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Research paper

A nonlinear-elastic constitutive model for soft connective tissue based on a histologic description: Application to female pelvic soft tissue

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ABSTRACT

To understand the mechanical behavior of soft tissues, two fields of science are essential: biomechanics and histology. Nonetheless, those two fields have not yet been studied together often enough to be unified by a comprehensive model. This study attempts to produce such model.

Biomechanical uniaxial tension tests were performed on vaginal tissues from 7 patients undergoing surgery. In parallel, vaginal tissue from the same patients was histologically assessed to determine the elastic fiber ratio.

These observations demonstrated a relationship between the stiffness of tissue and its elastin content. To extend this study, a mechanical model, based on an histologic description, was developed to quantitatively correlate the mechanical behavior of vaginal tissue to its elastic fiber content. A satisfactory single-parameter model was developed assuming that the mechanical behavior of collagen and elastin was the same for all patients and that tissues are only composed of collagen and elastin. This single-parameter model showed good correlation with experimental results.

The single-parameter mechanical model described here, based on histological description, could be very useful in helping to understand and better describe soft tissues with a view to their characterization. The mechanical behavior of a tissue can thus be determined thanks to its elastin content without introducing too many unidentified parameters.

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1. Introduction

The pelvic suspension system provides balance to the pelvic system, allowing it to support abdominal pressure, the weight of organs and mobility of individual organs. If this balance is perturbed, pelvic floor disorders can occur, the most frequent of which is genital prolapse (Samuelsson et al., 1999; Swift, 2000). The pathophysiological reasons for these disorders are assumed to be linked to abnormally large loadings combined with defective elastin synthesis. Indeed, in the literature, prolapsed tissues are always shown to present a lower level of elastin than healthy tissue (Kerkhof et al., 2009). Even though elastin is a relatively stable protein, it has been shown that its synthesis can restart in response to damage (Kerkhof et al., 2009; Mithieux et al., 2005) particularly in the pelvic area where its synthesis seems to be continuous (Liu et al., 2006). In these conditions it is therefore to hypothesize that prolapse could be caused by a combination of abnormally large loading damaging the tissue and a defect in elastin synthesis making it impossible to repair the damage. Pregnancy and vaginal childbirth may lead to genito-urinary trauma causing damage to tissues which could lead to a prolapse when elastin cannot be synthesized properly. Mechanical factors may combine to induce prolapse. Treatment of genital prolapse is essentially surgical, attempting to restore a normal functional anatomy using autologous or heterologous tissues to reinforce the vaginal tissue which is thought to play a major role in maintaining pelvic static. A few years after intervention, the rate of failure of surgical intervention can be up to 40% with autologous repair (Shull, 1999; Weber et al., 2001), while with prosthetic implants the rate of failure is close to 7% (Gauruder-Burmester et al., 2007; De Tayrac et al., 2007). This improved outcome explains the increasingly widespread use of prosthetics in surgery.

To improve the efficiency of prosthetic implants in the pelvic area, a large number of studies have been performed in the last decade to characterize the mechanical properties of soft pelvic tissues. Most of these studies focused on the vaginal properties which are the major contributors to pelvic static. Some papers defined an accurate experimental protocol (Ettema et al., 1998; Miller, 1963; Rubod et al., 2007). This protocol was used in experimental campaigns on large numbers of samples of most soft tissues making up the female pelvic system (Goh, 2002; Gabriel et al., 2011; Jean-Charles et al., 2010; Rubod et al., 2008). A non-linear elastic behavior of the pelvic soft tissue was revealed for vaginal tissue by Rubod et al. (2008) and also by co-workers Gabriel et al. (2011) and Jean-Charles et al. (2010). To perform statistical studies on these soft tissues, Jean-Charles et al. (2010) proposed a phenomenological approach to model their behavior based on Mooney–Rivlin models (Rivlin, 1948). Thanks to this modeling, the variation in behavior between healthy tissue and prolapsed tissue was revealed. However, the authors did not propose a histological, mechanical or medical interpretation of their results. This lack of interpretation mainly stems from the type of mechanical model developed, which was based on purely phenomenological approaches.

A classical phenomenological model used for non-linear elasticity (Rivlin, 1948; Mooney, 1940) – initially used to describe elasticity in rubber – might be useful in modeling the behavior of pelvic soft tissues (Jean-Charles et al., 2010) or any other soft tissues (Fung, 1973, 1981; Yin and Elliott, 2004). Most previous studies applying this model to biological tissues used exponential or power-law functions to model the non-linear behavior of tissues. These phenomenological approaches do not aim to link non-linearity of behavior to the physics of materials, which in the case of tissues could be histological properties. If links could be established between mechanical properties and chemical, bio-chemical or histological properties of tissues, a multi-physical description of tissue properties could be proposed. To attempt this, multi-scale approaches have been proposed, attempting to introduce histological information into the mechanical model (Holzapfel et al., 2000, 2002; Ciarletta et al., 2006) in order to interpret the impact of histology and morphology on the mechanical properties of the tissue. This type of approach offers a huge opportunity for a better understanding of the behavior of tissue.

