the original samples in the longitudinal (L) and transversal (T) directions (see Table 5.2 and Figure 5.1).

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Cd01_AV2L	1.909	1.691	0.606	4.013	1.647
Cd01_PV1L	2.174	1.875	0.967	4.772	1.435
Cd02_AV1L	1.307	2.147	0.929	3.651	0.652
Cd02_PV1L	1.958	1.366	0.323	11.275	1.519
Cd03_AV1L	1.849	2.082	0.904	2.703	1.431
Cd03_PV1L	3.574	1.647	1.049	9.413	2.819
Cd04_AV1L	1.640	1.532	0.371	5.832	0.992
Cd04_PV1L	3.355	1.431	0.465	14.306	3.274
Cd04_PV2T	2.127	2.118	1.302	5.323	1.163
Cd05_AV1L	1.590	2.498	1.055	1.664	0.973
Cd05_AV2T	1.590	1.719	0.398	4.413	1.531
Cd05_PV1L	5.350	1.620	1.251	15.496	4.897
Cd05_PV2L	6.663	1.841	2.077	13.114	5.899
Cd05_PV3T	1.187	1.592	0.275	4.045	0.961
Cd06_AV1L	3.388	1.499	0.756	11.213	2.780
Cd06_AV3T	2.525	1.688	0.714	5.880	2.015
Cd06_PV1L	4.130	2.024	1.492	8.427	3.734
Cd06_PV2L	4.096	1.750	1.140	13.596	3.333
Cd06_PV3T	5.340	1.759	1.631	12.919	4.702
Cd07_AV1L	1.072	1.912	0.516	2.674	0.609
Cd07_AV2T	1.771	1.675	0.466	4.681	1.495
Cd07_PV1L	3.590	1.607	0.696	12.100	3.397
Cd07_PV2T	1.357	1.692	0.506	3.957	0.818

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(Table 5.5, cont.)

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Cd08_AV1L	1.969	1.730	0.539	4.732	1.632
Cd08_AV2T	0.875	1.779	0.228	2.751	0.682
Cd08_PV1L	2.453	1.808	0.785	5.093	1.502
Cd08_PV2L	2.002	1.600	0.542	5.024	1.908
Cd08_PV3T	1.447	1.930	0.459	3.355	1.279
Cd09_AV1L	2.069	1.579	0.463	5.600	1.523
Cd09_PV1L	2.594	2.374	1.529	3.097	1.367
Cd09_PV2T	2.547	2.010	1.170	3.681	1.213
Cd10_AV1L	2.518	1.830	0.836	4.091	2.172
Cd10_AV2L	2.773	1.963	1.257	4.329	1.814
Cd10_PV2L	3.880	1.772	1.122	7.705	3.669
Cd10_PV3T	3.892	1.821	1.435	7.295	2.954
Cd11_AV1L	3.314	1.573	0.795	9.452	2.556
Cd11_PV1L	2.226	1.395	0.360	10.022	1.834
Cd11_PV2T	6.851	1.587	1.215	23.380	6.342
Cd12_AV1L	6.397	1.677	1.753	15.033	5.207
Cd12_PV1L	7.411	1.571	1.713	18.206	6.613
Cd12_PV2L	6.835	1.638	1.662	17.468	6.194
Cd12_PV3T	7.350	1.548	1.389	22.603	6.829
Cd13_AV1L	3.502	1.642	0.974	8.052	3.220
Cd13_AV2T	6.216	2.006	2.764	7.330	6.053
Cd13_PV1L	3.321	1.473	0.655	9.693	3.198
Cd13_PV2L	4.185	1.616	0.841	11.392	4.041
Cd13_PV3T	2.470	1.607	0.622	7.110	1.984
Cd15_AV1L	5.796	1.650	1.824	15.713	3.711
Cd15_PV1L	3.772	1.543	0.937	11.296	2.601
Cd15_PV2T	5.229	1.866	1.653	13.389	4.793
Cd17_AV1L	1.323	1.510	0.349	5.252	0.988
Cd17_PV1L	2.556	1.424	0.300	13.610	2.269

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(Table 5.5, cont.)

Sample	σ_{max} (MPa)	λ_U	U_S (nJ.m ⁻³)	E_t (MPa)	σ_Y (MPa)
Cd17_PV2T	1.005	1.375	0.144	4.780	0.907
Cd17_PV3L	2.614	1.449	0.366	12.043	2.255

5.2.3 Results

As illustrated in Figures 5.2 and 5.3, the presence of POP and the anatomical site were associated to modifications in E_t and σ_{max} distributions in vaginal tissue. Figure 5.2 presents the average mechanical behavior of the vaginal tissue for the POP group and Figure 5.3 does the same for the non-POP group. POP patients were associated to higher values of E_t (13.1 ± 0.8 versus 9.5 ± 0.7 MPa; $p < 0.001$) and σ_{max} (5.3 ± 0.5 versus 3.2 ± 0.9 MPa; $p < 0.001$) in anterior vaginal wall compared to the posterior vaginal wall (Figure 5.4). Non-POP women presented a different pattern, with lower values of E_t and σ_{max} in anterior vaginal wall compared to the posterior vaginal wall (6.9 ± 1.1 versus 10.5 ± 1.0 MPa; $p = 0.010$ and 2.6 ± 0.4 versus 3.5 ± 0.4 MPa; $p = 0.043$), respectively.

It was performed a univariate analysis of the factors that may influence E_t and σ_{max} in vaginal tissue (Table 5.7). Age, parity and the presence of POP were associated to higher E_t and σ_{max} in anterior vaginal samples. However, linear regression analysis (Table 5.8) revealed that the occurrence of POP was the only independent predictor of higher values of E_t and σ_{max} in anterior vaginal samples ($p = 0.003$ and $p = 0.008$; respectively). POP patients presented higher values of E_t (13.1 ± 0.8 versus 6.9 ± 1.1 MPa; $p < 0.001$) and σ_{max} (5.3 ± 0.5 versus 2.6 ± 0.4 MPa; $p = 0.001$) in anterior vaginal wall compared to non-POP women. Women with severe anterior vaginal prolapse presented higher levels of E_t and σ_{max} in anterior vaginal sample compared to those with lower POP stages ($p = 0.001$ and $p = 0.010$; respectively) (Figure 5.5).

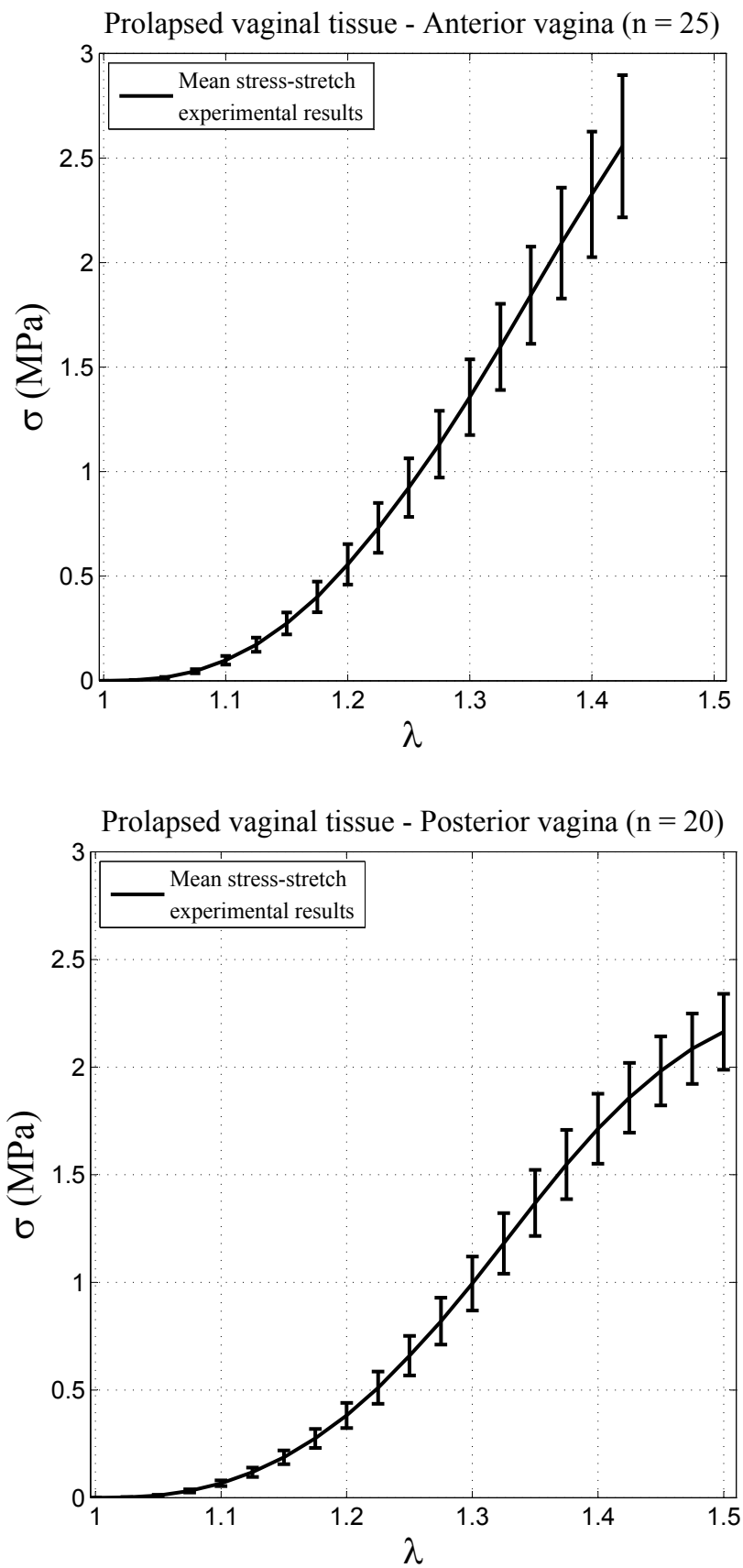


Figure 5.2: Typical mechanical behavior for the anterior and posterior vaginal wall. Prolapsed vaginal tissue, POP group. Longitudinal (fiber oriented) direction.

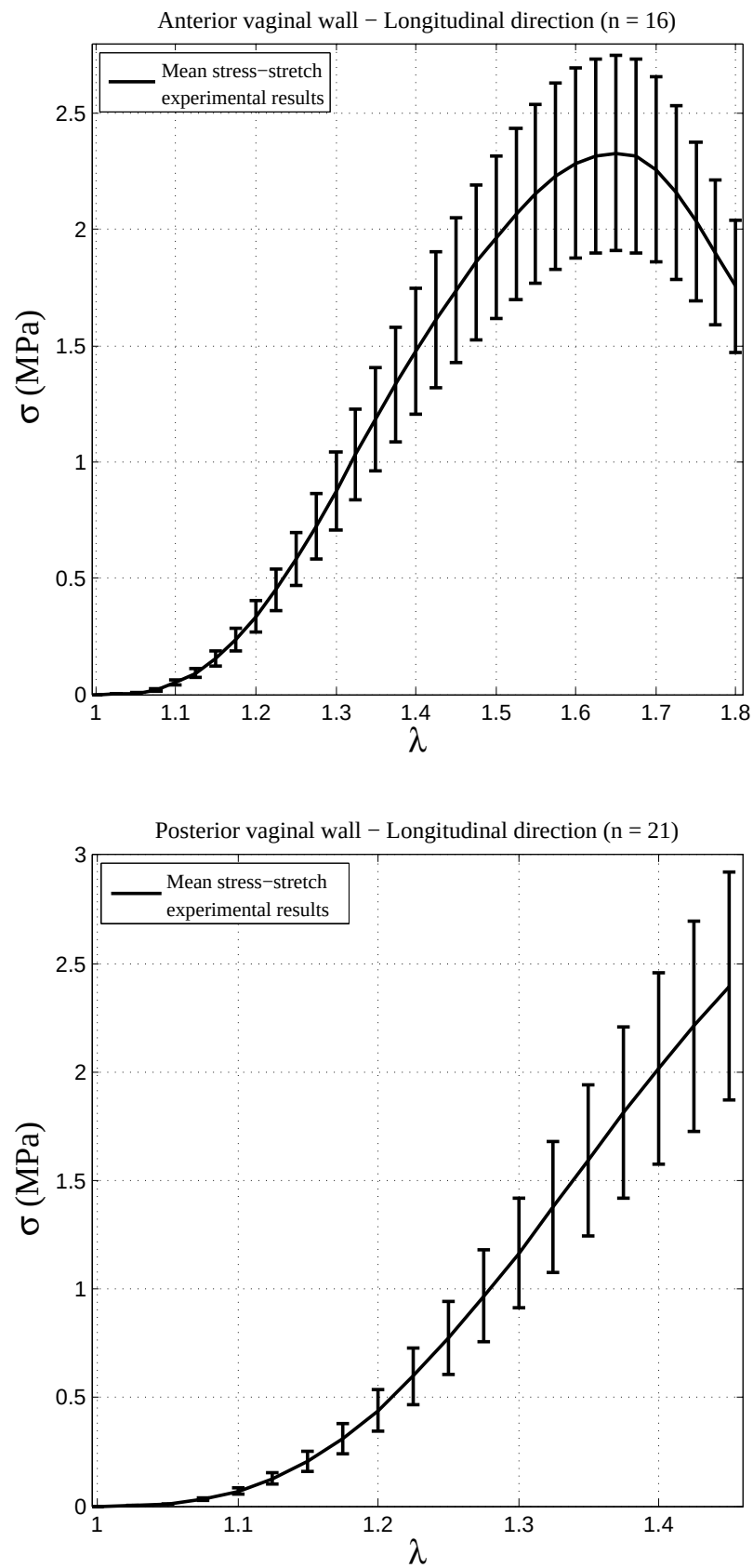


Figure 5.3: Typical mechanical behavior for the anterior and posterior vaginal wall. Cadaver vaginal tissue, non-POP group. Longitudinal (fiber oriented) direction.

Table 5.7: Univariate analysis of factors that may influence the biomechanical properties (E_t and σ_{max}) in vaginal tissue.

Variable	Anterior vaginal sample		Posterior vaginal sample	
	E_t (MPa)	p	E_t (MPa)	σ_{max} (MPa)
Age		0.013		0.023
< 50 years	7.9 ± 1.2		10.2 ± 1.4	3.9 ± 0.5
≥ 50 years	12.2 ± 0.9		9.8 ± 0.6	3.1 ± 0.2
Parity		0.005		0.013
< 2	7.6 ± 1.5		9.5 ± 1.4	3.3 ± 0.4
≥ 2	12.5 ± 0.8		10.0 ± 0.7	3.5 ± 0.3
BMI		0.458		0.793
< 25 Kg/m ²	7.2 ± 2.3		11.3 ± 2.1	3.4 ± 1.0
≥ 25 Kg/m ²	5.3 ± 0.6		10.6 ± 1.6	3.6 ± 0.4
Menopause		0.207		0.678
No	10.4 ± 0.9		9.4 ± 0.7	3.1 ± 0.2
Yes	12.5 ± 1.2		9.9 ± 1.3	3.1 ± 0.2
Prolapse		< 0.001		0.442
No	6.9 ± 1.1		10.5 ± 1.0	3.5 ± 0.4
Yes	13.1 ± 0.8		9.5 ± 0.7	3.2 ± 0.3

Table 5.8: Linear regression analysis of the factors that may influence the biomechanical properties (E_t and σ_{max}) in vaginal tissue; CI - Confidence interval.

Variable	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for B
	B	Std. Error				
E_t anterior vaginal sample						
	age	0.030	0.071	0.080	0.416	0.680
	parity	-0.092	0.358	-0.042	-0.257	0.798
	POP occurrence	-6.621	2.099	-0.594	-3.155	0.003
						-10.879 - -2.364
σ_{max} anterior vaginal sample						
	age	-0.035	0.039	-0.183	-0.910	0.369
	parity	0.127	0.190	0.111	0.669	0.507
	POP occurrence	-3.258	1.161	-0.560	-2.805	0.008
						-5.606 - -0.909

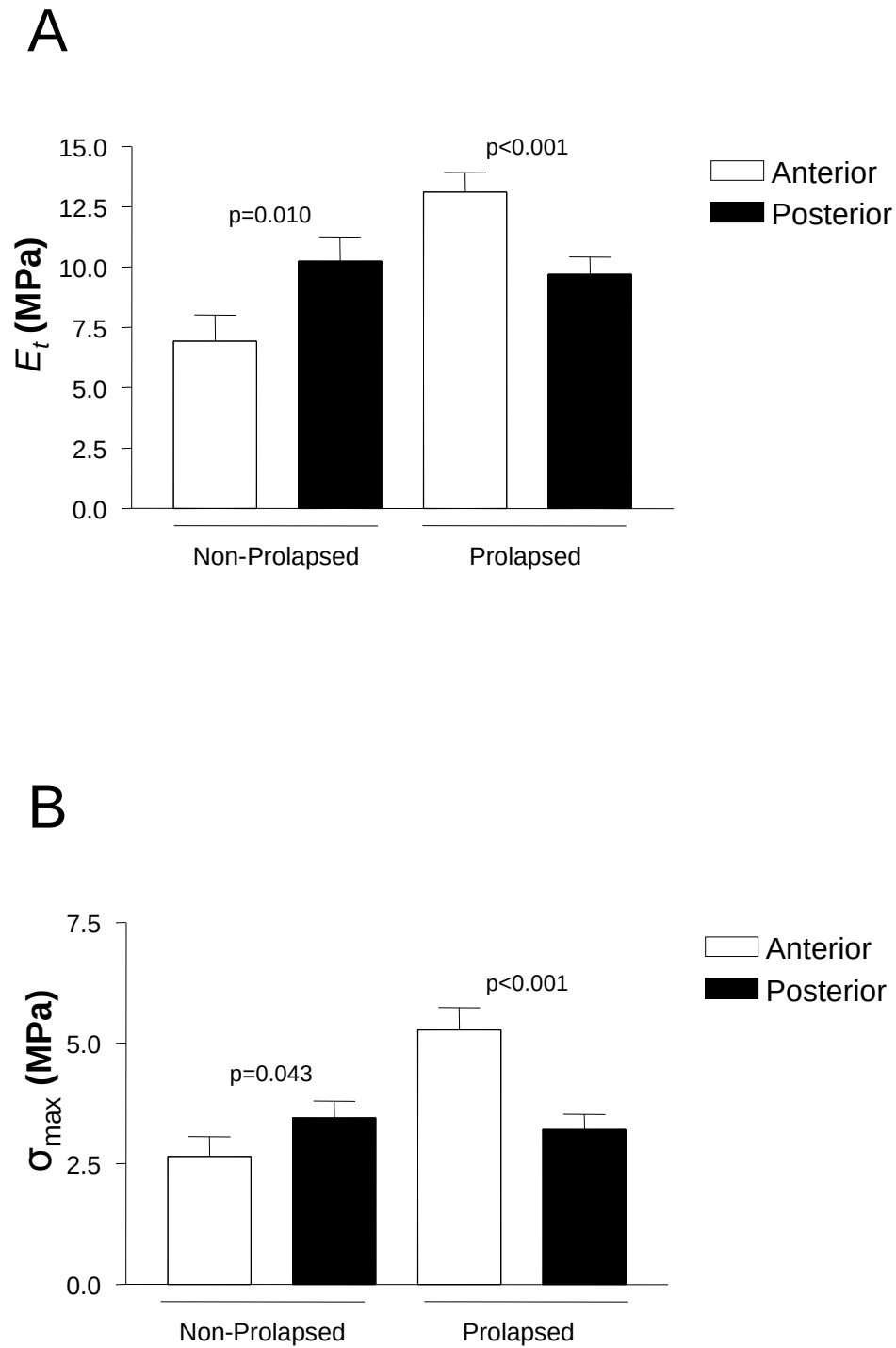
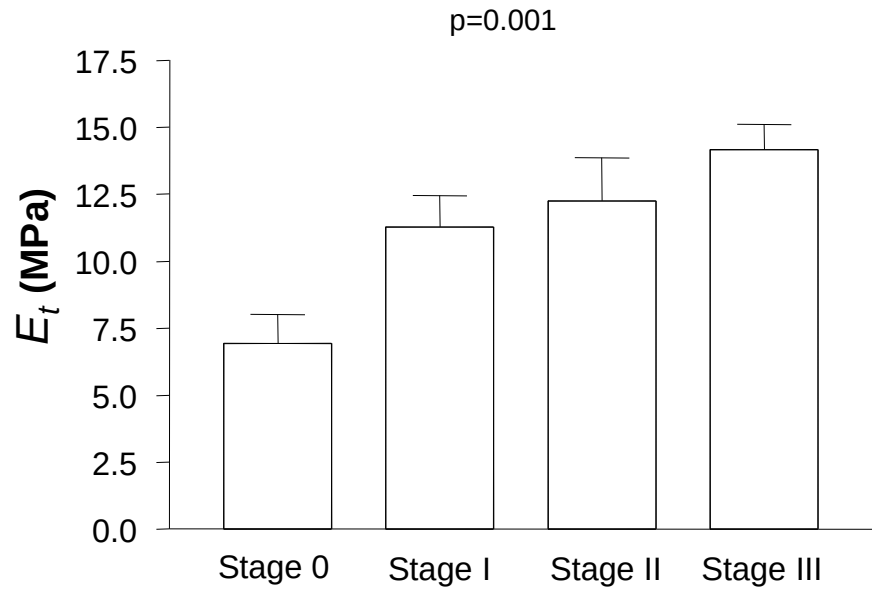


Figure 5.4: Comparison of E_t (A) and σ_{max} (B) between anterior and posterior vaginal samples in non-prolapsed and prolapsed women. Differences between groups were assessed by paired Student's t test.

A



B

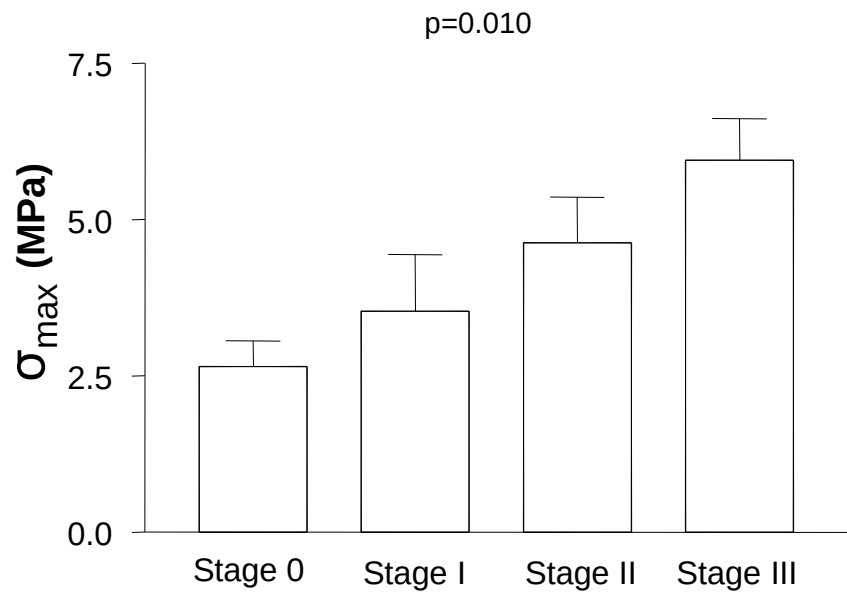


Figure 5.5: Association between anterior compartment prolapse stage and (A) E_t and (B) σ_{max} in anterior vaginal tissues. The prolapse stage was determined according to Pelvic Organ Prolapse Quantification (POP-Q) system 14. Differences between groups were assessed by ANOVA.

5.2.4 Discussion and conclusions

The translation of biomechanics research to clinical practice may improve the assessment and treatment of the pelvic floor dysfunctions [DeLancey, 2005; Epstein et al., 2008b; Goh, 2003]. In the work reported, the authors attempted to evaluate the biomechanical properties of vagina, providing mechanical parameters that are able to characterize the elastic regimen under uniaxial extension. Studying these tissues with linear elasticity theory constitutes a simplified way of analyzing this condition known to be more complex [Rubod et al., 2008]. The mechanical behavior of biological soft tissues is typically nonlinear with hyper and viscoelastic characteristics [Fung, 1993]. The tests were performed in both cadaver and ex-vivo specimens. Due to clinical, technical and ethical reasons it is not feasible to obtain samples from the middle third of the vagina in non-POP women. According to an animal model study freezing vaginal tissue has no consequences on the mechanical response during unidirectional tension testing [Rubod et al., 2008]. Therefore the refrigeration ($5^{\circ}C$ in saline solution) of the tissue samples during small time periods (4-6 hours) is assumed to be a suitable storage procedure for preserving the mechanical properties. The ideal measurement of the biomechanical properties would be done in-vivo conditions. Tissue suction and indentation techniques constitute non-destructive and less invasive tests that have been used to evaluate the mechanical properties of human tissues [Epstein et al., 2008a; Tay et al., 2006].

Recently, important efforts to perform in vivo measurements of biological material parameters have been performed [Kauer et al., 2002; Mazza et al., 2006]. However, for the purpose of deriving new constitutive material models, it is the authors understanding that whenever possible, the direct testing of the materials and/or structures involved, should be considered. In this sense, the biomechanical properties of pelvic tissues have been already evaluated [Cosson et al., 2003a, 2004; Goh, 2002; Lei et al., 2007; Rubod et al., 2008].

There was no association between age, parity, BMI and menopausal status with the vaginal biomechanical properties. A study comparing pre- and postmenopausal vaginal tissue from prolapsed patients also showed few differences in biomechanical assessment [Goh, 2002]. However, another study, involving 43 patients undergoing vaginal hysterectomy, noted association of elastic modulus, maximum elongation and maximum

fracture in vaginal tissues with the menopausal status [Lei et al., 2007].

The occurrence of POP was the only independent predictor of tangent modulus and tensile strength in vaginal tissues. Other studies have already showed this association, suggesting that changes of biomechanical properties in pelvic support construction might lead to the occurrence of prolapse [Lei et al., 2007; Rubod et al., 2008]. However, if these changes are related to POP pathogenesis or caused by the anatomical distortion and tissue overload remains unclear.

POP patients were associated to higher tangent modulus and tensile strength in anterior vaginal wall compared to the posterior vaginal wall. This evidence suggests that this site may be more vulnerable to deformations. It is in agreement with clinical data, which have demonstrated that anterior prolapse occurs more frequently than apical or posterior vaginal wall prolapse [Swift et al., 2005; Rooney et al., 2006; Ellerkmann et al., 2001]. There is an association between the POP stage and the stiffening of the vaginal tissue. The vaginal tissue exposed to an overload alters its biomechanical characteristics becoming more rigid. MRI studies also indicate more vaginal tissue in patients with prolapse compared to non-prolapsed women [Hsu et al., 2005]. Current research also suggests that the vagina and its supportive tissues actively remodel in response to different environmental stimuli [Alperin and Moalli, 2006]. The increase in collagen III and active MMP-9 expression in the vaginal tissues of patients with prolapse suggests that this tissue is actively remodeling to accommodate a progressively increasing mechanical load [Moalli et al., 2005]. This enforces the idea that biomechanical modifications in vagina may be repercussions of POP and not the cause of this condition. Significant advances have been made in characterizing the biomechanical properties of normal and injured pelvic floor structures. A new model for the viscoelastic mechanical properties of the human vaginal tissue was proposed. This structural model may be helpful in the design of surgical procedures with autologous tissue [Peña et al., 2010]. The mechanical damage process on prolapsed vaginal tissue associated with large strains have been demonstrated by a damage model [Calvo et al., 2009]. Tissue damage was simulated considering different damage behaviors for the matrix and the fibers.

The presented evidence suggests that anatomic distortion caused by POP is followed by modifications in mechanical proprieties of the vagina. The new field of functional

tissue engineering offer many possibilities which may contribute to understand the etiology of this complex condition. A combination of technologies will allow researchers to identify mechanisms of pelvic floor disorders, assume preventive strategies, improve rehabilitation protocols and optimize surgical procedures.

5.3 Uniaxial mechanical behavior of Human female bladder

5.3.1 Introduction

The bladder is considered the more important and more active part of the lower urinary tract [Abrams, 2006]. Normal voiding implies the ability to quickly and completely empty a bladder recognized as being normally full [Smith, 2010]. Detrusor underactivity is defined as a contraction of reduced strength and/or duration, resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span [Abrams et al., 2002]. This condition affects nearly 3% of the adult females, and the symptoms include urinary retention, poor urinary stream, and/or incontinence [Smith, 2010; Cucchi et al., 2008].

Voiding dysfunction is also affected by changes in the viscoelastic properties of the bladder wall [Vasavada et al., 2004]. Therefore, the biomechanical assessment of bladder tissue may provide the clinician a better understanding of this condition and improve clinical outcomes. The purpose of this study was to investigate the tensile biomechanical properties of female bladder tissues. For this purpose, based on a uniaxial tensile test the factors influencing the stiffness (E_t) and maximum stress (σ_{max}) of bladder were investigated (see Figure 4.3 and Table 5.1).

5.3.2 Materials and methods

For the preparation of the current work, it was collected bladder tissue from thirteen female cadavers without clinical noticed pelvic floor dysfunctions. The samples were collected in agreement with a procedure approved by the direction board of the Forensic Pathology Service of the *North Branch of INML, I.P.*. The main characteristics of the population studied were presented in Table 5.4. The mean age of the cadavers was 45.1 ± 3.0 years (ranging from 18 to 65 years) and parity of 0.8 ± 0.2 (ranging from 0 to 2). The samples were not formolised but refrigerated at $5^{\circ}C$ in saline solution bath for preservation.

Bladder tissue samples

The tissue samples were collected at the end of the normal forensic autopsy procedures. The samples were dissected from the bladder cuff and the test specimens, prepared following the procedure described in Section 4.4, had the approximate dimensions of $2\text{ cm} \times 1\text{ cm}$. They were preserved in a saline solution bath and kept at $5^{\circ}C$ until 15 minutes before the mechanical tests were performed. The storage time of the soft tissues (until testing) did not exceed 6 hours, and for most of the samples it was under 4 hours.

Stiffness and maximum stress assessment Geometrical properties were acquired through digital image analysis. Two USB webcams were connected to the data acquisition PC to capture front and side view shots. This procedure allows the measurement of specimen dimensions with the software ImageJ, as described in Section 4.4.3.

The biomechanical properties were evaluated by means of uniaxial tensile tests. The specimens were prepared in the longitudinal and transversal axis (Figure 5.1). For the particular case of bladder tissue, “...The muscle fibers of the bladder run in every direction (without distinct layers) until they approach the bladder neck.” [Junqueira and Carneiro, 2007, Ch. 19]. Therefore the specimens cut in different directions have the same biomechanical properties. To perform the tests, the tissue specimens were assembled on the testing machine using the techniques described in Section 4.4. The mechanical testing process has been validated for different hyperelastic materials on

a previous work [Martins et al., 2006b]. The load (N) and displacement (mm) data were acquired using a load cell; ($F_{max} = 200\text{ N}$) and a displacement sensor. A constant displacement rate of 5 mm.min^{-1} was maintained throughout the test and for all tests. Both load and displacement data were recorded till complete specimen rupture. Each mechanical test was filmed. This enabled the quality control of each test, eliminating those with inadequate alignment or slippage (the most common issues).

The biomechanical properties (see Figure 4.3, Table 5.1) considered in this study are the stiffness (E_t) and maximum stress (σ_{max}). The maximum stress is also known as ultimate stress and is the stress limit before sample rupture.

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software program version 16.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of the data was verified using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The variables were described as means with their respective ranges and standard error of the mean. The statistical differences between the groups were tested using Student's unpaired t-test. Correlations were calculated using the Pearson correlation coefficient, with a significance level set to $p < 0.05$.

5.3.3 Results

The biomechanical properties extracted from the testing of 26 bladder tissue specimens (corresponding to 13 female cadavers, Table 5.4) are presented in Table 5.9. An analysis of the global mechanical behavior of the tested samples (Figure 5.6), reveals small fluctuations around the mean stress-stretch curve (error bars in Figure 5.6). These results display the nonlinear mechanical behavior, characteristic of soft biological tissues, either Human or animal [Fung, 1993].

The bladder tissue stiffness (E_t) ranged from 1 to 4.1 MPa , with $mean \pm SEM = 1.9 \pm 0.2\text{ MPa}$. The mean maximum stress was, $\sigma_{max} = 0.9 \pm 0.1$, ranging from 0.5 to 2.6 MPa . There was a strong positive correlation between stiffness and maximum stress in bladder tissue ($\rho = 0.829$; $p < 0.001$) (Figure 5.7). It was carried out a

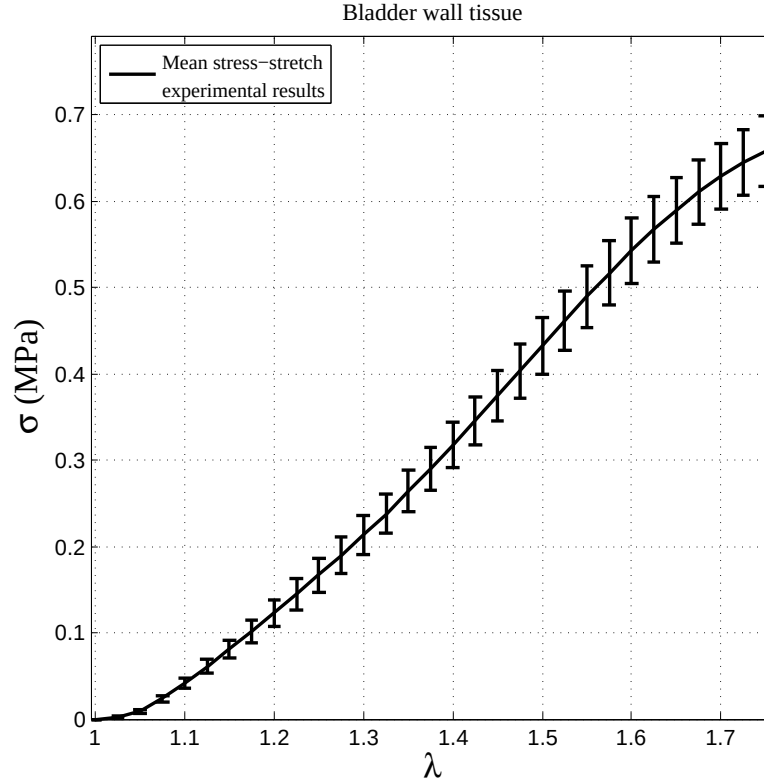


Figure 5.6: Typical mechanical behavior for bladder tissue.

univariate analysis of some of the factors (based on the available data) that may influence stiffness and maximum stress in female bladder tissue (Table 5.10). It was seen that women with less than 50 years presented higher bladder stiffness (2.1 ± 0.2 versus 1.3 ± 0.1 MPa; $p = 0.02$) compared to older subjects. The maximum stress was not associated with age (1.0 ± 0.2 versus 0.7 ± 0.1 MPa; $p = 0.349$). Body mass index and menopausal status were not associated to the biomechanical proprieties of bladder tissue.

5.3.4 Discussion and conclusions

In the investigations here reported, it was attempted an evaluation of the stiffness and maximum stress of bladder tissue, mechanical parameters used to characterize the uniaxial tensile state. A translation of biomechanics research to clinical practice may improve the assessment and treatment of the voiding dysfunctions.

In the present study, younger women were associated to higher bladder stiffness when compared to older subjects. This evidence may suggest that bladder stiffness decreases

Table 5.9: Experimental data extracted from cadaveric tissue. Bladder

Sample	σ_{max} (MPa)	λ_U	U_S (nJ.m ⁻³)	E_t (MPa)	σ_Y (MPa)
Cd03_B1d1	0.746	1.770	0.250	1.456	0.630
Cd04_B1d1	0.576	1.684	0.151	1.012	0.184
Cd04_B2d1	0.595	1.552	0.159	1.860	0.319
Cd04_B3d2	0.967	1.638	0.238	2.819	0.682
Cd05_B1d1	0.857	1.731	0.307	2.118	0.454
Cd05_B2d1	0.645	1.560	0.170	2.147	0.369
Cd06_B1d1	1.032	1.897	0.449	1.425	0.649
Cd06_B2d1	0.657	1.950	0.289	1.071	0.392
Cd06_B3d2	0.709	1.789	0.245	1.235	0.658
Cd07_B1d1	1.059	1.673	0.335	2.796	0.804
Cd07_B3d2	0.549	1.757	0.239	2.145	0.310
Cd08_B1d1	0.629	1.497	0.149	2.141	0.496
Cd08_B2d1	0.954	1.686	0.270	2.981	0.728
Cd09_B1d1	1.742	2.332	0.997	2.678	1.238
Cd09_B2d2	3.420	2.314	1.617	5.485	2.770
Cd10_B1d1	0.610	1.605	0.188	1.589	0.423
Cd10_B2d1	0.695	1.839	0.264	1.573	0.477
Cd10_B3d2	0.445	1.604	0.131	1.014	0.355
Cd11_B1d1	0.677	2.208	0.363	0.877	0.439
Cd12_B1d1	0.802	1.721	0.258	1.762	0.218
Cd13_B1d1	0.559	1.687	0.234	1.716	0.249
Cd13_B2d2	0.681	1.933	0.282	1.408	0.394
Cd15_B1d1	0.542	1.802	0.212	0.921	0.435
Cd17_B1d1	1.691	2.790	1.433	1.207	0.715
Cd17_B2d1	0.952	2.000	0.346	1.662	0.878
Cd17_B3d1	0.768	1.728	0.223	1.795	0.744

Table 5.10: Univariate analysis of the factors that possible influence stiffness and maximum stress in female bladder tissue (E_t and σ_{max}) in Bladder tissue; Differences between groups were assessed by unpaired Student's t test.

Variable	E_t	σ_{max}
	(MPa)	(MPa)
Age	0.027	0.349
< 50 years	2.1 ± 0.2	1.0 ± 0.2
≥ 50 years	1.3 ± 0.1	0.7 ± 0.1
BMI	0.893	0.492
< 25 Kg/m ²	1.9 ± 0.4	0.8 ± 0.1
≥ 25 Kg/m ²	2.0 ± 0.4	1.0 ± 0.1
Menopause	0.405	0.660
No	1.7 ± 0.4	0.8 ± 0.1
Yes	2.3 ± 0.5	1.1 ± 0.4

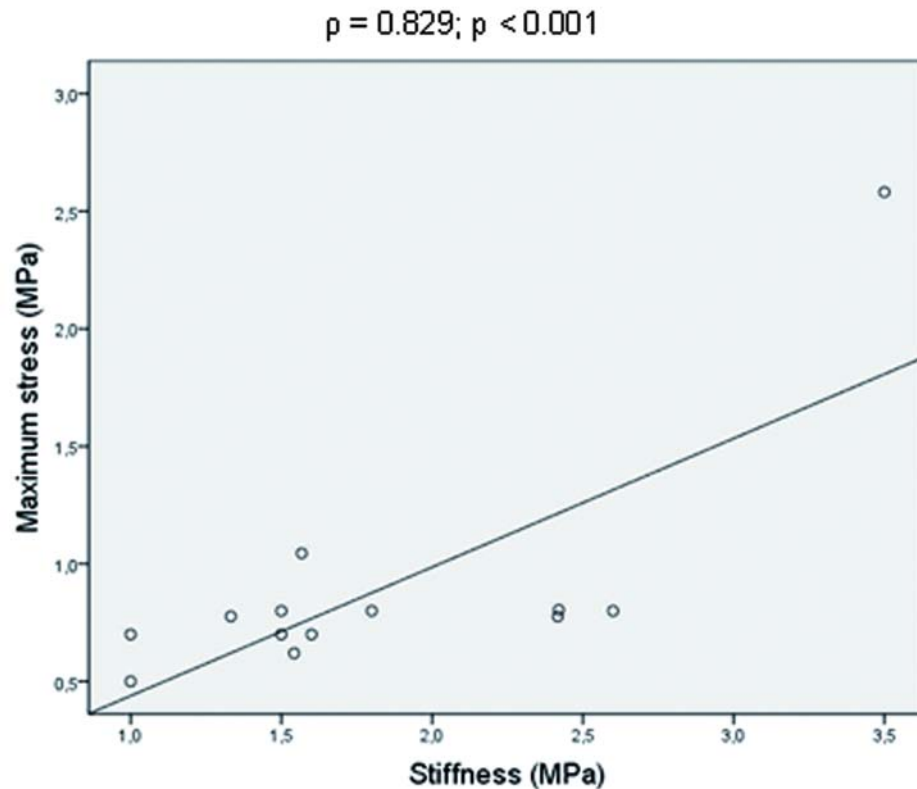


Figure 5.7: Correlation between stiffness and maximum stress in female human bladder. Correlations were calculated using the Pearson correlation coefficient.

with aging. Age have been linked to the presence of abnormalities in detrusor microstructure patterns [Elbadawi et al., 1993]. These structural and functional tissue changes accompanying aging have been associated to altered bladder function [Smith, 2010]. Voiding dysfunctions in the elderly are generally multifactorial, therefore any association with age or pathology related changes remain unclear [Smith, 2010]. Some urodynamic data associates the loss of bladder function and voiding efficiency with aging [Madersbacher et al., 1998]. An increase of postvoid residual volume, a decrease of flow rate, average flow rate, voided volume and bladder capacity was related with progressing age. However, another study evaluating urodynamic parameters in 32 postmenopausal women considered detrusor underactivity a pathological process rather than a natural evolution due to aging [Cucchi et al., 2008].

The limitations of the present study must be considered before interpreting the results. The ideal measurement of the biomechanical properties would be done in-vivo conditions. Tissue suction and indentation techniques constitute non-destructive and less invasive tests that have been used to evaluate the mechanical properties of human tissues [Epstein et al., 2008a; Tay et al., 2006]. The feasible animal models (rabbit,

guinea pigs, sheep, etc) are quadrupedal, factor which differentiates significantly the morphology and physiology of their pelvic cavity from the humans [Abramowitch et al., 2009].

The presented evidence suggests that age may influence the uniaxial mechanical behavior of female human bladder. Further studies are required to evaluate the biomechanical behaviour of female pelvic tissues. Biomechanical characterization of female pelvic tissues is an important step toward a more complete understanding of bladder function.

5.4 Round and Uterosacral ligaments strength

5.4.1 Introduction

The pelvic floor and the associated structures comprise one of the most complex regions of the human anatomy. Both the muscles of the pelvic floor and their intact attachments to the endopelvic fascia support the pelvic organs. The round ligaments originate at the uterine horns and maintain the anteversion of the uterus. The uterosacral ligaments are condensations of endopelvic fascia which hold the upper vagina and cervix over the levator plate [Nichols and Randall, 1996]. Biomechanical assessment of pelvic organs supports may provide the clinician a better understanding of this condition and improve clinical outcomes.

Therefore, the purpose of this study was to investigate the tensile biomechanical properties of round (RL) and uterosacral ligaments (USL). For this purpose, based on a uniaxial tensile test the factors influencing the stiffness (E_t) and maximum stress (σ_{max}) of pelvic ligaments were investigated.

5.4.2 Materials and methods

The ligament samples used for the present study were collected from fifteen female cadavers without clinical noticed pelvic floor dysfunctions or POP. The available data on the studied population is presented in Table 5.4. The cadavers were not formolised but refrigerated for preservation. The mean age of the cadavers was 45.1 ± 3.0 years (ranging from 18 to 65 years) and parity of 0.8 ± 0.2 (ranging from 0 to 2). The sample collection followed a procedure approved by the direction board of the Forensic Pathology Service of the *North Branch of INML, I.P.*.

Round and Uterosacral ligaments samples

The tissue samples were collected at the end of the normal forensic autopsy procedures. The specimens were preserved in a saline solution bath and kept at $5^{\circ}C$ until 15 minutes before the mechanical tests were performed. The storage time of the soft tissues (until testing) did not exceed 6 hours, and for most of the samples was inferior to 4 hours.

Stiffness (E_t) and maximum stress (σ_{max}) assessment

Given the anatomic function of the ligaments, it is very important to assess the biomechanical properties in the longitudinal axis i.e., in the fiber direction (Figure 5.1). Therefore, although some exploratory tests were performed with specimens cut in the transversal axis, the data used in this work (Table 5.11 and 5.12) is from uniaxial tensile tests in the longitudinal axis.

To perform the tests, the tissue specimens were prepared and assembled on the testing machine following the procedures described in Section 4.4. The testing conditions were identical to those of the previous studies.

The biomechanical properties considered in this study are the stiffness (E_t) and maximum stress σ_{max} . The round and uterosacral ligaments have nonlinear mechanical behavior as evidenced by the stress-strain curves in Figures 5.8 and 5.9.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences

(SPSS) software program version 16.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of the data was verified using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The variables were described as means with their respective ranges and standard error of the mean. The statistical differences between the groups were tested using Student's paired and unpaired t-test as required. Correlations were calculated using the Pearson correlation coefficient. Significance level was established as $p < 0.05$.

5.4.3 Results

There was a great variability in the measurements of stiffness and maximum stress in pelvic ligaments, as shown by the experimental data results (Tables 5.11 and 5.12). The round ligaments (Table 5.11 and Figures 5.8-(a) and 5.9) presented stiffness of $9.1 \pm 1.6 \text{ MPa}$ (*mean $\pm$ SEM*) (ranging from 2 to 25.6 MPa) and maximum stress of $4.3 \pm 0.7 \text{ MPa}$ (ranging from 1.2 to 11.5 MPa). For the uterosacral ligaments (Table 5.12 and Figures 5.8-(b) and 5.9) E_t was $14.1 \pm 1.4 \text{ MPa}$ (ranging from 5.7 to 26.1 MPa) and σ_{max} of $6.3 \pm 0.8 \text{ MPa}$ (ranging from 2.2 to 11.9 MPa).

There was a strong positive correlation between stiffness and maximum stress in female pelvic ligaments ($\rho = 0.851$; $p < 0.001$) (Figure 5.10). As illustrated in Figure 5.11, the uterosacral ligaments presented higher E_t and σ_{max} compared to the round ligaments ($p = 0.006$ and $p = 0.034$; respectively).

A univariate analysis of the factors that may influence stiffness and maximum stress in female pelvic ligaments was performed (Table 5.13). Age, body mass index and menopausal status were not associated to the round and uterosacral ligaments biomechanical proprieties. Nulliparous women were associated to lower uterosacral stiffness (15.5 ± 1.3 versus $10 \pm 1.8 \text{ MPa}$; $p = 0.033$) and maximum stress (8.2 ± 0.9 versus $4.2 \pm 1.1 \text{ MPa}$; $p = 0.028$) compared to parous women.

Table 5.11: Experimental data extracted from cadaveric tissue. Round ligaments (RL).

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Cd01_RL2L	6.247	1.684	1.432	21.047	5.698
Cd02_RL1L	2.718	1.782	0.759	5.415	2.447
Cd03_RL1L	6.214	1.764	1.871	13.640	5.403
Cd04_RL1L	4.831	1.594	1.066	12.378	4.181
Cd05_RL1L	2.532	1.443	0.431	11.037	2.227
Cd06_RL1L	4.753	1.970	1.728	8.533	3.811
Cd07_RL1L	3.840	1.739	1.365	8.130	2.410
Cd07_RL2L	11.718	2.410	6.296	12.060	5.311
Cd08_RL1L	1.181	1.490	0.276	3.497	1.026
Cd09_RL1L	2.192	1.922	0.817	3.911	1.835
Cd10_RL1L	3.652	1.909	1.535	7.772	2.480
Cd11_RL1L	3.279	2.322	1.535	6.141	2.353
Cd12_RL1L	1.147	1.956	0.467	2.030	1.068
Cd13_RL1L	4.319	1.747	1.573	9.350	2.417
Cd13_RL2L	4.398	1.840	1.465	9.295	3.753
Cd15_RL1L	11.525	1.691	3.537	25.513	8.247
Cd17_RL1L	6.590	1.576	1.677	18.043	5.475
Cd17_RL2L	3.479	1.798	1.040	7.182	2.994

Table 5.12: Experimental data extracted from cadaveric tissue. Uterosacral ligaments (USL).

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Cd01_USL2L	3.065	1.448	0.469	12.183	2.487
Cd02_USL1L	9.450	1.802	3.312	17.579	7.278
Cd02_USL2L	14.375	2.181	6.678	20.772	12.696
Cd03_USL1L	3.794	1.840	1.215	8.592	3.203
Cd04_USL1L	10.972	1.828	3.255	22.320	9.738
Cd04_USL2L	2.122	1.600	0.554	6.022	1.700
Cd05_USL1L	5.492	1.576	1.411	16.592	4.245
Cd06_USL1L	8.487	1.767	3.313	17.482	5.368
Cd07_USL1L	7.221	1.770	2.193	13.328	6.200
Cd08_USL1L	3.828	1.459	0.721	12.310	2.983
Cd09_USL1L	2.161	1.626	0.635	5.242	1.467
Cd10_USL1L	9.686	1.951	3.744	15.137	8.154
Cd10_USL2L	9.586	2.201	4.547	13.018	8.125
Cd11_USL1L	5.779	1.629	1.541	15.855	4.649
Cd12_USL1L	7.040	2.004	3.302	10.487	4.823
Cd13_USL1L	1.369	1.523	0.329	4.578	0.963
Cd15_USL1L	10.234	1.615	2.380	26.163	9.027
Cd17_USL1L	4.044	1.637	1.008	10.157	2.847
Cd17_USL2L	3.273	1.510	0.600	10.521	2.786

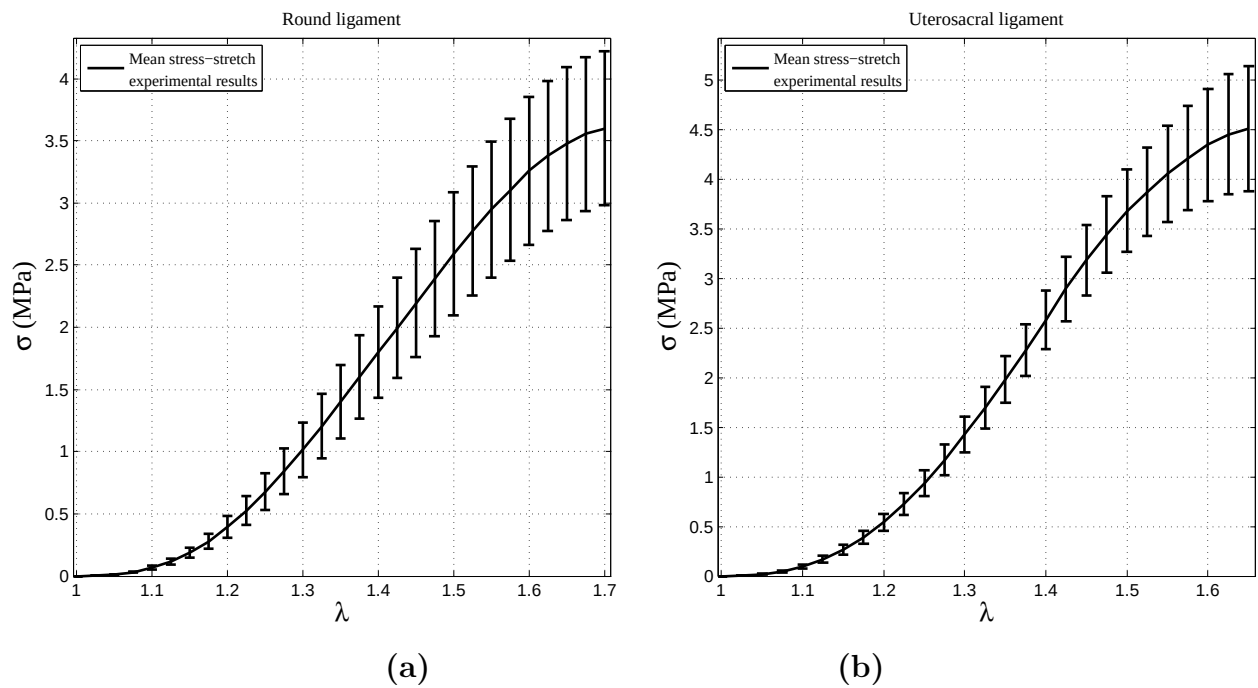


Figure 5.8: Typical mechanical behavior of (a) Round Ligaments (RL) and (b) Uterosacral Ligaments (USL). Longitudinal direction (aligned with fibers).

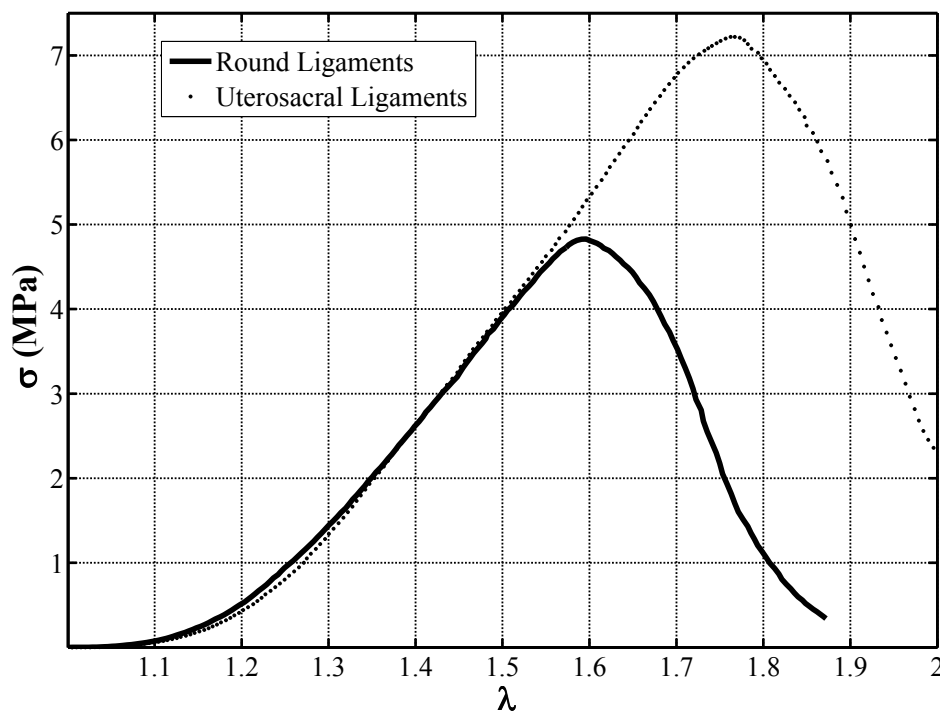


Figure 5.9: Typical mechanical behavior (mean) for round (full line) and uterosacral (dotted line) ligaments.

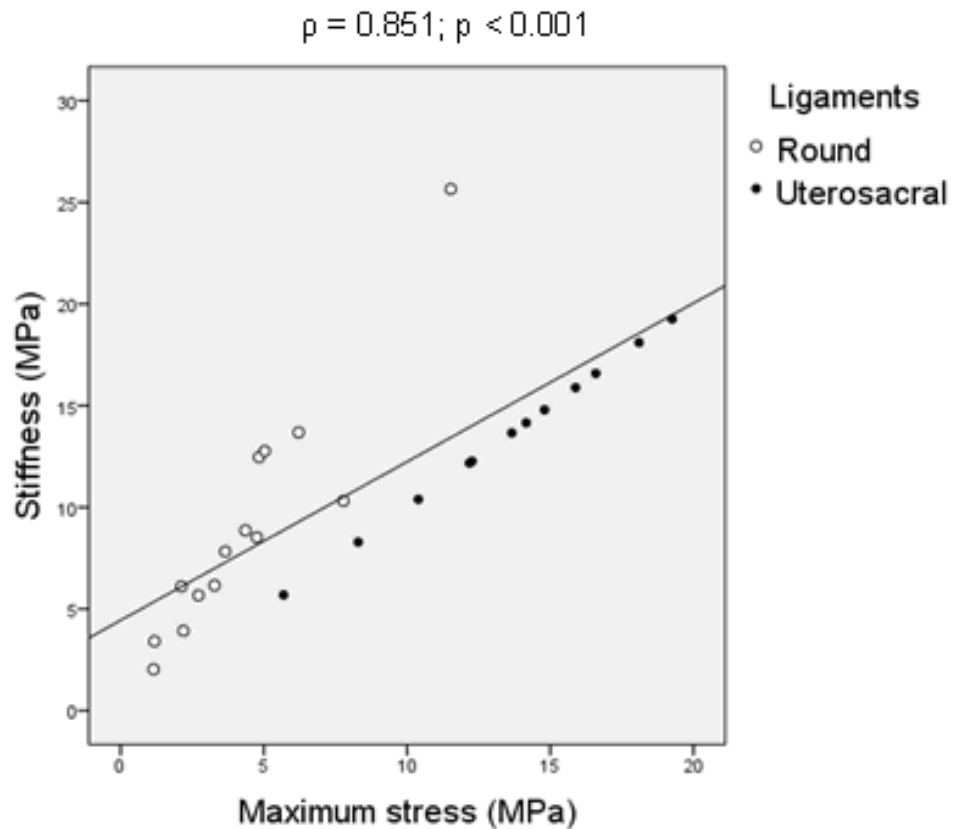


Figure 5.10: Correlation between stiffness and maximum stress in female pelvic ligaments. Correlations were calculated using the Pearson correlation coefficient.

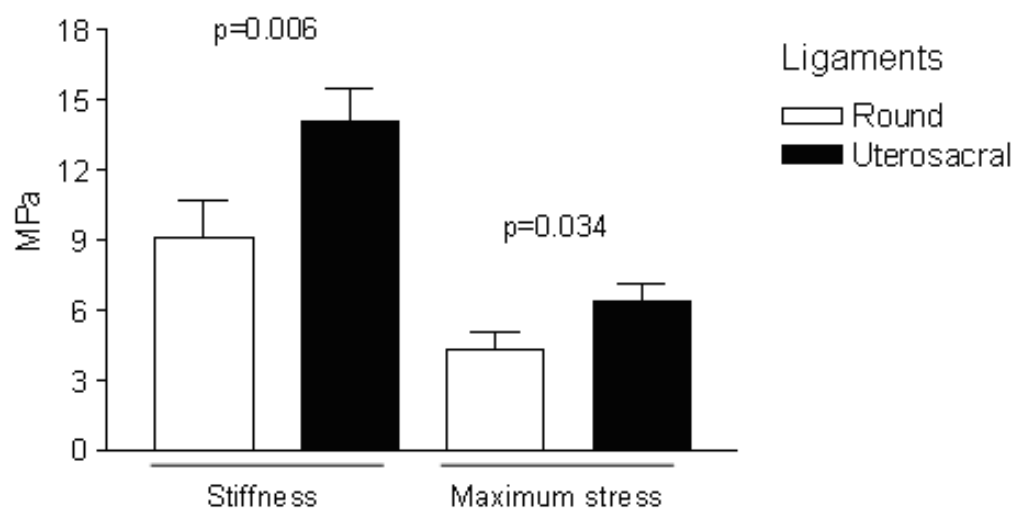


Figure 5.11: Comparison of stiffness and maximum stress between round and uterosacral ligaments. Differences between groups were assessed by paired Student's t test.

Table 5.13: Univariate analysis of factors that may influence the biomechanical properties (E_t and σ_{max}) in female pelvic ligaments (RL, USL). between groups were assessed by unpaired Student's t test.

Variable	Round Ligaments (RL)			Uterosacral Ligaments (USL)		
	E_t (MPa)	p	σ_{max} (MPa)	E_t (MPa)	p	σ_{max} (MPa)
Age						
< 50 years	6.8 ± 1.3		3.4 ± 0.8	13.2 ± 1.4		6.3 ± 0.9
≥ 50 years	12.1 ± 3.0		5.5 ± 1.3	15.4 ± 2.7		6.4 ± 1.5
		0.100			0.182	
						0.456
						0.950
Nulliparous						
No	7.3 ± 1.2		3.4 ± 0.5	15.5 ± 1.3		8.2 ± 0.9
Yes	7.8 ± 2.5		4.3 ± 1.6	10.0 ± 1.8		4.2 ± 1.1
		0.850			0.593	
						0.033
						0.028
BMI						
< 25 Kg/m ²	5.5 ± 1.4		3.2 ± 1.2	14.3 ± 1.5		7.1 ± 1.3
≥ 25 Kg/m ²	9.3 ± 1.4		4.1 ± 0.6	11.6 ± 1.7		5.2 ± 1.1
		0.089			0.292	
						0.282
Menopause						
No	10.5 ± 2.1		4.4 ± 0.5	13.7 ± 1.7		5.3 ± 0.9
Yes	10.2 ± 4.1		5.3 ± 1.9	14.4 ± 3.3		6.6 ± 1.6
		0.968			0.886	
						0.580

5.4.4 Discussion and conclusions

In the work reported, the authors attempted to evaluate the biomechanical properties of round and uterosacral ligaments, providing mechanical parameters that are able to characterize a state of uniaxial tension.

The uterosacral ligaments presented higher stiffness and maximum stress compared to the round ligaments. This is in accordance to clinical data, the round ligaments do not have a role in support of the pelvic organs [Nichols and Randall, 1996; Davila et al., 2006]. Otherwise, the uterosacral ligaments represent an important part of the pelvic support system and establish the level 1 support of the cervix and the upper vagina [Nichols and Randall, 1996; DeLancey, 1992]. The strength of iliopectineal, sacrospinous and arcus tendineus of pelvic fascia were evaluated in 29 female cadaveric specimens [Cosson et al., 2003a]. The iliopectineal ligament was significantly stronger than the sacrospinous and arcus tendineus of pelvic fascia. There was a correlation between subjective evaluation and objective strength measurements. In the present study, nulliparous women were associated to lower uterosacral stiffness and maximum stress when compared with parous women. This evidence apparently contradicts the widely accepted notion of vaginal parity being the strongest risk factor for pelvic organ prolapse [Swift et al., 2005; Turner et al., 2009; Ashton-Miller and Delancey, 2009]. The childbirth may result in pelvic floor laxity and genital hiatus enlargement. The pelvic organs must be held in place by the suspensory ligaments [Kerkhof et al., 2009]. Therefore, the ligaments will sustain these loads and the connective tissues will stretch. This enforces the idea that biomechanical modifications in uterosacral ligaments may be caused by pregnancy and parturition. A tissue more requested becomes stronger. A significantly higher elastic modulus was observed in postmenopausal vaginal tissue [Goh, 2002]. This age-related stiffening of vaginal tissues raises the possibility of a reduction in pelvic organ mobility with increasing age. Current research also suggests that supportive tissues actively remodel in response to different environmental stimuli [Alperin and Moalli, 2006]. Modifications in collagen content may suggest tissue repair after overstretching of the supportive connective tissue of the pelvic floor load [Kerkhof et al., 2009]. Supportive tissues are actively remodeling to accommodate a progressively increasing mechanical load [Alperin and Moalli, 2006; Moalli et al., 2005].

The limitations of the present study must be considered before interpreting the results. The ideal measurement of the biomechanical properties would be done in-vivo conditions. Tissue suction and indentation techniques constitute non-destructive and less invasive tests that have been used to evaluate the mechanical properties of human tissues [Epstein et al., 2008a; Tay et al., 2006]. However, by logical and ethical reasons, this kind of procedures cannot be applied to analyze these supportive structures. The feasible animal models (rabbit, guinea pigs, sheep, etc) are quadrupedal, factor which differentiates significantly the morphology and physiology of their pelvic cavity from the humans [Abramowitch et al., 2009]. Therefore, the tests were performed in human cadaver, whose availability is limited.

The presented evidence suggests that Uterosacral ligaments (USL) are stronger than round ligaments (RL). Parturition seems to enhance the stiffness and maximum stress of the uterosacral ligaments. Further studies are required to evaluate the factors influencing the biomechanical proprieties of female pelvic tissues. Biomechanical characterization of female pelvic tissues is an important step toward a more complete understanding of the pathology mechanisms leading to pelvic organ prolapse.

5.5 Mechanical properties of polypropylene mesh used in pelvic floor repair

5.5.1 Introduction

The contact between two materials with different hardnesses may cause erosion (damage) in the softer material as a result of mechanical abrasion [Czichos et al., 2006, Ch. 13-Friction and Wear]. The knots and thread folds in polypropylene mesh (Figure 5.13) used in surgeries for pelvic floor repair, may be perceived as small, harder particles in the contact region. If the mesh slides in contact with the surrounding tissue, occurs a process called sliding abrasion or “two-body” abrasion (Figure 5.12 (a)). If one of the knots is specially protruding the abrasion phenomena is called scratching. Figure 5.12 (b) shows the effect of long term (18 years) mesh erosion.

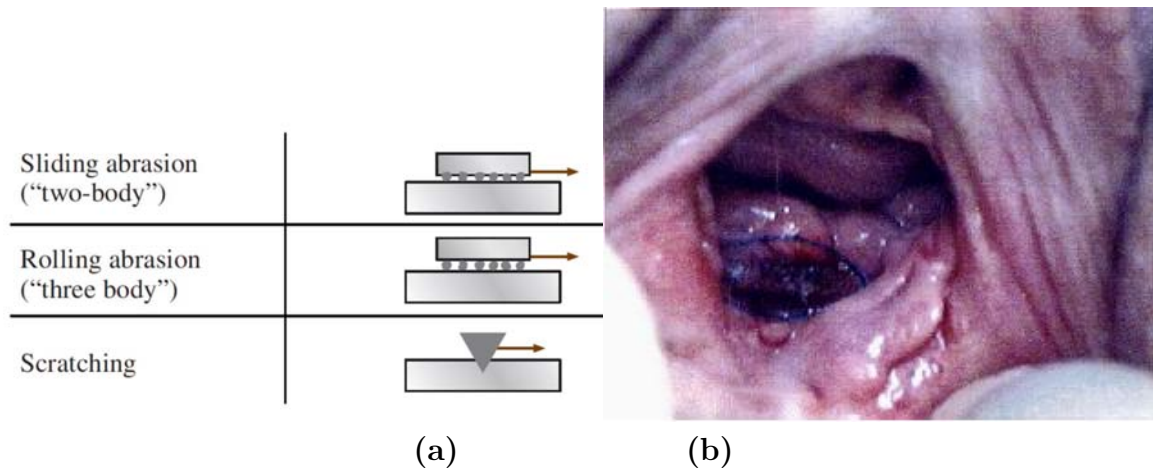


Figure 5.12: (a) The contact interface can have a significant role in this process [Wriggers, 2006, Ch.5], a consideration valid for biological systems [Weaver and Haut, 2005]. Abrasive wear due to the presence of hard particles in a sliding contact, and scratch damage. Adapted from [Czichos et al., 2006, Ch. 13-Fig.13.2]. (b) Mesh with prolene suture protruding through vagina due to mesh erosion 18 years after a rectocele repair. Adapted from [Putran et al., 2010].

In this sense, it is possible to infer that the vaginal and urethral erosion rates of synthetic sub-urethral slings may be related to the mechanical properties of the filament, among other characteristics of the meshes used, such as mesh geometry. In a work comparing the mesh erosion rates for two different meshes used for transvaginal repair of cystocele, Deffieux et al. [2007] did not find a significant difference in the incidence of vaginal erosion.

The erosion rate of polypropylene mesh used in stress incontinence surgery is approximately 1 – 3% [Petros, 2007, pp 108-156], but the low elasticity, micropores lower than 10 μm and multifilament polypropylene meshes may pre-dispose to an unacceptably high rate (17 – 20%) of defective vaginal healing and mesh extrusion [Abdel-Fattah et al., 2006; Siegel, 2005; Siegel et al., 2005].

Falconer et al. [2001] investigated the influence on the paraurethral connective tissue of different sling materials used in incontinence surgery. The authors reported a minimal inflammatory reaction, without significant change in collagen solubility, in the polypropylene group. Cosson et al. [2003b] reviewed the literature concerning all types of synthetic implants used in prolapse repair and in the treatment of stress urinary incontinence and analyzed the mechanical properties and the tolerance to the various products used. The most promising appears to be the polypropylene meshes, which offer mechanical properties of durability and elasticity. Mesh stiffness may not conform

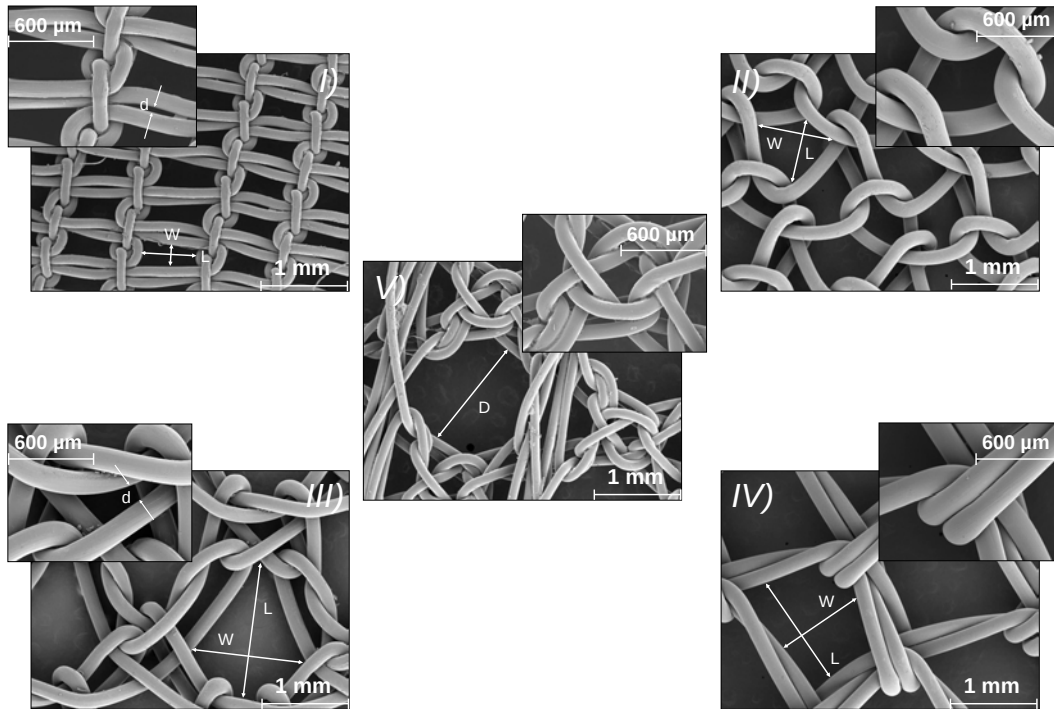


Figure 5.13: Details of the five different Meshes; D diameter, W width L length, d distance between filaments; I Aris™, II Auto Suture™, III Uretex™, IV TVTO™, V Avaulta™.

to the surrounding tissue as well as the more flexible sling, further interfering with integration [Siegel, 2005; Siegel et al., 2005; Falconer et al., 2001; Cosson et al., 2003b; Comiter and Colegrove, 2004]. The more flexible slings seem to adhere and conform to the underlying tissues better than the stiffer sling [Siegel, 2005]. The polypropylene mesh used specifically for the treatment of stress urinary incontinence may present 50% more flexibility than the standard mesh [Johnson&Johnson, 2003]. Differences of flexibility between different monofilament polypropylene tapes are related with mesh weave (Figure 5.13) and thickness of the surgical suture. This study aimed to compare the stiffness, under uniaxial tensile and flexural conditions, of different urogynecology meshes and one standard mesh.

5.5.2 Materials and methods

To understand what distinguishes the different mono-filament polypropylene tapes used as implants for pelvic floor repair, five different mesh types were mechanically tested (Aris™, TVTO™, Uretex™, Avaulta™ and Auto Suture™). Aris™, TVTO™

and UretexTM are indicated for the treatment of female stress urinary incontinence resulting from urethral hyper-mobility and/or intrinsic sphincter deficiency. AvaultaTM is indicated for tissue reinforcement and long-lasting stabilization of fascial structures of the pelvic floor in vaginal wall prolapse. Auto SutureTM (Surgipro Mesh Ref SPMM35) is not tested in the practical clinic for pelvic floor repair. For each type of synthetic sling under study, three specimens with total length of approximately 40 mm (± 0.5 mm) were prepared using a scalpel and cut from the original material. There were slight variations in width according to the technical specifications of each particular sling tested. For the Auto SutureTM mesh, samples of 40 mm in length and 10 mm in width were cut. The width dimension was chosen according to the values found for the other four meshes (between 10 and 11 ± 0.5 mm, according to our measurements). All the samples were tested with a constant displacement rate of $5 \text{ mm} \cdot \text{min}^{-1}$ in the mechanical testing machine described in Chapter 4 and according with the experimental protocol presented in [Martins et al., 2006b]. All the tests were performed at room temperature since as reported by Dietz et al. [2003], the temperature (room temperature, $\approx 20^\circ\text{C}$ or body temperature $\approx 37^\circ\text{C}$) did not affect the results significantly. Two types of mechanical tests were carried out, from which three different mechanical properties were extracted. The first test method used, consists on the uniaxial tensile test (Figure 5.14 (a)). The load and displacement values were recorded, and two stiffness parameters E_I and E_{II} can be extracted from this curve, according to the scheme illustrated in Figure 5.15. This parameters resemble modulus E_S and E_t defined in Section 4.2.1 for the stress-stretch curve.

The linear interpolations (with slopes E_I and E_{II}), used the intervals of load displacement data points with loads in the range of $0 \leftrightarrow 500 \text{ g}$ for E_I and loads in the range of $500 \leftrightarrow 2000 \text{ g}$ for E_{II} . These limits were taken because the load displacement curves of Figure 5.16 clearly present two different mechanical behavior regimens.

In a previous work studying the mechanical properties of urogynecologic implant materials, Dietz et al. [2003] [Schumpelick et al., 1999] have done the calculation of one stiffness parameter, accommodating all the physiological load range for the intra-abdominal pressure of $10 \leftrightarrow 16 \text{ N}$. However, the kind of non-linearity shown by the load displacement curves for the polypropylene meshes is also observed on load displacement experiment results of soft tissues [Fung, 1993, p.261]. In practice, the calculation of the

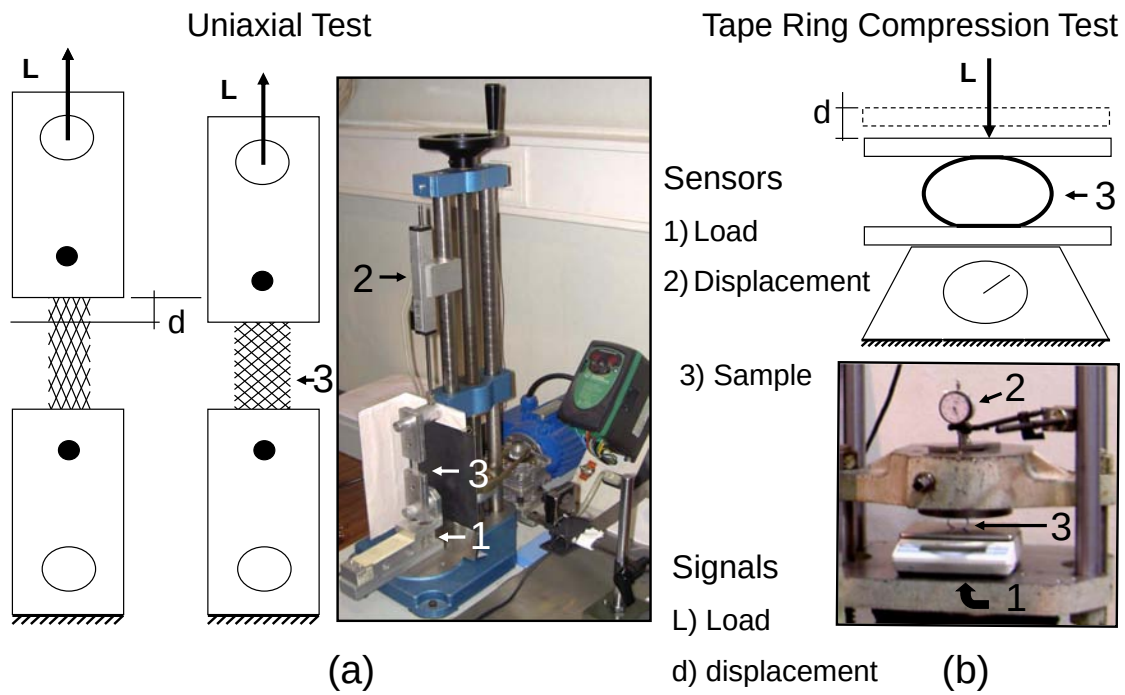


Figure 5.14: Experimental apparatus and mechanical test schemes a, b

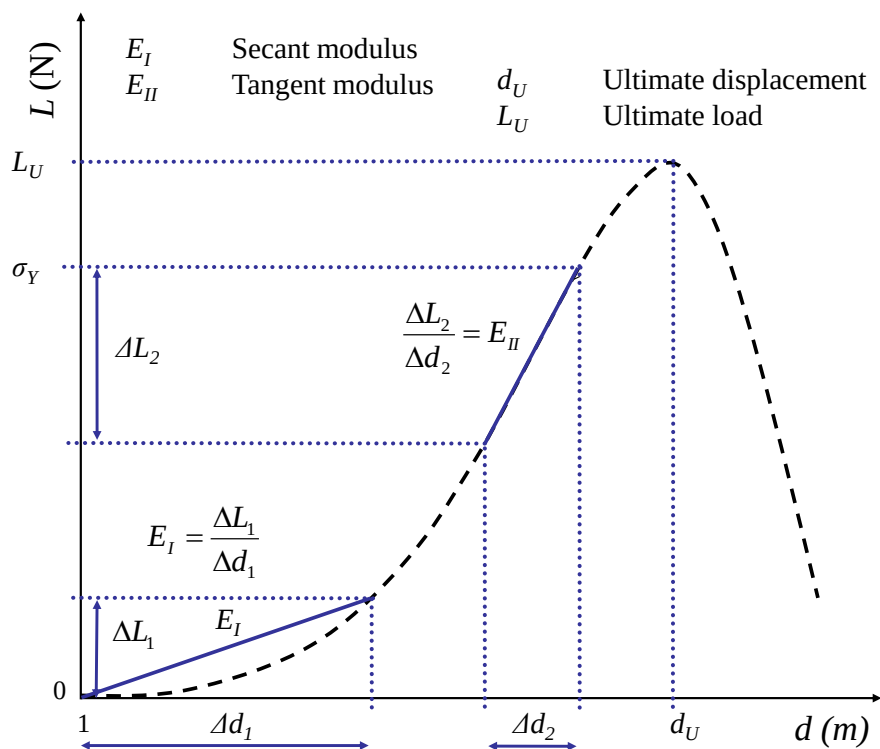


Figure 5.15: Typical load-displacement curve showing the definitions of stiffness quantities E_I and E_{II} .

Table 5.14: Fundamental geometrical properties for each mesh.

Tapes	$[dimension \pm SD (mm)]$			
	<i>length</i>	<i>width</i>	<i>diameter</i>	<i>fiber diameter</i>
Aris TM	0.575 ± 0.096	0.209 ± 0.014	—	0.113 ± 0.003
Auto Suture TM	0.900 ± 0.028	0.730 ± 0.048	—	0.164 ± 0.013
Avaulta TM	—	—	1.379 ± 0.071	0.105 ± 0.007
TVTO TM	1.290 ± 0.227	1.093 ± 0.170	—	0.179 ± 0.009
Uretex TM	1.470 ± 0.144	1.218 ± 0.147	—	0.170 ± 0.018

two stiffness parameters can correspond to an approximation of the load displacement curve's complex behavior. The other experiment performed allowed the comparison of the behavior of mesh samples in a mechanical situation where flexural mechanical properties are dominant (Figure 5.14-(b)).

The mechanical test devised by the authors is inspired on the compression tests performed in pipe sections [Moser, 2001, p.267]. The mechanical testing machine was used with a constant displacement rate, allowing in this way a continuous compression of the mesh loops. Load and displacement values were taken for each 1 *mm* deformation in diameter (Figure 5.17). The main geometrical characteristics of each mesh, the polypropylene monofilament diameter and the pore size were measured using the electron microscope images from Figure 5.13. The average results from these measurements are presented in Table 5.14.

5.5.3 Results

In the present section, results of the uniaxial tensile and tape ring tests are presented. Both tests were performed using batches of three samples for each of the five mesh types studied. All the results displayed graphically, in Figures 5.16 and 5.17, represent average results for the respective sample batches (three samples). In Figure 5.16, the results for the uniaxial tensile tests (Tables 5.15 and 5.16) are presented graphically. For deformations superior to 15 *mm* there is a strong increase on the variations around the mean Load-displacement curve. This increase on the dispersion of the experimental results is also verified in the tissues from the female pelvic cavity (see Figure 5.3 for instance).

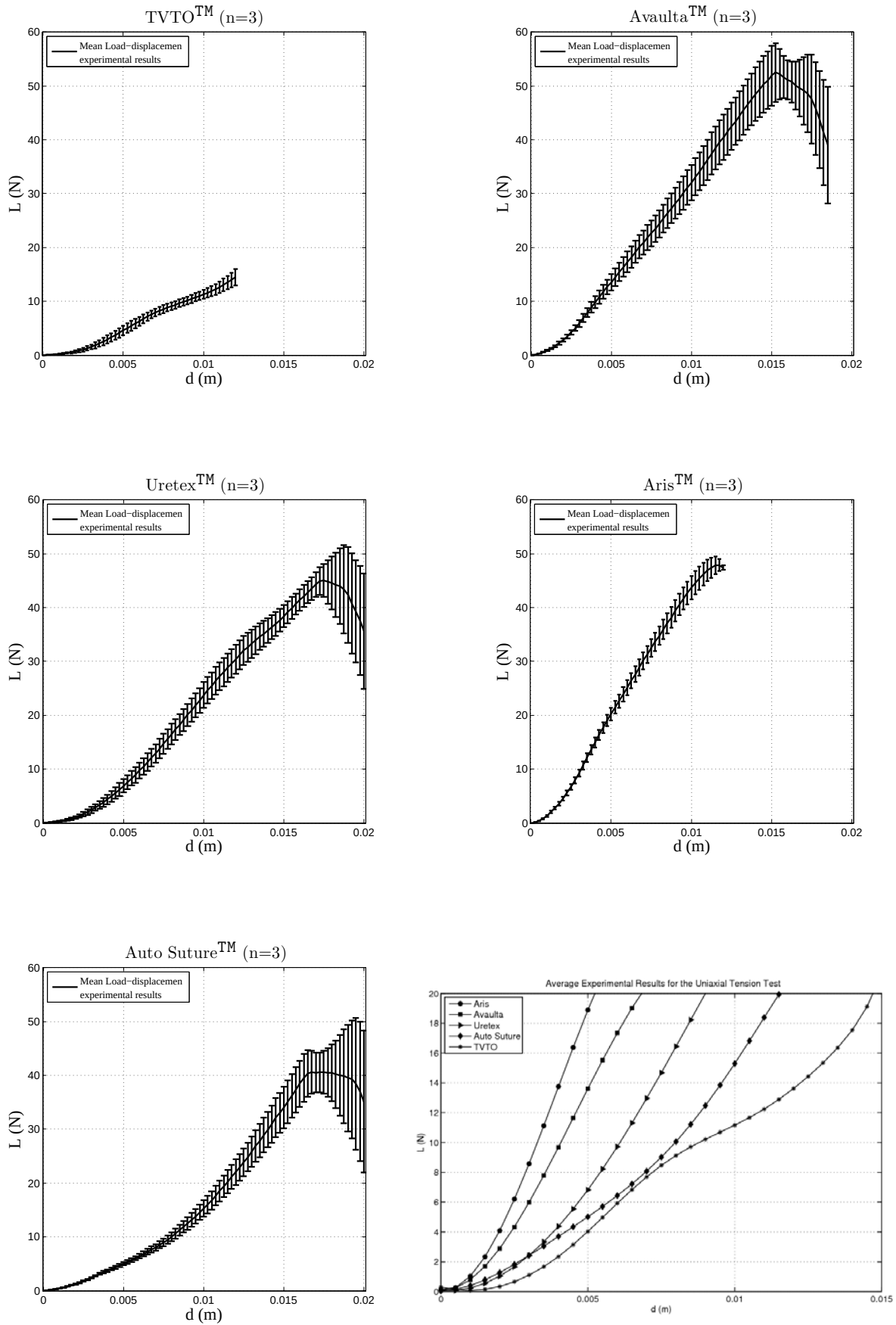


Figure 5.16: Uniaxial test results for the five different meshes tested: Load $L(N)$ vs displacement $d(m)$. Typical force-displacement behavior for each mesh.

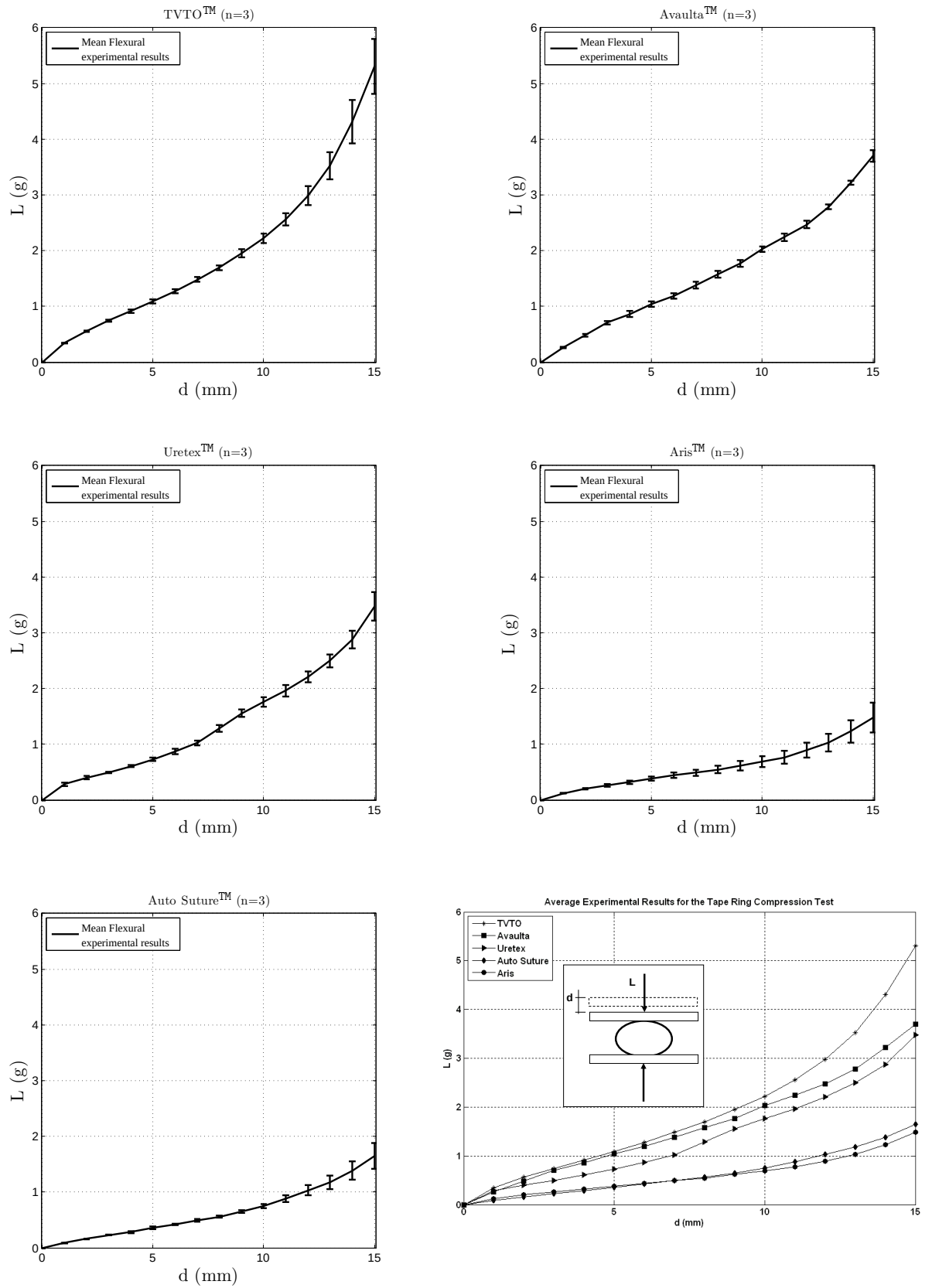


Figure 5.17: Experimental results for the tape ring compression test: Load $L(g)$ vs displacement $d(mm)$. Typical force-displacement behavior (mean $\pm$ SEM) for each mesh.

The analysis of Figure 5.16 shows two different regimens in the load-displacement curve, evolving from a non-linear to a quasi-linear regimen. To understand the differences between the materials for both the linear and non-linear regimen, the stiffness parameters E_I and E_{II} were calculated according to the criteria presented in the last section. The mean values and standard deviations of E_I and E_{II} , for each type of mesh tested, are presented on Table 5.7. The most significant difference of mechanical behavior appears between Aris™ and TVTO™ and is verified on the E_I ($p=0.001$) and E_{II} ($p=0.0003$) regions. In fact, the only significantly similar ($p=0.42$) mechanical behaviour occurs for Auto Suture™ and TVTO™ on E_I . The tape ring compression test results are shown in Figure 5.5. On this test, the flexural properties of the material play the most important part. The test conditions were maintained throughout the testing procedures, providing in this way the empirical means for a direct comparison between meshes. From the analysis of the experimental data, we classified the mechanical behaviour (for this particular test conditions), in two main groups. The first group includes Auto Suture™ and Aris™ meshes. The two meshes seem to have a similar flexural behaviour, from the analysis of the graphic in Figure 5.5 ($p=0.65$ for $d=5$ mm; $p=0.66$ for $d=15$ mm). The second group includes TVTO™, Uretex™ and Avaulta™. The difference between these two groups is clearly evident comparing TVTO™ (maximum load values) and Aris™ (minimum load values). The statistical analysis of the load values for a displacement of 5 ($p=0.0002$) and 15 mm ($p=0.0024$) supported these conclusions. Main geometrical properties (porosity-length and width and monofilament diameter) of each mesh are presented in Table 5.14. These measurements were taken from the electron microscope pictures presented in 5.13, using the freeware application ImageJ [Open-Source, 2007]. The interstices between filaments measured an average 0.04 mm , being of the same order of magnitude for all the monofilament meshes, as illustrated in Figure 5.13.

5.5.4 Discussion and conclusions

In this study, only the in vitro uniaxial tensile and flexural stiffness properties were examined. All the meshes tested are regularly used in the practical clinic; in consequence, no additional clinical tests were performed. Material stiffness has been associated with

Table 5.15: Experimental data extracted from polypropylene urogynecologic mesh implants: ArisTM, TVTOTM, UretexTM, AvaultaTM, Auto SutureTM. Uniaxial tensile test.

Sample	d_U (m)	L_U (N)	$[0 \leftrightarrow 500] g$	$[500 \leftrightarrow 2000] g$
			$E_I [(N/m) \times 1000]$	$E_{II} [(N/m) \times 1000]$
Mh_Aris1	0.0117	47.9680	2.5023	5.6225
Mh_Aris2	0.0147	51.2433	2.5257	5.7419
Mh_Aris4	0.0121	46.6369	2.1414	4.7471
Mh_Avlt1	0.0210	56.9668	1.5961	3.0235
Mh_Avlt2	0.0174	61.5389	1.8206	3.7711
Mh_Avlt3	0.0151	61.4672	1.9791	4.5485
Mh_TVTO1	0.0138	18.2526	0.8862	1.3548
Mh_TVTO2	0.0199	56.7951	1.1566	1.4886
Mh_TVTO3	0.0120	11.9069	0.7855	1.0815
Mh_AuSt1	0.0165	45.5966	1.0796	2.5230
Mh_AuSt2	0.0198	44.4710	0.9638	1.9917
Mh_AuSt3	0.0194	56.6424	1.1086	2.2725
Mh_Urtx1	0.0171	43.0396	1.0915	3.1829
Mh_Urtx2	0.0219	53.6410	0.9715	3.0612
Mh_Urtx3	0.0187	55.9835	1.3471	3.4252

Table 5.16: Uniaxial results. E_I , E_{II} , d_U and L_U (mean $\pm$ SD).

Tapes	$E_I \pm SD [(N/m) \times 1000]$	$E_{II} \pm SD [(N/m) \times 1000]$
Aris TM	2.3898 ± 0.2154	5.3705 ± 0.5432
TVTO TM	0.9428 ± 0.1919	1.3083 ± 0.2075
Uretex TM	1.1367 ± 0.1918	3.2231 ± 0.1853
Avaulta TM	1.7986 ± 0.1925	3.7810 ± 0.7626
Auto Suture TM	1.0507 ± 0.0766	2.2624 ± 0.2658
	$d_U \pm SD$ (m)	$L_U \pm SD$ (N)
Aris TM	0.0128 ± 0.0016	48.6161 ± 2.3706
TVTO TM	0.0152 ± 0.0041	28.9849 ± 24.2924
Uretex TM	0.0192 ± 0.0024	50.8881 ± 6.8971
Avaulta TM	0.0178 ± 0.0030	59.9910 ± 2.6192
Auto Suture TM	0.0186 ± 0.0018	48.9033 ± 6.7258

the likelihood of tissue erosion [Fenner, 2000]; however, in the present work, no clinical studies have been carried out to confirm this preposition. Dietz et al. [2003] consider that the stress transmission at the tissue/implant interface will be influenced by the biomechanical properties of implant and native tissue, respectively, and the biomechanical properties of synthetic meshes is important in choosing appropriate materials for a variety of urogynecologic procedures, particularly sub-urethral slings. These authors conclude that the lowest initial stiffness, i.e. low resistance to deformation at forces below the elastic limit, may be at least partly responsible for the apparent clinical success of the implants. This observation seems to be supported by other authors. Feifer and Corcos [2007] referred that tensile strength of synthetic meshes is not a differentiating factor among products since it is one order of magnitude higher than normal fibrous tissue. Neymeyer et al. [2007] also recommended that the most appropriate mesh properties should be as close as possible to natural human tissue. For the same material, stiffness is related with mesh geometry, mesh manufacturing and mesh thickness. Alternatively, for the same mesh (same manufacturing and same thickness), the stiffness can be changed by using different materials. The sample's stiffness is directly proportional with thickness values obtained from the tensile test. However, considering the tape ring compression test, stiffness varies in an inversely cubic manner with thickness [Popov, 1990, Ch. 6]. From solid mechanics considerations, we can assume that polypropylene filament thickness has more influence (mechanically) on the tape ring compression test than on the uniaxial tensile test. This study shows that there are significant differences on the mechanical properties between urogynecology meshes. The TVTO™ mesh as stated by the manufacturer is approximately 50% more flexible than the standard polypropylene mesh, and the bi-directional elastic properties allow adaptation to various stresses encountered in the body [Johnson&Johnson, 2003]. The test results obtained in the present work confirmed that the referred mesh has an inferior tensile stiffness when compared to the other studied meshes. Although the experimental values of tensile and flexural stiffness are low, Auto Suture™ is currently not used in the practical clinic for pelvic floor repair. Considering the stress urinary incontinence meshes, Aris™ presented a tensile stiffness significantly higher than Uretex™ and TVTO™; however, the flexural stiffness was found to be significantly smaller. For these meshes, the decrease in flexural stiffness is followed by a reduction in monofila-

ment diameter. All these meshes are made of polypropylene monofilament fibres and present macropores [Abdel-Fattah et al., 2006; Deval et al., 2006]. The tested mesh used on pelvic floor repair of vaginal wall prolapse (Avaulta™) shows high tensile and flexural stiffness values. Additional clinical studies are necessary to evaluate the real importance of the flexural stiffness. A comparison of the present results with previous related works is consistent with the behaviour of TVTO™ mesh stiffness. In the current work as well as in Dietz et al. [2003], the TVT presents the lowest tensile stiffness values.

In conclusion, this research revealed significant differences on the mechanical properties between different urogynecology meshes.

5.6 Conclusions

This chapter represents a significant part of this thesis. Here were presented experimental results and clinical discussion and conclusions. In case medical doctors are not familiar with numerical methods, they can correlate the results of this chapter with their clinical practice. Experimental results in this chapter are mostly unique and presented here for the first time in a consistent way. We restricted the results to uniaxial static tensile tests on bladder, vaginal tissues, Uterosacral and round ligaments. The experimental data concerning *levator ani* complex muscles and *Scarpa's* fascia can be consulted in Appendix A. It is the author's belief that this chapter has successfully proved that the experimental set-up can produce adequate and useful results to be used in practical clinic. It was shown that the experimental curves are as expected although scatter is evident.

5.6.1 The Influence of POP on the biomechanical properties of vaginal tissue

The factors influencing the tensile biomechanical properties of vaginal tissue in prolapsed (POP) and non-POP women were investigated. Forty patients submitted to

surgery for POP and 15 non-POP female cadavers were prospectively evaluated. The uniaxial tensile test to obtain stiffness (E_t) and maximum stress (σ_{max}) was applied to vaginal wall samples. POP patients were associated to higher values of E and Smax in anterior vaginal wall compared to the posterior vaginal wall. In contrast, non-POP women presented lower values of E and Smax in anterior vaginal wall. The occurrence of POP was the only independent predictor of higher values of E and Smax in anterior vaginal samples. Women with severe anterior vaginal prolapse presented higher levels of E and Smax in anterior vaginal sample compared to those with lower POP stages. It can be concluded that anatomic distortion caused by POP is followed by significant changes of biomechanical properties in the vagina.

5.6.2 Uniaxial mechanical behavior of Human female bladder

The tensile biomechanical properties of female human bladder were investigated. Tissue samples were obtained from 13 cadavers without clinical noticed pelvic floor dysfunctions. Uniaxial tensile tests to obtain stiffness and maximum stress of bladder tissue were performed. There was a strong positive correlation between stiffness and maximum stress in bladder tissue. Women with less than 50 years presented higher bladder stiffness compared to older subjects. The bladder maximum stress was not associated with age. Body mass index and menopausal status were not associated with biomechanical proprieties. There was a great variability in the measurements of stiffness and maximum stress in bladder tissues. In conclusion, age may influence the uniaxial mechanical behavior of female human bladder.

5.6.3 Round and Uterosacral Ligaments Strength: A Biomechanical Study

The tensile biomechanical properties of round and uterosacral ligaments were also investigated. The tissue samples were obtained from 15 non-prolapsed female cadavers. Uniaxial tensile tests to obtain stiffness and maximum stress of round and uterosacral

ligaments were performed. The uterosacral ligaments presented higher stiffness and maximum stress compared to the round ligaments. There was a strong positive correlation between stiffness and maximum stress in female pelvic ligaments. No correlations were found between the biomechanical properties of round and uterosacral ligaments and age, body mass index and menopausal status. Nulliparous women were associated to lower uterosacral stiffness and maximum stress when compared to parous women. It seems that uterosacral ligaments are significantly more resistant than round ligaments. Parturition seems to enhance the stiffness and maximum stress of the uterosacral ligament.

The presented evidence suggests that Uterosacral ligaments (USL) are stronger than round ligaments (RL). Parturition seems to enhance the stiffness and maximum stress of the uterosacral ligaments.

5.6.4 Mechanical properties of polypropylene mesh used in pelvic floor repair

Five different mesh types were mechanically tested in order to compare the stiffness of different meshes under two types of mechanical tests. The methods used consisted on uniaxial tensile test (tensile stiffness) and tape ring tests, experimental continuous compression of the mesh loops (flexural stiffness). This study shows that there are significant differences on the mechanical properties between urogynecology meshes.

Chapter 6

Constitutive modeling of biological soft tissues

6.1 Introduction

This chapter introduces the theoretical framework used for modeling the complex (non-linear) mechanical behavior experimentally observed on highly hydrated soft tissues. The nonlinear theory of elasticity, being a fundamental tool for the treatment and interpretation of the experimental results, was used to model the mechanical behavior of soft biological tissues using constitutive materials models.

The basis of the work here presented was object of publication by the author and colleagues [Martins et al., 2006b, 2010b].

6.2 From continuum mechanics to nonlinear solid mechanics

In order to study materials with nonlinear mechanical properties, which usually undergo large strains with small applied stresses, a nonlinear version of the classical

elasticity theory was developed [Ogden, 1984; Holzapfel, 2000; Lai et al., 1996]. To understand the deformation of solids and generalize it to a possible nonlinear behavior, requires a knowledge of the kinematic relations, the equations of balance and the constitutive equations.

Continuum mechanics as a starting point

Continuum mechanics allows the study of deformation and flow in a continuous medium subjected to forces, either internal or external. The continuous medium takes a portion of the 3D space, and it is confined to a given volume. The possibility of volume and boundary change with time i.e., the continuous change (flow) between two configurations is in fact the motion of the continuum [Sachse, 2004, Ch. 4].

For the modeling of biological soft tissues, elasticity theory applied to continuous media, is specially important. Elasticity theory constitutes a means for the quantitative description of structurally complex biological soft tissues, under the influence of external forces.

6.2.1 Kinematics of a deformable body

If a body is considered a continuum of material particles (Figure 6.1), in respect to an initial ($t = 0$) reference coordinate system, $\mathbf{E}_A$, $A = \{1, 2, 3\}$, the coordinates of a material point Q on its initial state are given by $\mathbf{X} = X_1\mathbf{E}_1 + \dots + X_3\mathbf{E}_3$ (on a cartesian system). In a deformed configuration at time t the coordinates of the deformed point q on the local cartesian system $\mathbf{e}_a$, $a = \{1, 2, 3\}$ are given by the position vector $\mathbf{x} = x_1\mathbf{e}_1 + \dots + x_3\mathbf{e}_3$. The motion of the set of material points Ω (the continuum) may be mapped as a one-to-one continuous transformation between the initial and deformed configurations by the transformation $\phi : \Omega \rightarrow \mathbf{R}^3$. So the position of a given particle on the position $\mathbf{X}$ at $t = 0$ and $\mathbf{x}$ at t is,

$$\mathbf{x} = \phi(\mathbf{X}, t) \quad ; \quad x_a = \phi(X_A, t) \quad ; \quad a, A = 1, 2, 3 \quad (6.1)$$

As the flow of each point from the continuum Ω describes a trajectory in space, this curve is described by the parametric equation (6.1). Assuming the continuity conditions

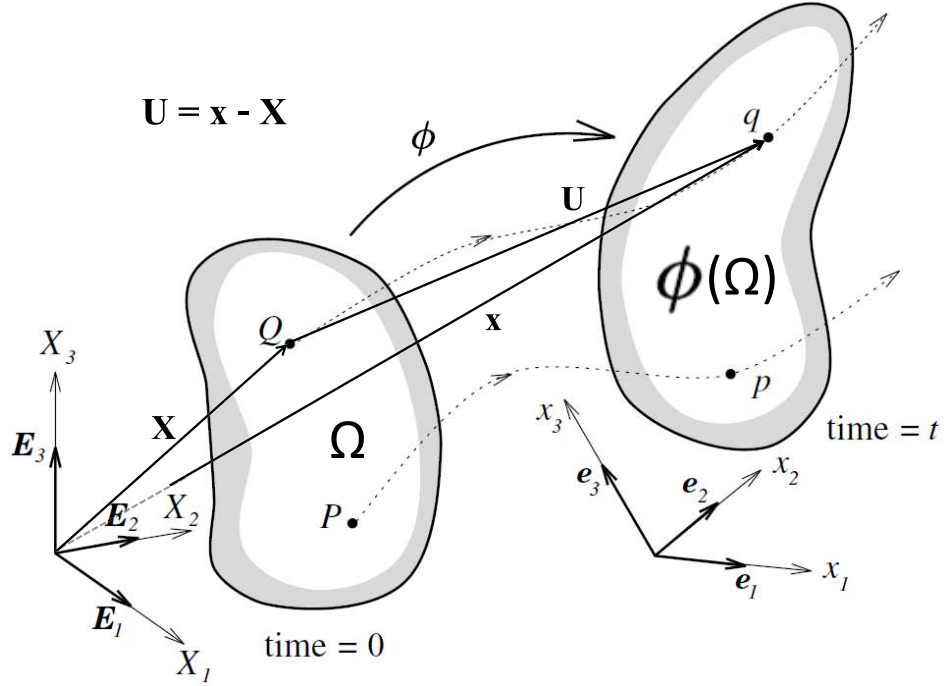


Figure 6.1: General motion of a deformable body. Displacement of the material point Q through the transformation $\mathbf{x} = \phi(\mathbf{X}, t)$ i.e., $\mathbf{U} = \mathbf{x} - \mathbf{X}$. Adapted from [Bonet and Wood, 1997, Ch. 3].

are guaranteed for $\forall Q : Q \in \Omega$, it is possible to define the inverse transformation of Q into q ,

$$\mathbf{X} = \phi^{-1}(\mathbf{x}, t) \quad ; \quad X_A = \phi^{-1}(x_a, t) \quad ; \quad a, A = 1, 2, 3 \quad (6.2)$$

6.2.2 Eulerian (spatial) and Lagrangian (material) descriptions

In finite deformation analysis the representation of physical quantities, may be done using the material coordinates (X_1, X_2, X_3) and time t using 6.1. This is called the *material* or *Lagrangian* description of continuous media [Kojic et al., 2008, Ch. 2]. The *Eulerian* description uses the spatial coordinates (x_1, x_2, x_3) and time t i.e., relation 6.2 and is typically used in fluid mechanics.

There are two types of *Lagrangian* formulations, the *total Lagrangian* formulation and the *updated Lagrangian* formulation. It is going to be used the *total Lagrangian* formulation i.e., the derivatives and integrals of the *Lagrangian* physical quantities (strains, stretches, stresses, etc.) are computed in respect to the material coordinates, $\mathbf{X}$,

6.2.3 From infinitesimal deformations to finite deformations

In finite deformation analysis the amplitude of the material point's displacement, $\mathbf{U} = \mathbf{x} - \mathbf{X}$ may exceed (without any restriction) the dimensions of the domain Ω [Bonet and Wood, 1997, Ch. 3]. However, if an infinitesimal deformation is considered so that instead of $\mathbf{U}(\mathbf{X})$ we have $\mathbf{U}(\mathbf{X} + d\mathbf{X})$ (see [Lai et al., 1996, Ch. 3] for details), the mapping of the transformation of the material line element $d\mathbf{X} = (dX_1, dX_2, dX_3)$ into the spatial line element $d\mathbf{x} = (dx_1, dx_2, dx_3)$ is made by the deformation gradient tensor, $\mathbf{F}$. The relation between the two elements is simply given by,

$$d\mathbf{x} = \mathbf{F}d\mathbf{X} \quad (6.3)$$

Using the transformation ϕ , defined previously, $\mathbf{F}$ can be defined as [Holzapfel, 2000, Ch. 2],

$$\begin{aligned} \mathbf{F} &= \nabla \phi(\mathbf{X}, t) = F_{aA} \mathbf{e}_a \otimes \mathbf{E}_A = \frac{\partial x_a}{\partial X_A} \mathbf{e}_a \otimes \mathbf{E}_A \\ \mathbf{F} &= \frac{\partial \mathbf{x}}{\partial \mathbf{X}} \quad ; \quad F_{aA} = \frac{\partial x_a}{\partial X_A} \quad ; \quad a, A = 1, 2, 3 \end{aligned} \quad (6.4)$$

$\mathbf{F}$ is a second order tensor and is a linear operator. Preserving the original assumption of a one to one mapping of the material points, $\forall P : P \in \Omega, P \xrightarrow{\phi} p$, requires a preservation of every point in the continuum. This reflects into $\mathbf{F}$ by the imposition of its non-singularity, in practice:

$$J = \det(\mathbf{F}) \neq 0, \quad (6.5)$$

The Jacobian determinant J must be further conditioned, if there is mass preservation, so that $J > 0$. This condition guarantees that the inverse line element transformation exists $d\mathbf{X} = \mathbf{F}^{-1}d\mathbf{x}$, meaning that we can establish the inverse deformation gradient $\mathbf{F}^{-1}$,

$$\mathbf{F}^{-1} = \frac{\partial \mathbf{X}}{\partial \mathbf{x}} \quad ; \quad (F_{aA})^{-1} = \frac{\partial X_A}{\partial x_a} \quad ; \quad a, A = 1, 2, 3 \quad (6.6)$$

The transformation of area is given by [Parente, 2008],

$$d\mathbf{a} = J\mathbf{F}^{-T}d\mathbf{A} \quad (6.7)$$

and the volume deformation by,

$$d\mathbf{v} = J d\mathbf{V} \quad (6.8)$$

6.2.4 Incompressible hyperelastic materials under uniaxial stress conditions

Given the extensive uniaxial testing in this work we detail the formulation for incompressible hyperelastic materials under uniaxial stress conditions.

The particular form of $\mathbf{F}$ in the case of hyperelastic materials subjected to an uniaxial tension is,

$$\mathbf{F} = \begin{bmatrix} \lambda & 0 & 0 \\ 0 & \frac{1}{\sqrt{\lambda}} & 0 \\ 0 & 0 & \frac{1}{\sqrt{\lambda}} \end{bmatrix} \quad (6.9)$$

assuming that the stress is applied along the $\hat{x}_1$ direction (see figure 6.2), taking $\lambda_1 = \lambda$ and noticing that the incompressibility condition

$$J = \prod_{i=1}^3 \lambda_i = \det(\mathbf{F}) = 1 \quad (6.10)$$

requires that, $\lambda_2 = \lambda_3 = \frac{1}{\sqrt{\lambda}}$.

The Right (C) and Left (b) Cauchy-Green tensors can be obtained from (6.9).

$$\mathbf{C} = \mathbf{F}^T \mathbf{F} = \begin{bmatrix} \lambda^2 & 0 & 0 \\ 0 & \frac{1}{\lambda} & 0 \\ 0 & 0 & \frac{1}{\lambda} \end{bmatrix} \quad (6.11)$$

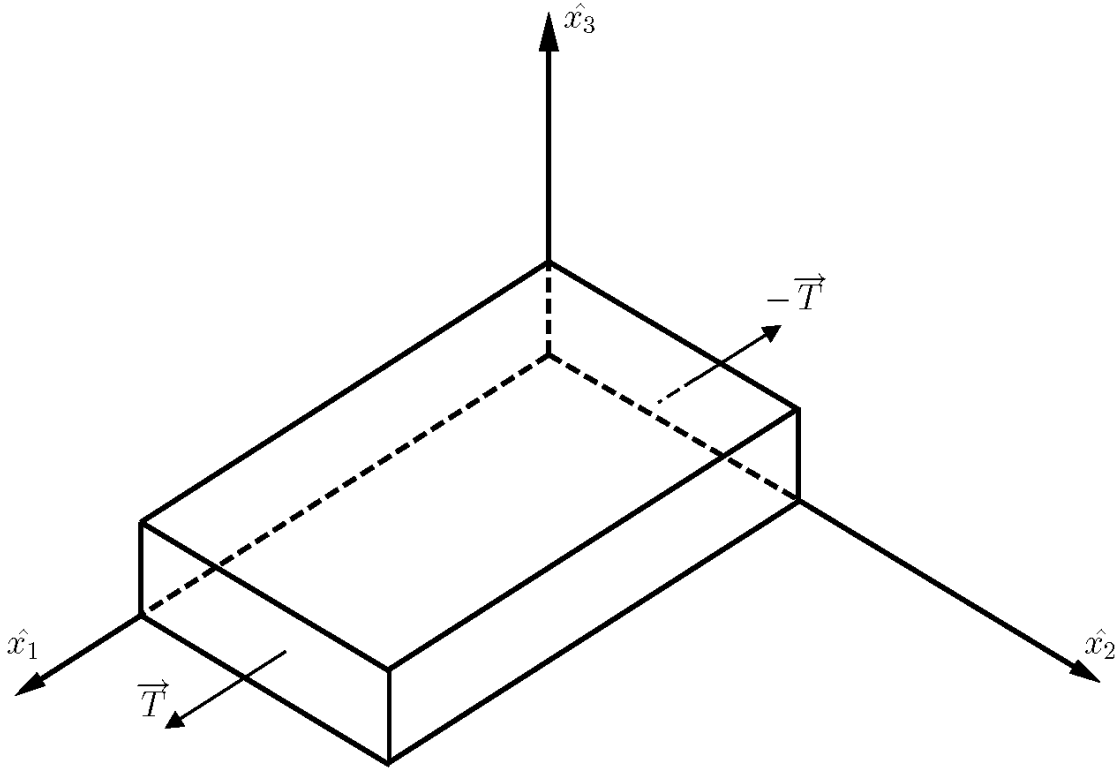


Figure 6.2: Uniaxial deformation. Tension state of a uniaxial tensile test.

$$\mathbf{b} = \mathbf{F}\mathbf{F}^T = \begin{bmatrix} \lambda^2 & 0 & 0 \\ 0 & \frac{1}{\lambda} & 0 \\ 0 & 0 & \frac{1}{\lambda} \end{bmatrix} \quad (6.12)$$

and in this particular case are equal. Now it is possible to define the invariants (6.13) from the Right Cauchy-Green tensor (6.11)

$$I_1 = \text{tr}(\mathbf{C}) \quad (6.13)$$

$$I_2 = \frac{1}{2} \left((\text{tr}(\mathbf{C}))^2 - \text{tr}(\mathbf{C}^2) \right)$$

$$I_3 = \det(\mathbf{C})$$

In this particular case they take the form

$$I_1 = \lambda^2 + \frac{2}{\lambda} \quad (6.14)$$

$$I_2 = 2\lambda + \frac{1}{\lambda^2}$$

$$I_3 = 1$$

6.3 Material models for soft tissues

The mechanical behavior of materials evidencing a nonlinear stress-stretch relation, and in particular materials that show large deformations for small applied stresses, can be described using hyperelastic material models. The early works of [Rivlin, 1948] give the strain energy function (SEF) the form of a polynomial expansion in terms of the strain invariants. Mooney [1940], contributed by limiting the Rivlin polynomial to a two term expansion assuming absolute incompressibility. Together, these two contributions constitute a seminal example of hyperelastic material model.

The strain energy function may also be expressed in terms of the principal stretches. An important example of this approach is the Ogden [Ogden, 1984] material model. To move from these classical examples, to strain energy functions that are capable of accommodating the specificity of biological materials requires, requires the process described in Sections 6.3.1 to 6.3.2.

The following model formulation, was based upon the work of Dr. Peña and Dr. Begoña and published by the author as colleagues in [Martins et al., 2010b].

6.3.1 Anisotropic hyperelastic response of soft tissues

As a first step toward a prediction of the nonlinear behavior of vaginal tissue, this section deals with the finite strain material model formulation. To better understand the approach taken it is necessary to consider finite strain hyperelasticity in terms of invariants with uncoupled volumetric/deviatoric responses, first suggested in Flory [1961], generalized in Simo and Taylor [1991] and employed for anisotropic soft biological tissues in Weiss et al. [1996]; Holzapfel et al. [2000]; Peña et al. [2007] among others. Based on histologic results and the nature of the tissue (see Section 3.3.1, Figure 3.6),

the vaginal tissue was assumed to behave as a transversely isotropic hyperelastic material.

A common way to formulate an elastic constitutive law for fibered soft tissues is to postulate the existence of a strain energy function that depends on the direction of the family of fibers at a point $\mathbf{X}$ that is defined by the unit vector field $\mathbf{m}_0$ [Peña et al., 2007]. The fiber moves with the material points of the continuum body, so the stretch λ_m of the fibers defined as the ratio between its lengths at the deformed and reference configurations can be expressed as

$$\lambda_m^2 = \mathbf{m}_0 \mathbf{C} \mathbf{m}_0 \quad (6.15)$$

where $\mathbf{m}$ is the unit vector of the fibers in the deformed configuration, $\mathbf{F} = \frac{\partial \mathbf{x}}{\partial \mathbf{X}}$ (equation (6.9)) and $\mathbf{C} = \mathbf{F}^T \mathbf{F}$ are the standard deformation gradient and the corresponding right Cauchy-Green strain measure. In order to handle more easily the quasi-incompressibility constraint, a multiplicative decomposition of $\mathbf{F} = J^{\frac{1}{3}} \bar{\mathbf{F}}$ and $\mathbf{C} = J^{\frac{2}{3}} \bar{\mathbf{C}}$ into *volume-changing (dilational)* and *volume-preserving (distortional)* parts is usually established as in [Simo and Taylor, 1991].

To characterize isothermal processes, we postulate the existence of a unique decoupled representation of the strain-energy density function Ψ [Simo and Taylor, 1991] that explicitly depends on both the right Cauchy-Green tensor $\mathbf{C}$ and the fibers direction $\mathbf{m}_0$

$$\Psi(\mathbf{C}, \mathbf{M}) = \Psi_{vol}(J) + \bar{\Psi}(\bar{\mathbf{C}}, \mathbf{M}) = \Psi_{vol}(J) + \bar{\Psi}(\bar{I}_1, \bar{I}_2, \bar{I}_4) \quad (6.16)$$

where $\Psi_{vol}(J)$ and $\bar{\Psi}$ are given scalar-valued functions of J , $\bar{\mathbf{C}}$, $\mathbf{M} = \mathbf{m}_0 \otimes \mathbf{m}_0$ respectively that describe the volumetric and isochoric responses of the material [Holzapfel, 2000], $\bar{I}_1$ and $\bar{I}_2$ are the first and second modified strain invariants of the symmetric modified Cauchy-Green tensor $\bar{\mathbf{C}}$ (Note that $I_3 = J$ and $\bar{I}_3 \simeq 1$). Finally, the pseudo-invariant $\bar{I}_4$ characterizes the constitutive response of the fibers [Spencer, 1954]:

$$\bar{I}_4 = \mathbf{C} : \mathbf{M} \quad (6.17)$$

From the Clausius-Planck inequality, we now define the constitutive equation for compressible hyperelastic materials

$$\mathbf{S} = 2 \frac{\partial \Psi(\mathbf{C}, \mathbf{M})}{\partial \mathbf{C}} = \mathbf{S}_{vol} + \bar{\mathbf{S}} = Jp\mathbf{C}^{-1} + \bar{\mathbf{S}} \quad (6.18)$$

where the second Piola-Kirchhoff stress $\mathbf{S}$ consists of a purely volumetric contribution $\mathbf{S}_{vol}$, a purely isochoric one $\bar{\mathbf{S}}$ and $p = \frac{d\Psi_{vol}(J)}{dJ}$ is the hydrostatic pressure.

The Cauchy stress tensor $\boldsymbol{\sigma}$ is $1/J$ times the push-forward of $\mathbf{S}$ ($\boldsymbol{\sigma} = J^{-1}\boldsymbol{\chi}_*(\mathbf{S})$)

$$\begin{aligned} \boldsymbol{\sigma} = & p\mathbf{1} + \frac{2}{J} \left[\left(\frac{\partial \bar{\Psi}}{\partial \bar{I}_1} + \bar{I}_1 \frac{\partial \bar{\Psi}}{\partial \bar{I}_2} \right) \bar{\mathbf{b}} - \frac{\partial \bar{\Psi}}{\partial \bar{I}_2} \bar{\mathbf{b}}^2 + \bar{I}_4 \frac{\partial \bar{\Psi}}{\partial \bar{I}_4} \mathbf{m} \otimes \mathbf{m} \right. \\ & \left. - \frac{1}{3} \left(\frac{\partial \bar{\Psi}}{\partial \bar{I}_1} \bar{I}_1 + 2 \frac{\partial \bar{\Psi}}{\partial \bar{I}_2} \bar{I}_2 + \frac{\partial \bar{\Psi}}{\partial \bar{I}_4} \bar{I}_4 \right) \right] \end{aligned} \quad (6.19)$$

with $\mathbf{1}$ the second-order identity tensor, $\bar{\mathbf{b}} = \bar{\mathbf{F}}\bar{\mathbf{F}}^T$ the modified left Cauchy-Green tensors.

6.3.2 Parameter estimation

The experimental data showed several relevant features of the vaginal tissue. First, specimens experienced finite strains for small loads. Second, a strongly marked non-linearity was found in the tissue. Therefore a suitable mechanical framework has to be adopted in order to properly model its mechanical behavior. Moreover, the tissue was assumed as incompressible, that is $I_3 = J^2 = 1$, [Holzapfel et al., 2005]. If we consider a simple tension test in the direction of the fibers with $\mathbf{n} = \mathbf{e}_z$, then $\lambda_z = \lambda$, $\lambda_x = \lambda_y = \lambda^{-\frac{1}{2}}$ and $I_4 = \lambda^2$, the Cauchy stress tensor $\boldsymbol{\sigma}_e$ becomes diagonal with $\sigma_{zz} = \sigma$, $\sigma_{xx} = \sigma_{yy} = 0$ [Merodio and Goicolea, 2007].

Three different strain-energy functions (SEF), representing the elastic energy stored in the material per unit volume, were used for mimicking vaginal tissue's mechanical behavior. The first is the well-known Quadratic exponential SEF for blood vessels (QExp) proposed by Holzapfel et al. [2000], shown in (6.20). In this equation I_1 represents the first invariant of the Cauchy-Green tensor [Spencer, 1954], $I_4 > 1$ characterizes the

mechanical response of the fibers, $k_0 > 0$ and $k_1 > 0$ are stress-like parameters, and $k_2 > 0$ is dimensionless.

$$\bar{\Psi} = k_0(\bar{I}_1 - 3) + \frac{k_1}{2k_2} \{ \exp[k_2(\bar{I}_4 - 1)^2] - 1 \} \quad (6.20)$$

Another exponential SEF was proposed by Pioletti [1997] for isotropic materials and it was modified in order to consider the anisotropy of the soft tissues by Natali et al. [2003]. It consists in an exponential function where $a_1 > 0$ and $a_2 > 0$ are stress-like parameters, and $a_3 > 0$ is dimensionless.

$$\bar{\Psi} = a_1(\bar{I}_1 - 3) + \frac{a_2}{a_3} (\exp^{a_3(\bar{I}_4 - 1)} - a_3(\bar{I}_4 - 1) - 1) \quad (6.21)$$

In addition to the SEFs proposed by Holzapfel et al. [2000] and Natali et al. [2003], the particular characteristics of the stress-stretch curves obtained from the tests leaded the authors to consider a new anisotropic SEF trying to reproduce the vaginal mechanical behavior. In this sense, the well-known Weiss's SEF [Weiss et al., 1996] was modified in order to obtain analytical expressions of the strain energy function shown in (6.22). In those equations $\bar{I}_1$ represents the first modified invariant of the modified Cauchy-Green strain tensor [Spencer, 1954] and $I_4 > \bar{I}_{40}$ characterizes the mechanical response on the fiber direction.

$$\bar{\Psi} = c_1(\bar{I}_1 - 3) + \bar{\Psi}_f$$

$$\bar{\Psi}_f = 0 \quad I_4 < \bar{I}_{40}$$

$$\bar{\Psi}_f = \frac{c_3}{c_4} (\exp^{c_4(\bar{I}_4 - \bar{I}_{40})} - c_4(\bar{I}_4 - \bar{I}_{40}) - 1) \quad \bar{I}_4 > \bar{I}_{40} \text{ and } \bar{I}_4 < \bar{I}_{4ref}$$

$$\bar{\Psi}_f = 2c_5\sqrt{\bar{I}_4} + c_6 \ln(\bar{I}_4) + c_7 \quad \bar{I}_4 > \bar{I}_{4ref} \quad (6.22)$$

$\bar{I}_{4ref}$ characterizes the stretch at which collagen fibers start to be straightened, $c_1 > 0$, $c_3 > 0$, $c_5 > 0$, and $c_6 > 0$ are stress-like parameters, $c_4 > 0$ is dimensionless and c_7 is a strain parameter. Moreover, it has been assumed that the strain energy corresponding to the anisotropic terms only contributes to the global mechanical response of the tissue when stretched, that is $\bar{I}_4 > \bar{I}_{40}$. Note that $c_5 > 0$, $c_6 > 0$ and $c_7 > 0$ are not

independent parameters that enforce strain, stress and stress derivative's continuity.

6.3.3 Applying the new SEF to vaginal prolapsed tissue

The experimental data fitting was performed using an implementation of the Levenberg-Marquardt (LM) (see Appendix B) minimization algorithm [Marquardt, 1963]. This algorithm, widely used for experimental data fitting [Holzapfel et al., 2000], is based upon the minimization of an objective function that took the form represented in (6.23) for the uniaxial tensile test.

$$\chi^2 = \sum_{i=1}^n [\sigma - \sigma^\Psi]^2 \quad (6.23)$$

In order to access the quality of the new SEF, its results were compared with the results of the QExp SEF 6.20 and those of the exponential SEF 6.21. These results were published by the author and colleagues in [Martins et al., 2010b]. Eight patients with pelvic organ prolapse were considered. The vaginal tissue used to perform the mechanical tests reported in this paper was excised exclusively from post-menopausal women, during prolapse-correcting surgeries. All the patients, living in the north region of Portugal, were referred to the Urogynecology Unit of S. João Hospital.

Sample collection and specimen preparation followed the methods described in Section 5.2, with all the specimens cut in the longitudinal direction (see Figure 5.1). The mechanical tests were performed following the procedures and techniques established in Section 4.4.

The stress-strain curves (Figure 6.3) show that along the longitudinal axis, vaginal tissue presents a highly nonlinear mechanical behavior associated with large deformations ($\approx 38 \leftrightarrow 80\%$). Two different curve groups were observed. The first reaching maximal stress values near $3.0 \div 4.0$ MPa, the second near $6.0 \div 8.0$ MPa. Despite these differences, the linear region is approximately constant for all specimens. The principal difference resides on the initial slopes, which lead to different stretches. The quality of the fittings was evaluated by computing the coefficient of determination R^2 ,

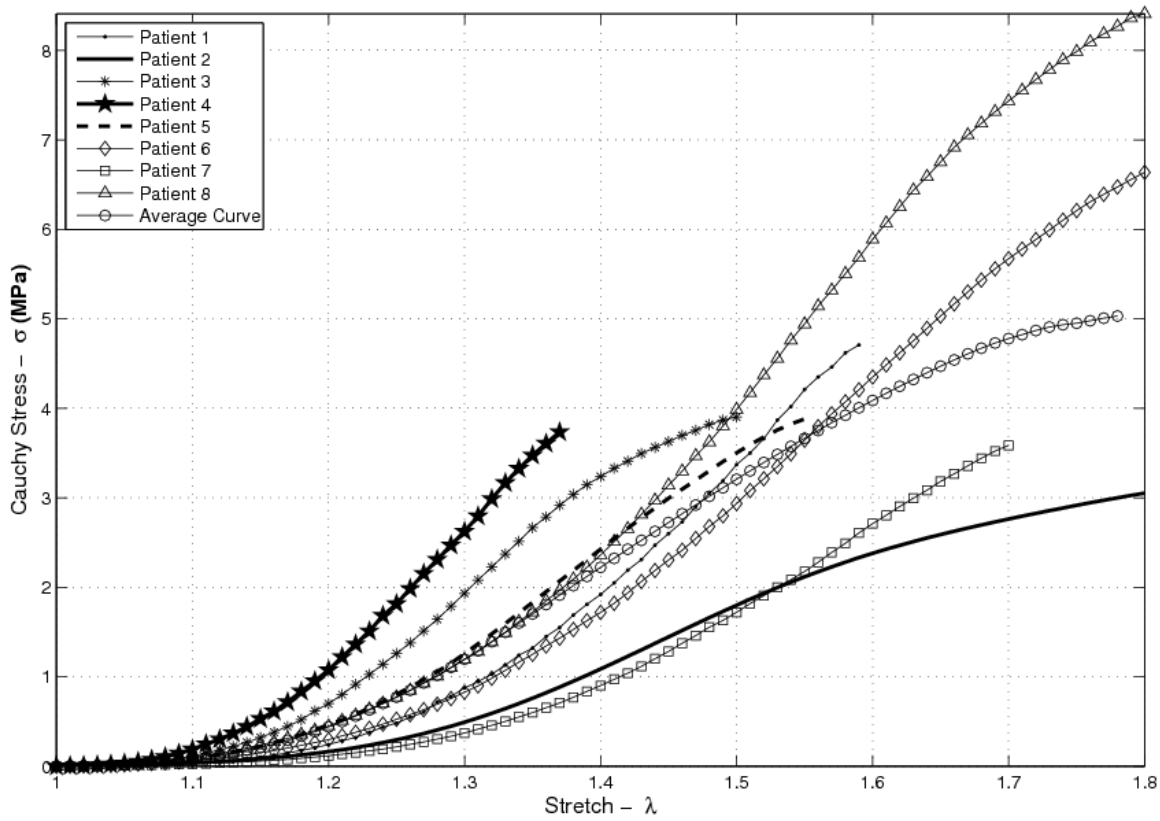


Figure 6.3: Experimental data considered.

defined as

$$R^2 = 1 - \frac{\sum_{i=1}^n [\sigma - \sigma^\Psi]_i^2}{\sum_{i=1}^n [\sigma - \mu]_i^2} \quad (6.24)$$

The normalized mean square root error ε_n (6.25) was also computed for each fitting. In this equation q is the number of parameters of the SEF, being $n - q$ the number of degrees of freedom, and μ the mean stress given by $\mu = \frac{1}{n} \sum_{i=1}^n [\sigma]_i$.

$$\varepsilon_n = \frac{\sqrt{\frac{\chi^2}{n-q}}}{\mu} \quad (6.25)$$

The experimental data obtained from simple tension tests was fitted for those specimens whose samples provided valid results. The average curve for each sample was fitted following the procedure explained in section 6.3.2. Convergence to global minima of all three models was difficult to achieve and the final results depended on the initial guess of the parameters. This problem has been noted earlier and reported by Fung

[1993]. For this reason, the fits were performed multiple times starting with randomized parameters. The results presented are the parameters that gave minimal values for χ . The fitting results obtained for QExp SEF (6.20), are shown in Table 6.1. The obtained values of R^2 are close to 1 ($R^2 = 0.9844$), however ε_n values are not low ($\varepsilon_n = 0.1319$). In Figures 6.4(a) and 6.5(a) we can observe how the fitting constants provide an accurate approximation of the experimental data. The average value of the parameters was 0.0034 ± 0.0016 MPa, 0.3746 ± 0.1297 MPa and 0.4112 ± 0.2949 for k_0 , k_1 and k_2 , respectively. The fitting results relative to the exponential SEF (6.21) are shown in Table 6.2. R^2 values are close to 1 ($R^2 = 0.989$) and ε_n values are low ($\varepsilon_n = 0.1086$), showing the experimental data fitting's good quality. In Figure 6.5(b) we can observe how exponential SEF is able to capture the vaginal tissue's me-

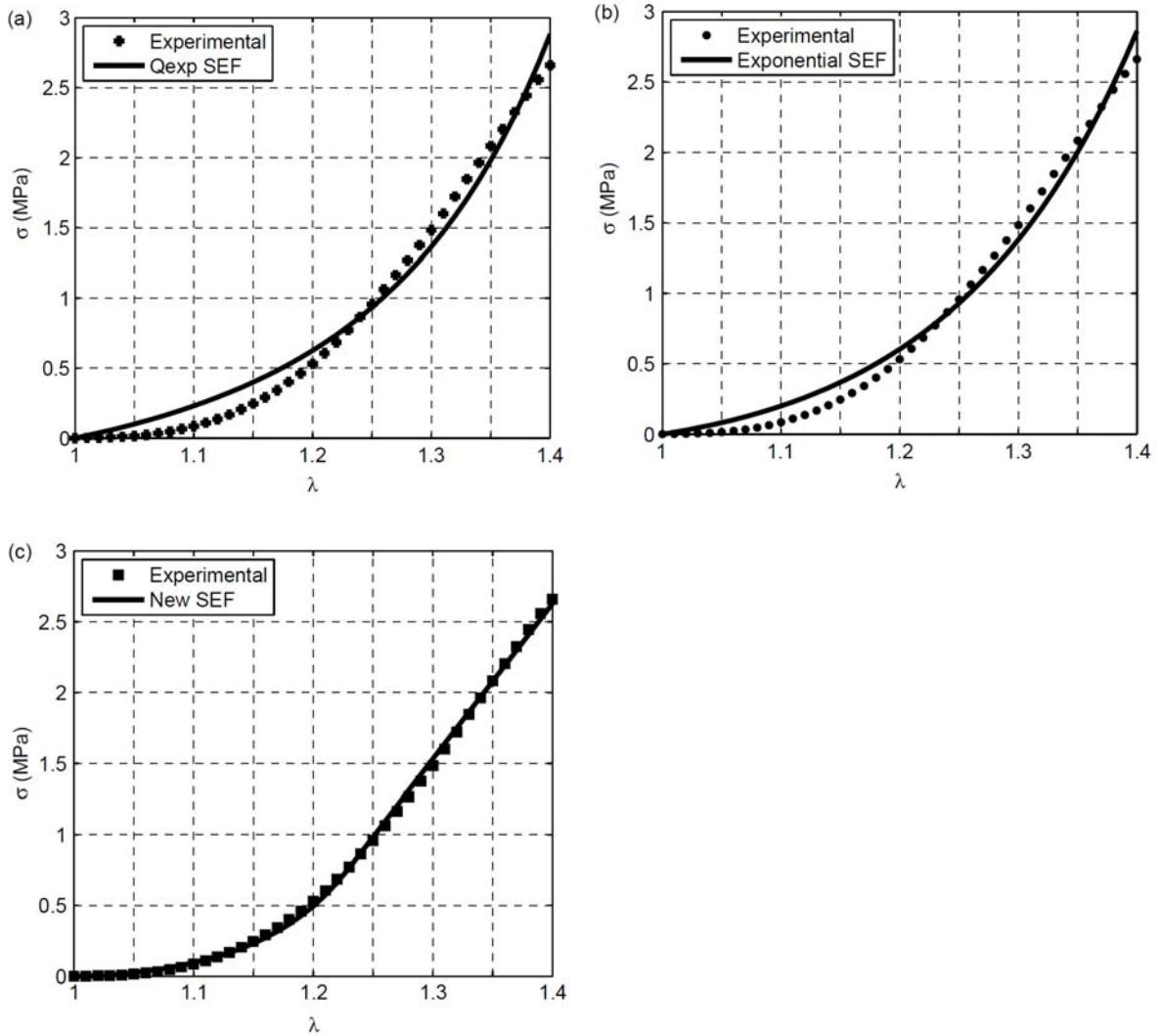


Figure 6.4: Fittings of the experimental data for individual *P4*. (a) QExp SEF, (b) exponential SEF and (c) new SEF.

Table 6.1: Material constants obtained for the QExp SEF. k_0 and k_1 values in MPa

	k_0	k_1	k_2	R^2	ε_n
$P1$	0.0061	0.2987	0.4789	0.9831	0.1358
$P2$	0.0022	0.2422	0.1226	0.9776	0.1515
$P3$	0.0016	0.5216	0.7937	0.9811	0.1525
$P4$	0.0039	0.4353	0.6097	0.9811	0.1493
$P5$	0.0012	0.3482	0.7756	0.9882	0.1217
$P6$	0.0047	0.4441	0.0622	0.9874	0.1118
$P7$	0.0043	0.1763	0.2961	0.9857	0.1374
$P8$	0.0033	0.5307	0.1512	0.9913	0.0960
Mean	0.0034	0.3746	0.4112	0.9844	0.1319
SD	0.0016	0.1297	0.2949	0.0045	0.0205

chanical properties. The average values of the parameters are 0.0037 ± 0.0017 MPa, 0.3588 ± 0.5868 MPa and 1.1909 ± 0.5868 for a_1 , a_2 and a_3 , respectively.

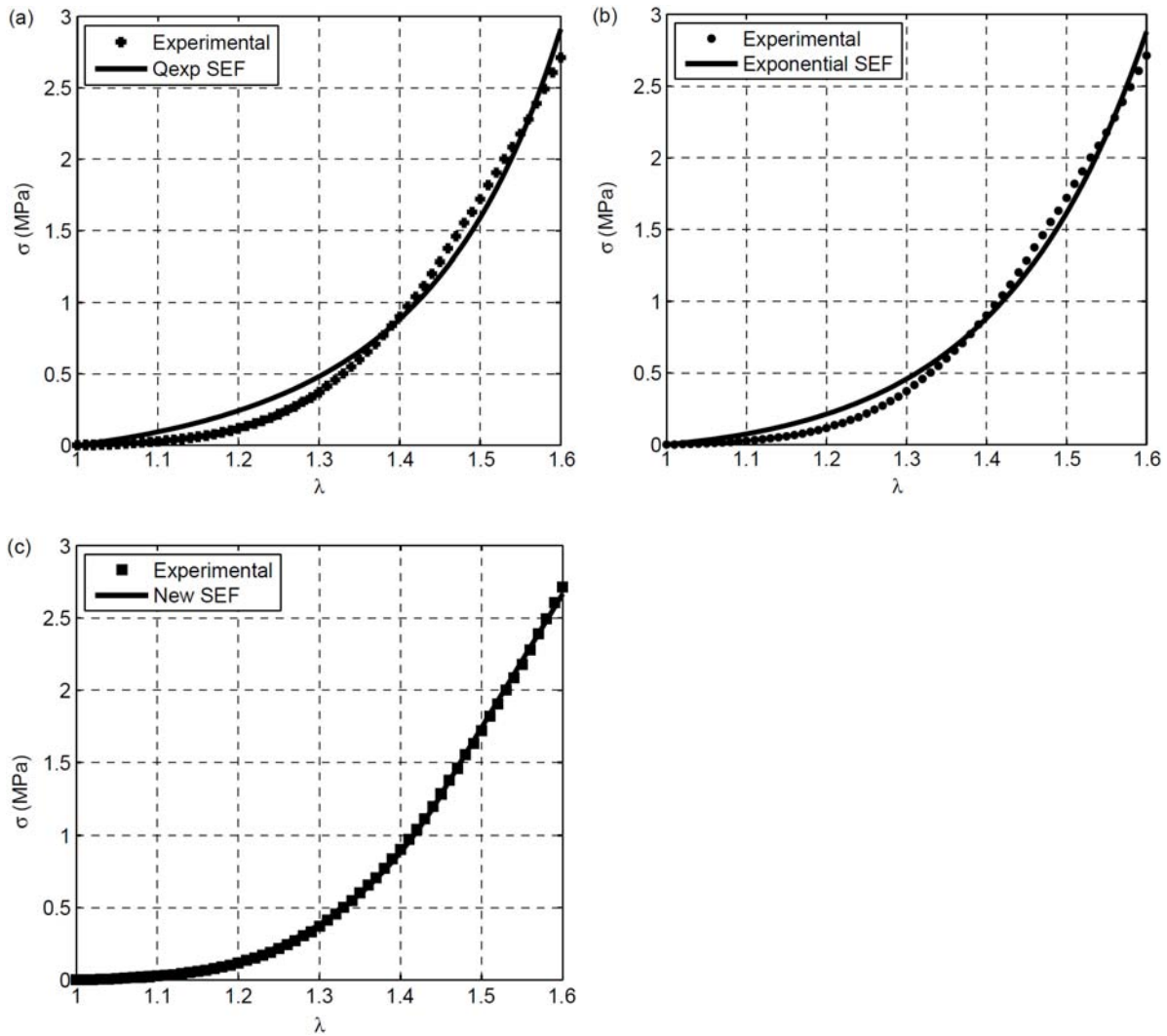
**Figure 6.5:** Fittings of the experimental data for individual $P7$. (a) QExp SEF, (b) exponential SEF and (c) new SEF.

Table 6.2: Material constants obtained for the Exponential SEF. Constant values in MPa

	a_1	a_2	a_3	R^2	ε_n
$P1$	0.0067	0.1362	1.5261	0.9897	0.1074
$P2$	0.0028	0.3527	0.5730	0.9823	0.1364
$P3$	0.0014	0.2129	1.8175	0.9878	0.1239
$P4$	0.0034	0.2130	1.5476	0.9876	0.1228
$P5$	0.0019	0.1362	1.8517	0.9935	0.0917
$P6$	0.0051	1.0636	0.3631	0.9900	0.1004
$P7$	0.0052	0.1050	1.1816	0.9912	0.1088
$P8$	0.0036	0.6513	0.6671	0.9943	0.0780
Mean	0.0037	0.3588	1.1909	0.989	0.1086
SD	0.0017	0.3356	0.5868	0.0037	0.0188

The fitting results for modified SEF proposed herein (6.22), are shown in Table 6.3. The obtained values of R^2 are very close to 1 ($R^2 = 0.9992$) and ε_n values are very low ($\varepsilon_n = 0.0286$) for all the fitted data pointing to the fitting quality. The fitted constants for the new SEF results in a theoretical stress-stretch relation very close to the experimental results (Figure 6.5(c)). The average values of the parameters were 0.0243 ± 0.0181 MPa, 0.1315 ± 0.1452 MPa, 2.6641 ± 1.7465 , 1.115 ± 0.0944 and 1.8235 ± 0.2505 for c_1 , c_3 , c_4 , $\bar{I}_{4_0}$ and $\bar{I}_{4_{ref}}$, respectively.

6.3.4 Discussion and conclusions

In this study it was verified that prolapsed vaginal tissue presents a non-linear mechanical behavior associated to large strains. The main results presented in the literature on the characterization and modeling of vaginal tissue assume a linear elastic mechanical behavior and present estimations of the associated Young modulus [Goh, 2003; Lei et al., 2007]. It means that these authors are assuming a linear and elastic mechanical behavior of vaginal tissue [Goh, 2003; Lei et al., 2007]. However, the mechanical behavior of the vaginal tissue (an highly hydrated soft tissue) has to be modeled with laws that can allow an accurate fitting of the mechanical response which means that hyperelasticity has to be considered [Rubod et al., 2008].

On the consulted literature the authors did not find fittings of hyperelastic model parameters concerning prolapsed vaginal tissue, therefore it is not possible to compare the results here presented. However, information relative to Young's modulus can be

Table 6.3: Material constants obtained for the SEF here proposed. Constant values in MPa

	c_1	c_3	c_4	c_5	c_6	c_7	$\bar{I}_{4_0}$	$\bar{I}_{4_{ref}}$	R^2	ε_n
$P1$	0.050	0.1145	2.1673	13.1708	-16.6090	-12.7165	1.20	1.8769	0.9991	0.0324
$P2$	0.010	0.0745	1.7978	6.8149	-8.4852	-6.5970	1.10	1.8769	0.9992	0.0303
$P3$	0.0494	0.0231	5.9509	12.6892	-14.6244	-12.5340	1.05	1.4641	0.9989	0.0385
$P4$	0.0187	0.0309	4.7146	10.8418	-12.5977	-10.6901	1.05	1.5129	0.9991	0.0333
$P5$	0.010	0.0877	2.5869	11.3115	-13.4535	-11.0877	1.05	1.6641	0.9993	0.0303
$P6$	0.010	0.1231	1.6431	13.1496	-16.7432	-12.6285	1.05	1.9881	0.9994	0.0240
$P7$	0.0370	0.1204	1.5131	8.9940	-11.8673	-8.5428	1.31	2.1025	0.9997	0.0205
$P8$	0.010	0.4781	0.9392	18.1826	-23.2681	-17.4097	1.11	2.1025	0.9996	0.0200
Mean	0.0243	0.1315	2.6641	11.8943	-14.7060	-11.5257	1.115	1.8235	0.9992	0.0286
SD	0.0181	0.1452	1.7465	3.3602	4.3729	3.2149	0.0944	0.2505	0.0002	0.0065

extracted from the linear part of the experimental stress-stretch curves (Figure 6.3). Comparing Young's modulus values obtained for the average data (Figure 6.3), ≈ 9.7 MPa, with those reported by Lei et al. [2007] for post-menopausal women with prolapse, 12.1 MPa, it is seen that they have the same magnitude. Furthermore, the shapes of the nonlinear stress-stretch curves here presented are very similar to those reported recently by Rubod et al. [2008].

The determination of constitutive equations is an inverse problem. A specific form of the constitutive relation is assumed, and based on computed values of stress and strain from loading and deformation experiments, the material properties that allow a good agreement (ideally the best agreement) between the theoretical model and the experimental data are determined. In the present work, we considered the mechanical properties of prolapsed vaginal tissue obtained from uniaxial tensile tests. The experimental data was used to fit a non-linear hyperelastic constitutive model. Three different functions were considered and their ability in reproducing the experimental data were evaluated. They all supplied good fittings of the experimental data. However, some remarks are worthy to be made for each of them.

QExp SEF has proved to reproduce the arterial behavior accurately [Holzapfel et al., 2000]. For the case of prolapsed vaginal tissue, it was possible to fit the experimental curves with this SEF very accurately (see Figure 6.5(a)). Although R^2 values were always over 0.96, ε_n values were higher than 0.1 for most of the fittings. The reason for these ε_n values relies in the strong exponential character of QExp SEF. Nevertheless, the obtained curves show a soft exponential character for small strains and strong linear regions at large strains (Figure 6.3). A similar result was obtained when exponential SEF was used. This exponential function has proved to reproduce the ligament and tendon behavior accurately [Pioletti, 1997; Natali et al., 2003]. The values of R^2 are close to 1 ($R^2 = 0.989$) and ε_n values low ($\varepsilon_n = 0.1086$) for the fitted data. These fitting results stress out the quality of exponential SEF when compared with QExp SEF. However, the same drawbacks of QExp SEF were observed with exponential SEF, mainly due to the linear mechanical behavior observed at large strains.

The specific characteristics displayed by the experimental results lead to the proposal of a new SEF (Equation 6.22). Unlike the two previous SEFs, it accounts for the mechanically distinctive regions (exponential and linear) and is able to fit the exper-

imental data accurately showing good fitting indicators, that were in fact the best of the three fits ($R^2 = 0.0.9992$, $\varepsilon_n = 0.0286$). This SEF is convex which ensures the existence and the uniqueness of the solution, we verified that the material constants do not violate the physical requirements of hyperelasticity; i.e., the condition that the material must be stable under loading and we confirmed that the second derivative of strain energy function with respect to strain is positive definite. Moreover, it presents several disadvantages with respect to the other two SEFs. First, it is defined by five material constants instead of the three parameters needed for QExp and exponential SEFs. Second, $c_5 > 0$, $c_6 > 0$ and $c_7 > 0$ are not independent parameters. In fact they enforce strain, stress and stress derivative continuity.

The average stiffness of the tissues in the low loading domain at which the non-collagenous matrix material is mainly active (k_0 , a_1 and c_1) is similar for the QExp and Exponential SEFs and lower for the new SEF. This fact is due to the exponential character of the QExp and exponential SEFs which presents itself in all strain ranges, so the neo-hookean term is not relevant. However, in the new SEF, this term associated to the parameters c_1 and $\bar{I}_{40}$ is the only present in the SEF for low strain ranges. The exponential parameters k_1 , a_2 are similar for QExp and Exponential SEFs, but k_2 , a_3 differ due to the quadratic character of the exponential term in QExp SEF which is not present in the Exponential SEF.

For the new SEF, the average stiffness of the individual tissue in the high loading domain, dominated due to the recruitment of collagenous fibers, is governed by the parameters c_3 , c_4 for the exponential region and c_5 and $\bar{I}_{4_{ref}}$ for the linear region. $\bar{I}_{40}$ is similar in all patients (1.115 ± 0.0944), but $\bar{I}_{4_{ref}}$ is specimen dependent (1.8235 ± 0.2505). Some limitations of this study can be remarked. Only the elastic properties of the vaginal tissue have been determined in this paper. Damage and viscoelastic vaginal tissue properties were not included in the scope of this research and remain as the aim of subsequent works [Peña et al., 2008]. More tests are needed to determine the plastic and viscoelastic properties, as well as the stiffness loss due to damage.

In spite these limitations, the obtained results could help to design surgical procedures with autologous tissue or prosthesis. Prostheses employed on the treatment of pelvic organ prolapse should have similar mechanical properties to vaginal tissue. In addition, the proposed constitutive model can be used to study vaginal tissue mechanics.

The structural model seems to be sufficiently accurate to guarantee its application in predicting reliable stress distributions, and is suitable for finite element computations.

6.4 Conclusions

In this chapter we presented the main theoretical aspects of nonlinear modeling of soft tissues. We restricted this work to hyperelastic materials, although we are well aware of the importance of viscoelasticity (as recently published by the author and colleagues in [Peña et al., 2010]).

It was proposed a new strain energy function (SEF) adapted for the specificity of prolapsed vaginal tissues. The material model parameters were fit to the experimental data using the Levenberg-Marquardt optimization algorithm. The new SEF proposed allowed accurate fittings of the experimental results, although a typical criticism of "any model with so many parameters can adapt to any material" is of course understandable. This is also a fundamental step towards the improvement of therapeutic techniques such as surgery. Issues like the efficiency of using autologous tissue in pelvic reconstruction may be addressed. The realism of FEM simulations of the pelvic cavity may also improve by using an adequate nonlinear material model.

This is an example of the strong collaboration with the Zaragoza group that produced a journal paper [Martins et al., 2010b].

Chapter 7

Conclusions and Future Work

7.1 Conclusions

The main purpose of this thesis was to contribute for the body of knowledge on the female pelvic cavity, by filling some gaps on the biomechanical properties of soft tissues and polypropylene (PP) meshes and on the understanding of their nonlinear mechanical behavior.

To achieve meaningful contributions on this domain, a multidisciplinary effort between engineers and medical doctors was required. The (ongoing) collaborations established, are one of the most promising results of this thesis:

- The beginning of this research, on the mechanical properties of prolapsed vaginal tissue, was possible due to Dr. Teresa Mascarenhas¹ (medical doctor).
- The hyperelastic model for prolapsed vaginal tissue, later expanded to an hyper-viscoelastic model and the study of damage phenomena were only possible due to the partnership established with Dr. Begoña Calvo and Dr. Estefanía Peña

¹Urogynecology department of Hospital S. João (Porto, Portugal)

(engineers) from University of Zaragoza².

- The study of the mechanical and physical properties of prostheses used in pelvic surgery (PP meshes), was proposed by Dr. José Sebastião Afonso³ (medical doctor).
- For the study of pelvic tissues from cadavers it was established a collaboration (and a research protocol with ethics committee approval) with Dr. Agostinho Santos and Dr. Liliana Santos⁴ (medical doctors).
- The partnership with Dr. Agnaldo Silva Filho⁵ (medical doctor), allowed a significant insight on pelvic floor problems and on the statistical interpretation of medical data.

The author feels that this thesis cannot be viewed as closed work, but rather as the beginning of several research fronts. The most relevant contributions of this thesis were:

1. A very large number of tests on cadaver tissues, to consistently characterize the tissues from the female pelvic cavity (Chapter 5). To our knowledge, this was the first comprehensive set of experimental results on the subject. Round and Uterosacral ligaments, vaginal and bladder wall tissues were considered as well as polypropylene meshes (urogynecologic prosthesis). More interesting than the extensive characterization, the clinical conclusions obtained may be helpful for clinical practice.
2. An extensive research of the mechanical behavior of prolapsed vaginal tissues was conducted (Chapters 5 and 6). An extension of existing hyperelastic models for soft tissues was proposed and compared with previous hyperelastic analytical

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solutions (see previous works from of Dr. Peña and Dr. Calvo [Peña et al., 2006; Calvo et al., 2007]) and our experimental results [Martins et al., 2010b]. This initial approach easily allows the inclusion of viscoelastic behavior as well as damage phenomena, as recently published by the author and colleagues [Peña et al., 2010; Calvo et al., 2009].

3. The development of an experimental set-up to carry on all uniaxial tests on a wide range of biological soft tissues (Chapter 4). This setup fills the following gap: a commercial setup was not available for this particular low stress applications. This in-house setup allow us to modify it in the future for other applications. Furthermore lessons learned will allow us to build a biaxial setup. In fact this biaxial setup is already under construction.
4. The creation of a test protocol for the mechanical testing of biological soft tissues (Chapter 4). Because there was not enough information available, we were forced to design a test protocol suitable for this type of materials. Using the same velcro fixation from other groups we were able to develop smaller and effective grips. Problems related to storage, handling, preparation of samples and testing, had to be solved under this protocol.
5. It is not practical to obtain strain from extensometers. One possible solution is the integration of non-contact imaging techniques to assess the evolution of strains (Chapter 4). In fact this approach proved to be a fast and flexible solution to extract strains. This non-contact technique was design and developed by the author trough the placement of webcams.
6. Custom software written by the author for the analysis and treatment of experimental data (Chapters 5 and 6). Our in-house experimental setup produces a large amount of raw data. We developed software to treat this data in order to obtain the stress-strain curves. The software consists of Matlab scripts based on digital signal processing.
7. A modular software for the calculation of optimal material parameter sets of

nonlinear material models (Chapter 6) was developed by the author in Matlab® (Appendix C), based on the Levenberg-Marquardt algorithm (see Appendix B).

8. An insight on the influence of the PP mesh used on pelvic floor repair, achieved by testing the mechanical properties of various types of meshes (Chapter 5). We learned that some meshes present a better mechanical behavior for long term application as prosthesis used in pelvic floor repair. Given the thinness of the meshes, we performed ring tests in order to obtain information on its flexural properties.

7.2 Future Work

This research subject is still in its infancy, so many research directions could be proposed. We select some of the areas that could follow this thesis:

1. The experimental studies here presented were one-dimensional. A better understanding of the soft tissues behavior could be produced by designing a new biaxial set-up (ongoing). Difficulties related to stress balance and image acquisition will be taken into account.
2. In uniaxial tensile tests, it is important to improve the estimation of sample's cross sectional area. Errors in area estimation produce a significant influence in stress calculations. An ideal solution would provide a 3D profile of the specimen during the mechanical test.
3. The elastic properties of soft tissues are an important subject. However, the consideration of damage and the effects of viscoelastic vaginal tissues would be an important subject, as already noticed by [Calvo et al., 2009; Peña et al., 2010]. In this subject the collaboration of the Zaragoza group is of the utmost importance given their capacity in dynamic tests.
4. The design of prosthesis would be a desirable sequence of the present work, given

the knowledge of the mechanical properties acquired. It is very important that the prosthesis material properties can mimic the vaginal tissue properties.

5. Further studies are required to evaluate the etiological factors influencing the biomechanical proprieties of female pelvic tissues, such as age, body mass index or hormonal status (menopause).

Appendices

Appendix A

Levator ani and *Scarpa's* Fascia experimental results

This appendix gathers the experimental results for *Scarpa's* Fascia and the *Levator ani* muscle complex. These tissues were taken from the pelvic cavity of female cadavers without any known pelvic trauma or pathology (Table 5.4).

This experimental data should be seen as a complement to the studies and experimental data presented in Chapters 5 and 6. Together they constitute a more complete description of the mechanical properties of the female pelvic cavity.

A.1 *Levator ani*

Table A.1 contains the experimental results for the *Levator ani* muscle complex. The tensile strength (σ_{max}) and tangent modulus (E_t) were significantly smaller when compared to all the soft tissues tested. The mean results of the mechanical behavior (stress-stretch diagram) are shown in Figures A.1 and A.2. Despite the small number of samples on the transversal direction ($n = 4$), directional differences (Longitudinal versus Transversal) are significant (Figure A.1). This result is consistent with tissue morphology, since muscle fibers are highly oriented (locally) in every region of the *le-*

vator ani (*puborectalis*, *pubococcygeus* and *iliococcygeus*). The dispersion of the results on the transversal direction (Figure A.2) has an anomalous behavior ($1.0 \leq \lambda \leq 1.2$) this effect should “dilute” with the inclusion of more samples.

Table A.1: Experimental data extracted from cadaveric tissue. Levator ani muscle.

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Cd01_LA1L	0.580	1.906	0.286	1.277	0.289
Cd03_LA1L	0.500	1.786	0.192	0.983	0.322
Cd03_LA2T	0.204	1.642	0.057	0.520	0.156
Cd04_LA1L	0.546	1.659	0.155	1.549	0.396
Cd04_LA2T	0.299	1.610	0.067	0.975	0.260
Cd05_LA1L	0.466	1.817	0.170	0.845	0.255
Cd06_LA1L	0.455	1.852	0.172	0.949	0.322
Cd07_LA1L	0.702	2.069	0.268	1.290	0.578
Cd08_LA1L	1.343	1.567	0.346	4.133	1.031
Cd09_LA1L	0.419	1.546	0.095	1.337	0.271
Cd09_LA2T	0.177	2.102	0.103	0.282	0.060
Cd10_LA2L	0.357	1.437	0.058	1.548	0.303
Cd10_LA3L	0.315	1.548	0.069	0.983	0.241
Cd11_LA1L	0.607	1.466	0.112	2.500	0.511
Cd12_LA1L	0.990	1.461	0.186	3.802	0.797
Cd12_LA2T	0.112	1.137	0.008	1.500	0.103
Cd13_LA1L	0.276	1.276	0.041	1.690	0.205
Cd15_LA1L	0.271	1.453	0.048	1.109	0.202
Cd17_LA1L	0.263	1.251	0.032	1.906	0.211
Cd17_LA2L	0.267	1.284	0.039	1.738	0.216

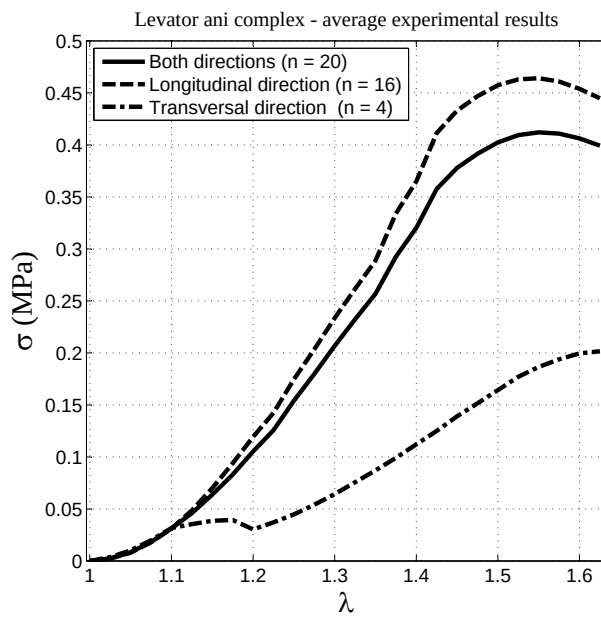


Figure A.1: Typical mechanical behavior for the levator ani complex. Global results.

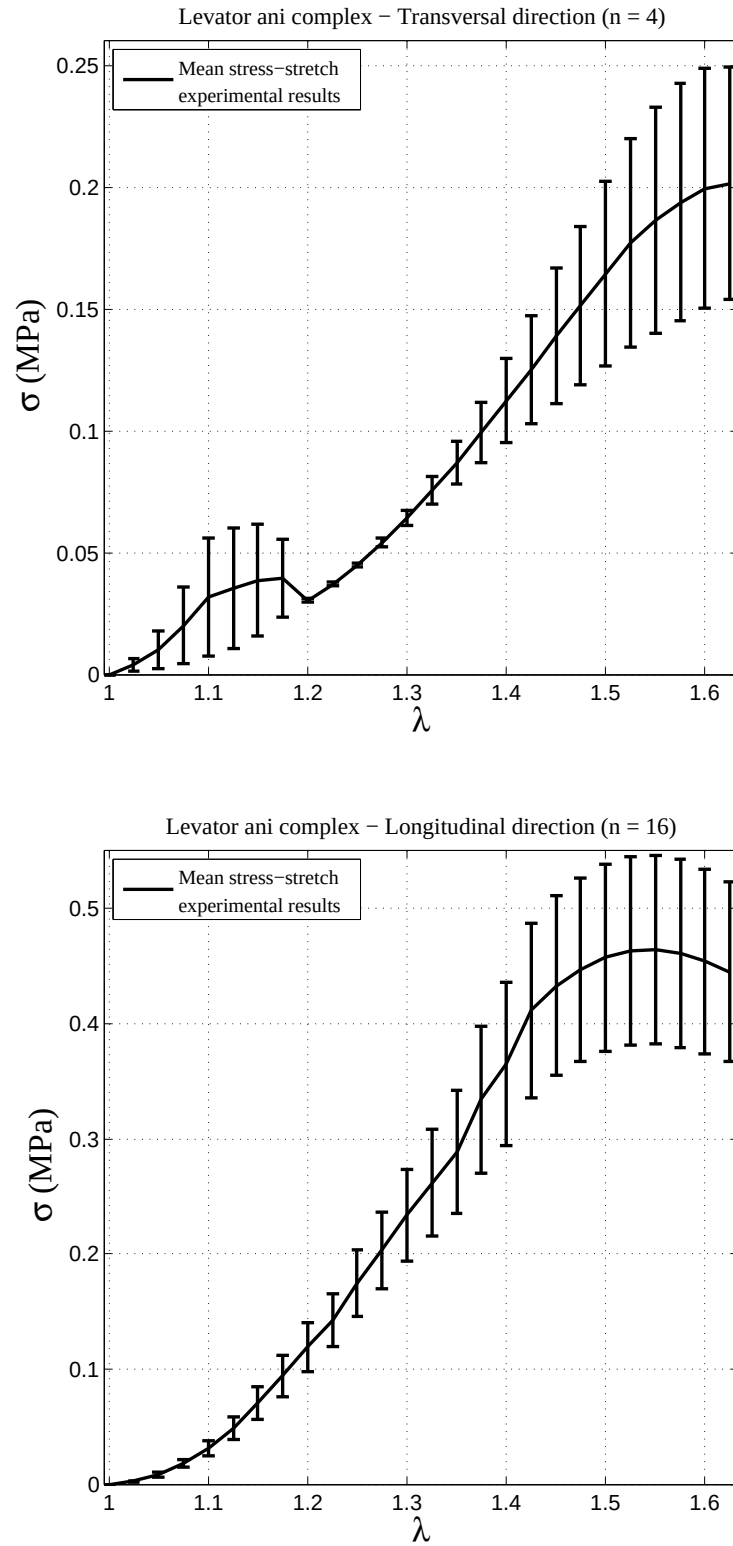


Figure A.2: Typical mechanical behavior for the levator ani complex. Comparison between orthogonal directions, i.e., longitudinal (fiber oriented) and transversal directions.

A.2 Scarpa's Fascia

Table A.2 contains the experimental results for the Scarpa's Fascia. The tensile strength (σ_{max}) and tangent modulus (E_t) were significantly higher when compared to all the soft tissues tested. The mean results of the mechanical behavior (stress-stretch diagram) for the fiber direction 1 (fd1) are shown in Figure A.3. This direction was established observing the samples's dominant fiber direction (see Figure 3.12). In several samples it was observed more than one dominant direction and/or layer. In figure A.4 it is clear that the mechanical behavior changes according dominant fiber direction. It is also difficult to guarantee that the samples were collected always from the same anatomical site.

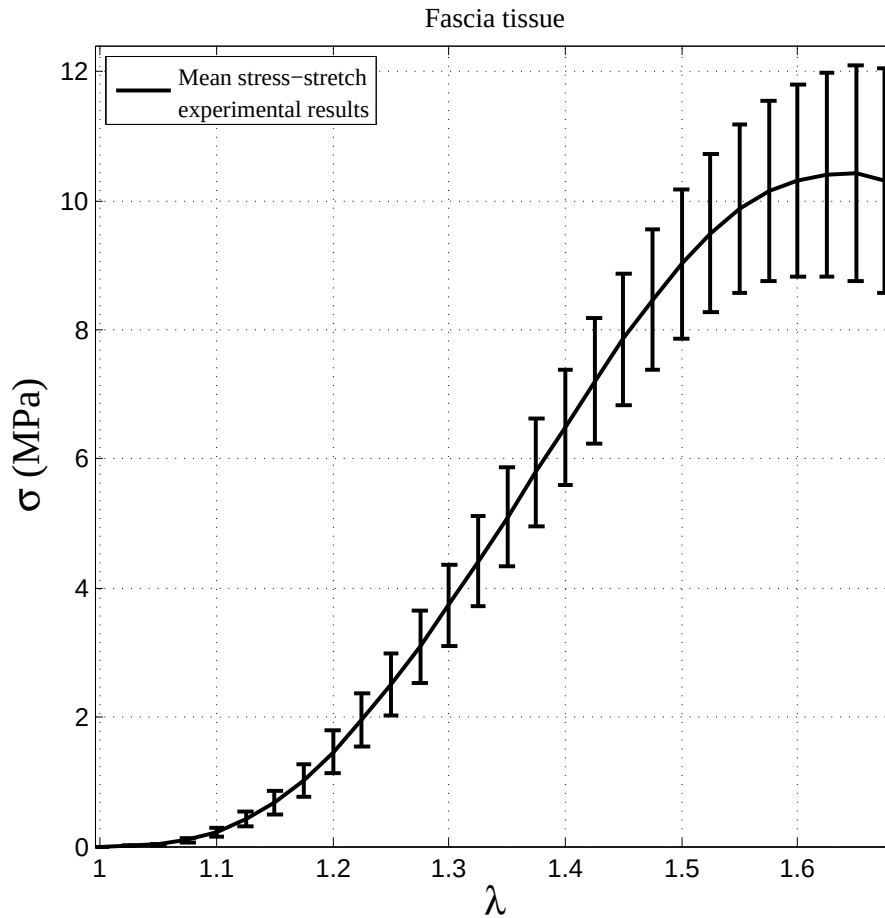


Figure A.3: Typical mechanical behavior of Scarpa's Fascia. Fiber direction fd1.

Table A.2: Experimental data extracted from cadaveric tissue. *Scarpa's Fascia*

Sample	$\sigma_{max} (MPa)$	λ_U	$U_S (nJ.m^{-3})$	$E_t (MPa)$	$\sigma_Y (MPa)$
Cd01_F1L	20.509	1.699	5.998	41.107	16.973
Cd01_F2T	20.968	1.722	6.002	51.401	17.341
Cd02_F1L	6.617	1.858	1.897	15.409	5.620
Cd02_F2T	1.197	1.489	0.248	4.354	0.944
Cd03_F1T	1.691	1.864	0.559	3.700	1.472
Cd04_F1L	16.270	1.645	4.244	39.582	14.184
Cd04_F2L	6.969	1.706	2.197	20.204	4.736
Cd04_F3T	3.584	1.891	1.354	8.116	3.095
Cd05_F1L	18.901	1.860	5.746	33.396	17.358
Cd05_F2L	9.509	1.596	2.392	27.597	7.503
Cd05_F3T	5.018	2.255	2.011	10.617	3.291
Cd08_F1L	7.584	1.492	1.669	26.239	5.187
Cd08_F2L	8.289	1.590	1.995	23.443	6.148
Cd09_F1L	11.187	1.900	4.317	19.234	8.746
Cd09_F2T	3.217	2.254	2.200	4.471	2.210
Cd10_F1L1	6.301	1.734	1.639	17.977	5.178
Cd10_F2L2	5.366	1.575	1.319	13.922	4.591
Cd11_F1L1	16.640	1.640	4.550	39.208	13.760
Cd11_F2L2	1.751	1.330	0.250	8.913	1.116
Cd12_F1L1	6.466	1.473	1.306	19.744	5.522
Cd12_F2L2	12.386	1.665	3.771	30.660	9.249
Cd13_F1L1	11.662	1.709	3.412	27.010	7.035
Cd13_F2L2	7.152	1.368	1.013	29.742	6.263
Cd15_F1L	22.149	1.628	7.046	50.515	15.546
Cd17_F1L1	10.116	1.495	2.047	31.716	9.057
Cd17_F2L2	14.174	1.494	2.807	43.023	12.356
Cd17_F3T	3.849	1.385	0.649	17.571	2.841

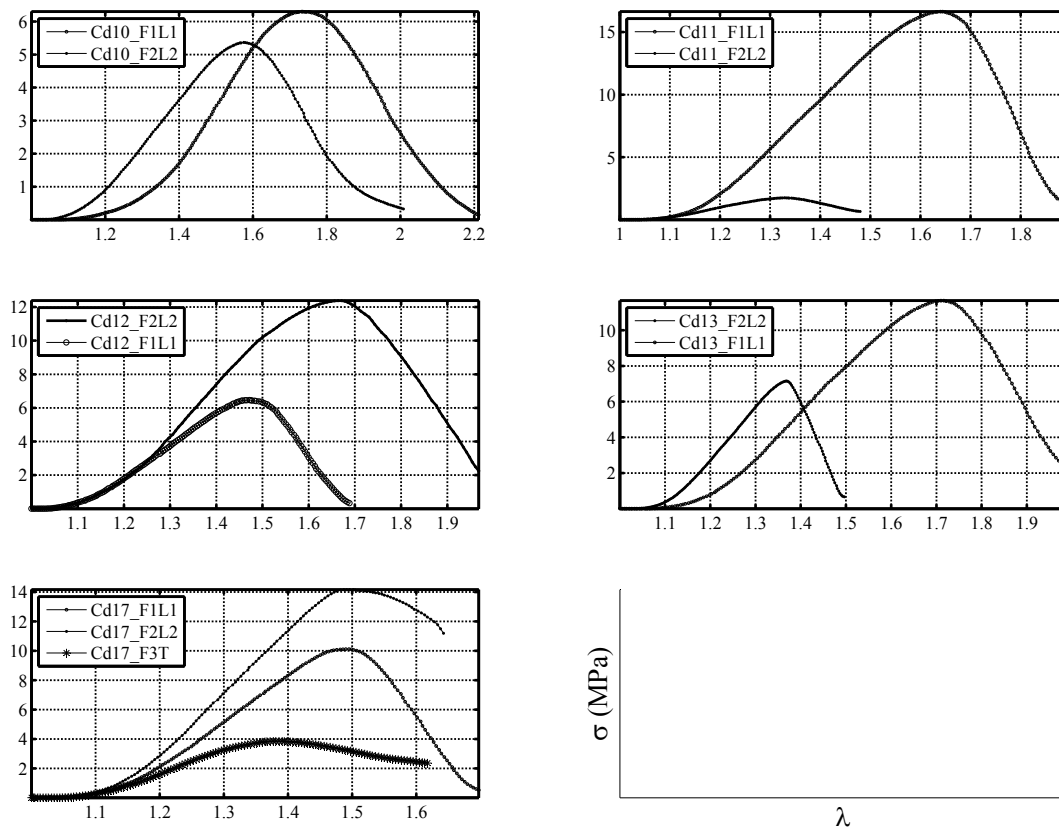


Figure A.4: Mechanical behavior of *Scarpa's* Fascia in different fiber directions. Results for 5 patients.

Appendix B

An implementation of the Levenberg-Marquardt optimization algorithm

B.1 Introduction

A FORTRAN 90 code based on the LM algorithm was developed [Martins et al., 2006b]. Although several implementations of this algorithm exist on commercial mathematical libraries (eg. NAG, IMSL), we decided to implement our own version of the algorithm. The aim of this code is to find the optimal value of some set $\{c_1, c_2, \dots, c_n\}$ of n unknown parameters. This method is based on the minimization of a cost function that depends on the analytical model that we are using.

If one needs to fit a generic set of parameters $\{c_1, c_2, \dots, c_n\}$, simplified in the vectorial form $\vec{C}$ a cost function that compares the squared differences between the analytical model results and the experimental results, must be defined.

B.2 The Objective function

A common method to build objective functions $O(\vec{C})$ is to generalize χ^2 of 6.23, the new form is given by:

$$O(\vec{C}) = \sum_{i=1}^m \omega_i^2 [\sigma^{an}(\vec{C}) - \sigma^{exp}]_i^2 \quad (\text{B.1})$$

Where:

- The summation index m is the number of experimental measurements
- ω_i is the weighting coefficient of the i^{th} measurement
On the tension experiments considered here, we assume that all measurements are equally "important", so $\omega_i = 1, \forall i : i \in \{1, \dots, m\}$
- σ_i^{exp} is the stress measurement of the i^{th} experimental (*stress, stretch*) point
- $\sigma_i^{an}(\vec{C})$ is the analytic value of stress for a material model (SEF).

The objective function (B.1) can be expressed using a function $\Sigma(\vec{C})$ for each measurement.

$$\Sigma_i(\vec{C}) = \sigma_i^{an}(\vec{C}) - \sigma_i^{exp} \quad (\text{B.2})$$

assuming the form:

$$O(\vec{C}) = \sum_{i=1}^m \Sigma_i(\vec{C})^2 \quad (\text{B.3})$$

B.3 Mathematical formulation of the Algorithm

To use the LM algorithm, the 1st and 2nd derivatives of the objective function (B.3) must be computed,

$$\frac{\partial O}{\partial c_k}(\vec{C}) = 2 \sum_{i=1}^m \Sigma_i \frac{\partial \Sigma_i}{\partial c_k} \quad (\text{B.4})$$

$$\frac{\partial^2 O}{\partial c_k \partial c_l}(\vec{C}) = 2 \sum_{i=1}^m \left(\frac{\partial \Sigma_i}{\partial c_l} \frac{\partial \Sigma_i}{\partial c_k} + \Sigma_i \frac{\partial^2 \Sigma_i}{\partial c_k \partial c_l} \right), \quad k, l = 1, \dots, n \quad (\text{B.5})$$

if $\vec{C}$ is close to the optimal set of parameters (which minimizes the value of $O(\vec{C})$), we can assume with little error that $\Sigma_i \approx 0$, allowing:

$$\frac{\partial^2 O}{\partial c_k \partial c_l} = 2 \sum_{i=1}^m \frac{\partial \Sigma_i}{\partial c_l} \frac{\partial \Sigma_i}{\partial c_k}, \quad k, l = 1, \dots, n \quad (\text{B.6})$$

Writing the set of functions Σ_i in a vectorial form, $\vec{\Sigma} = (\Sigma_1, \Sigma_2, \dots, \Sigma_m)$, we can analyze the variation of $\vec{\Sigma}$ with $\vec{C}$ using a gradient approach :

$$\nabla \vec{\Sigma} = \begin{pmatrix} \nabla \Sigma_1 \\ \dots \\ \nabla \Sigma_m \end{pmatrix} = \begin{pmatrix} \frac{\partial \Sigma_1}{\partial c_1} & \dots & \frac{\partial \Sigma_1}{\partial c_n} \\ \dots & \dots & \dots \\ \frac{\partial \Sigma_m}{\partial c_1} & \dots & \frac{\partial \Sigma_m}{\partial c_n} \end{pmatrix} \quad (\text{B.7})$$

For simplicity, let $\nabla \vec{\Sigma} = [A]$.

Now, in a similar manner, the variation of the objective function is calculated:

$$\nabla O(\vec{C}) = \left(\frac{\partial O}{\partial c_1}, \dots, \frac{\partial O}{\partial c_n} \right) = \left(2 \sum_{i=1}^m \Sigma_i \frac{\partial \Sigma_i}{\partial c_1}, \dots, 2 \sum_{i=1}^m \Sigma_i \frac{\partial \Sigma_i}{\partial c_n} \right) \quad (\text{B.8})$$

From equations (B.7) a useful form of equation B.8 is found:

$$\nabla O(\vec{C}) = 2[A]^T \cdot \vec{\Sigma} \quad (\text{B.9})$$

Considering equations (B.6), (B.7) and (B.9), the Hessian matrix of the objective function is obtained:

$$\nabla^2 O(\vec{C}) \simeq 2[A]^T[A] \quad (\text{B.10})$$

Considering an iterative process in which a set of parameters $\vec{C}$ approaches an ideal set of parameters (which minimizes the objective function $O(\vec{C})$), is possible to write the following iterative relation:

$$\vec{C}_{k+1} = \vec{C}_k + S_k \vec{d}_k \quad (\text{B.11})$$

where:

- $\vec{C}_{k+1}$ is the $k + 1^{th}$ set of parameters
- $\vec{C}_k$ is the k^{th} set of parameters
- S_k is the step size
- $\vec{d}_k$ is the displacement direction

Taking the step size $S_k = 1$ and applying a Newton-Raphson scheme:

$$\vec{C}_{k+1} = \vec{C}_k - [\nabla^2 O]_k^{-1} \cdot (\nabla O)_k \quad (\text{B.12})$$

Now, rearranging the terms of equation B.12 and considering $\overrightarrow{\Delta C}_k = \vec{C}_{k+1} - \vec{C}_k$ we can write the parameter variation expression:

$$([A]_k^T[A]_k) \cdot \overrightarrow{\Delta C}_k = -[A]_k^T \cdot \vec{\Sigma}_k \quad (\text{B.13})$$

The left side term of equation (B.13) is commonly near singular matrix, so in order to overcome this problem, an additional term proportional to the identity matrix $[I]$ is added to the left side term of (B.13).

$$([A]_k^T[A]_k + \lambda_m[I]) \cdot \overrightarrow{\Delta C}_k = -[A]_k^T \cdot \vec{\Sigma}_k \quad , \quad \lambda_m \in \mathbb{R} \quad (\text{B.14})$$

Equation (B.14) is the Levenberg-Marquardt method principal operation since we calculate the next set of parameters with

$$\vec{C}_{k+1} = \vec{C}_k + \overrightarrow{\Delta C}_k \quad (\text{B.15})$$

B.4 The complete Algorithm

The complete algorithm (as implemented in FORTRAN 90) is:

```

1:  $O(\vec{C}_k), \vec{\Sigma}_k \leftarrow \vec{C}_k$ , (initial guess  $\leftarrow k = 1$ )
2:  $\nabla \vec{\Sigma}_k = [A]_k \leftarrow \vec{C}_k$ 
3:  $[A]_k^T [A]_k + \lambda_m [I] \leftarrow ([A]_k, \lambda_M)$ 
    $[A]_k^T \cdot \vec{\Sigma}_k \leftarrow ([A]_k, \vec{\Sigma}_k)$ 
4:  $\overrightarrow{\Delta C}_k \leftarrow ([A]_k^T [A]_k + \lambda_m [I]) \cdot \overrightarrow{\Delta C}_k = -[A]_k^T \cdot \vec{\Sigma}_k$ 
5:  $\vec{C}_{k+1} \leftarrow \vec{C}_k + \overrightarrow{\Delta C}_k$ 
6:  $O(\vec{C}_{k+1}) \leftarrow \vec{C}_k$ 
7: if  $|O(\vec{C}_{k+1}) - O(\vec{C}_k)| \leq \epsilon$  then
8:   SAVE  $\vec{C}_{k+1}$ 
9:   EXIT PROGRAM
10: end if
11: if  $O(\vec{C}_{k+1}) \leq O(\vec{C}_k)$  then
12:    $\vec{C}_k \leftarrow \vec{C}_{k+1}$ 
13:    $O(\vec{C}_k) \leftarrow O(\vec{C}_{k+1})$ 
14:    $\lambda_M \leftarrow \frac{\lambda_M}{10}$ 
15:   GOTO 2
16: else
17:    $\lambda_M \leftarrow 10 \times \lambda_M$ 
18:   GOTO 2
19: end if

```

Notice that the stop criteria presented here is the difference of the objective function in two consecutive iterations, ϵ . Other criteria were included, like a maximum number of iterations, but for simplicity, they were omitted in the algorithm presented above.

Appendix C

A modular software for nonlinear optimization

The numerical optimization of material parameters for an hyperelastic SEF (Chapter 6), can be a repetitive and time consuming problem. Optimization algorithms such as the Levenberg-Marquardt algorithm do not produce a *unique* set of optimal parameters. This means that given a (numerical) stop criteria (ex. $|O(\vec{C}_{k+1}) - O(\vec{C}_k)| \leq \varepsilon$, Appendix B), $\vec{C}$ may not converge to an optimal and stable solution.

To expedite the process of optimization, it was created a software based on two previous Matlab[®] scripts, one by the author and other by Dr. Peña (from the Zaragoza group, Section 1.1).

C.1 Interactive approach

The main window of the application enables data input (Figure C.1, left), data selection and model selection (Figure C.1, right) using the the combo and text boxes available. The input data, may have different sources like experimental data files or Matlab's workspace variables. Information such as data limits, can be extracted directly from the curve using mouse input.

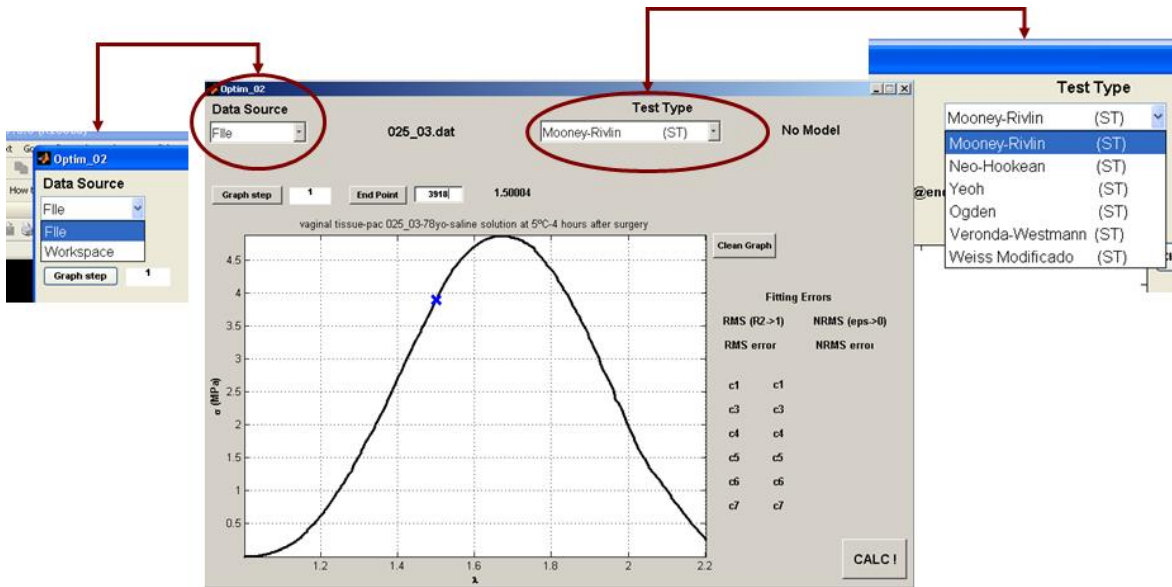


Figure C.1: The main window of the nonlinear optimization software.

C.2 Modularity

In Figure C.1, on the right the models to be fitted can be directly called, provided that some data was loaded previously. These models are implemented as functions external to the main application. The following example is the implementation of Yeoh's hyperelastic material model [Holzapfel, 2000] assuming isotropy and incompressibility conditions ($J = 1$), for a uniaxial tension state [Martins et al., 2006b]. The code is stored in an *.m* file called *Yeoh_ST.m* i.e., with the same name as the function.

```

1 function stress = Yeoh_ST(c,stretch1)
2 % Hyperelastic materials
3 %
4 % Stress state      : uniaxial (simple tension -> ST)
5 % Material          : isotropic / incompressible
6 % Strain-En. funct.: Yeoh
7 %
8 % Pedro Martins: palsm@fe.up.pt
9 %
10 % last updated: 2010-05-06
11 %
12 % Simple tension stress for Yeoh model

```

```

13 %
14 % Material parameters have physical meaning if:
15 % mu = mu(1) + mu(2) + mu(3)
16 % mu = 2*c(1) + 4*c(2)*(I1 - 3) + 6*c(3)*(I1 - 3)^2 > 0
17 % mu > 0 -> c(2) < 0; c(1) > 0; c(3) > 0%
18
19 % 1st stress invariant of the left
20 % cauchy-green strain tensor
21 inv01 = stretch1.^2 + 2./stretch1;
22 %
23 stress = 2.*(stretch1.^2 - 1./stretch1).*(c(1) + ...
24         2.*c(2).*(inv01 - 3) + 3.*c(3).*(inv01 - 3).^2);

```

The concept of modularity was extended to the graphical components of the application as well. When a particular SEF is selected on the main window, it calls the appropriate graphical interface enabling the user to input the optimization parameters (parameter seeds, stop criteria, etc.). Figure C.2 shows the optimization configuration window for an implementation of the new SEF proposed for prolapsed vaginal tissue (Chapter 6, [Martins et al., 2010b]). In Figure C.2, on the left, the label **Elastic** indicates the

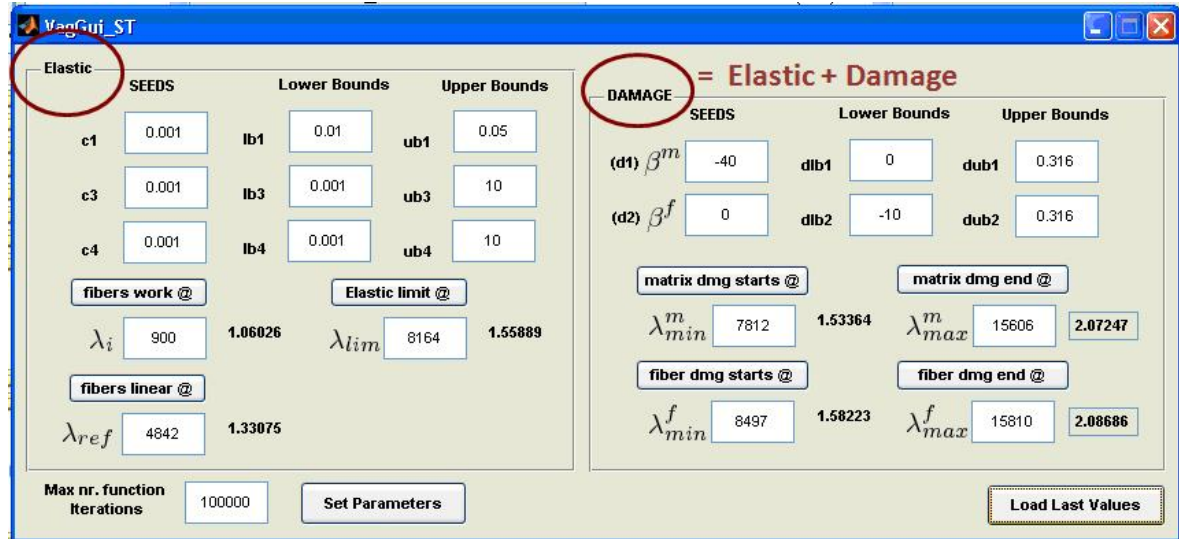


Figure C.2: The main window of the nonlinear optimization software.

data required to produce a fit constrained to the elastic limit of the stress-strain curve. On the right, labeled **Damage**, appears an extension of the model that includes (soft) damage proposed by Calvo et al. [2009]. When all the required parameters are set (for elastic or elastic+damage situations), the user must use the button **Set Parameters**.

The application returns to the main window, and the optimization process can start by pressing **CALC!** button.

C.3 Result production

The fittings' results are shown in Figure C.3(a) and (b) for the elastic and damage cases, respectively. The fitted parameters and errors appear directly on the graphical display of the results. As a consequence, users can evaluate the optimization results easily. It is possible to refine the optimization using the parameters of the last optimization. The results are also sent to a text file and to the clipboard, enabling the user to collect them in an excel sheet, for instance.

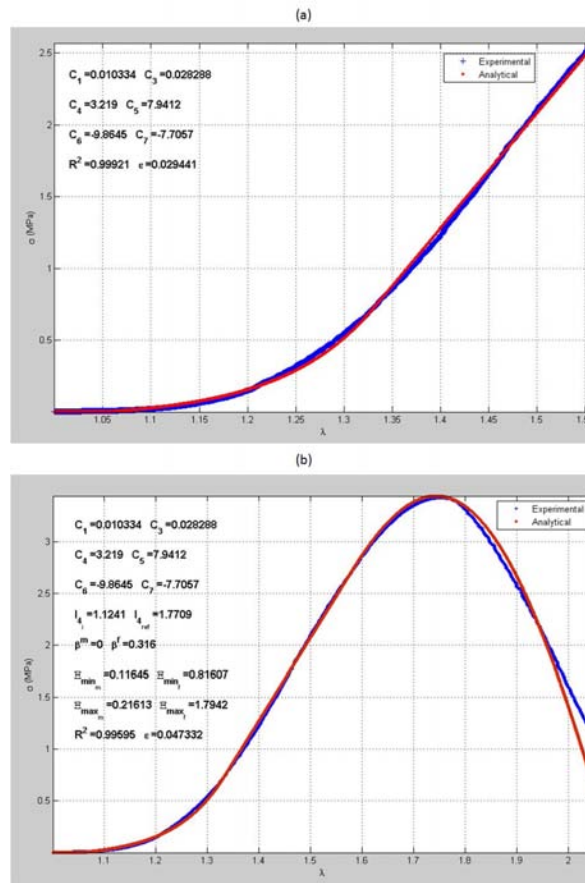


Figure C.3: The main window of the nonlinear optimization software.

Future developments: As is, the software proved versatile in producing fast results for the hyperelastic SEFs implemented. However, it is desirable a generalization of the modularity approach. This means that, optimally the material characteristics and the load conditions,¹ would be detached from the *.m* file representing the hyperelastic SEF. This would be a significant improvement as it would give the package a significant flexibility.

¹Load conditions such as simple, biaxial or equibiaxial tension.

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