However, the studies attempting to combine modeling with histology so far have focused only on semi-rigid tissues such as arterial or tendon tissues where collagen seems to play a key role. In addition, the models proposed required a very large number of difficult-to-determine parameters if a precise description of the histology of the tissues was to be obtained (Holzapfel et al., 2000, 2002; Ciarletta et al., 2006; Holzapfel and Ogden, 2010). With soft tissues, such as pelvic organs, collagen is associated with elastin, which has a much more non-linear behavior and is highly deformable. Soft tissues have not yet been studied with a mechanical approach combining mechanical modeling and histological or morphological information. Considering this type of soft tissue, made up of elastin and collagen, should not result in a model with an unreasonable number of unidentified parameters. Pelvic tissues, like all soft tissues, are non-specialized tissues. They are made up of cells linked by extracellular matrix (ECM). It is assumed that the ECM in these tissues provides the mechanical properties, while the cells provide the bio-chemical functions. ECM is itself mainly composed of macromolecules of biopolymers including collagen and elastin (Albert et al., 2012). Collagen fibers are considered to be very rigid and difficult to deform (Fung, 1973; Elliott, 1967; Shen et al., 2008), while elastin fibers have a very low stiffness and a high capacity to stretch without breaking (Gartner and Hiatt, 2014). Collagen and elastin fibers are both macromolecular three-dimensional polymeric chains forming cross-linked networks. Because vaginal tissue is assumed to be the major contributor to pelvic static, and because it is also the simplest to collect from patients, only vaginal tissue will be characterized, modeled and studied in this paper. However, the proposed model might easily be extended to other connective tissues.

The aim of this study was to model the behavior of soft tissue, taking into account differences in stiffness between collagen and elastin as well as the volume fraction of each phase, but trying to maintain a reasonable number of parameters to produce an accurate predictive model. The first part of this paper presents the results of an experimental study and a comparison of morphometric analysis and mechanical testing on vaginal soft connective tissue. These results highlight the main contribution of the respective proportions of elastin and collagen to the rigidity of soft tissue. The second part of the paper presents a model taking into account the macromolecular behavior of each component of the soft connective tissue and the non-linear elastic response of composite materials where each component can be modeled by macromolecular approaches (Treloar and Riding, 1979; Wu and Van Der Giessen, 1993; Gillibert et al., 2010; Arruda and Boyce, 1993). Both macromolecular networks are considered. A homogenization technique (Brieu and Devries, 1999; Ponte Castaneda and Tiberio, 2000; Brun et al., 2007) developed specifically to deal with non-linear elasticity under large strain makes it possible to propose a single-parameter model defined solely by the volume fraction of elastin. This model is a phenomenological model based on a physical description of the tissue composition. Finally, the third part of the paper compares the proposed model to experimental results and an interpretation of the contribution of collagen versus elastin is proposed.

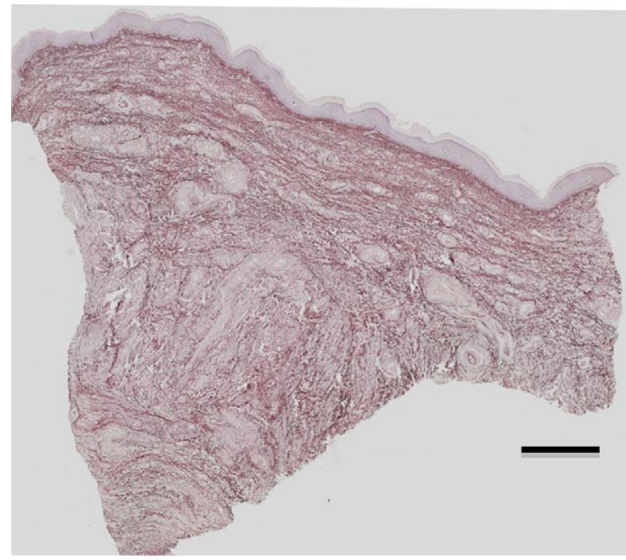

Fig. 1 – Orcein staining of tissue for morphometric measurements – Orcein-stained histological full-thickness section revealing elastin fibers in the anterior vaginal wall (original magnification ×10, scale bar 500 μm).

2. Histological and mechanical characterization

2.1. *Tissue collection*

Vaginal tissue was collected from patients for histological morphometric analysis and mechanical tests. Patients were recruited among patients consulting for gynecological surgery between July 2010 and May 2012 at the department of Gynecological Surgery at Jeanne de Flandre University Hospital, Lille, France. Review Board approval was obtained prior to the start of the study (CCPPBR CP 03/81; DRC 0315). All patients were duly informed of the aims of the study and gave written consent for participation before inclusion in this study. All patients were assessed to determine their Pelvic Organ Prolapse (POP) stage according to the International Pelvic Organ Prolapse Quantification system (Bump et al., 1996). Patients were included in the study only if they required surgical anatomic restoration to correct genital prolapse, and if such restoration involved a resection of vaginal tissue.

Tissue samples were obtained from 7 women who underwent vaginal surgical procedures for POP. Demographic characteristics (age, parity, BMI, menopausal status, POP-Q stage, type of surgery) were collected prospectively and stored in a dedicated database.

Full-thickness vaginal tissue samples were obtained with Metzenbaum scissors during POP repair, after sagittal midline section of the anterior or posterior vaginal wall.

2.2. *Histological analysis*

Immunohistochemistry, image processing and analysis were performed in the Laboratory of Tumor and Developmental Biology, GIGA-Cancer, University of Liège, Belgium. The study was also approved by the local Ethics Committee.

Histologically, the vaginal wall is composed of four layers. The epithelial layer is a superficial non-keratinized squamous epithelium. The subepithelial layer (lamina propria) is a dense connective tissue layer mainly composed of fibrillar collagens and elastin. The muscularis is mainly composed of smooth muscle cells embedded in connective tissue. The adventitia is a loose connective tissue layer. To distinguish between these different layers and examine their composition, human biopsy specimens were fixed in buffered 4% formalin, embedded in paraffin and serially sectioned at 5 μm thickness. Specimens were then stained with routine dyes (hematoxylin–eosin) for conventional histopathological examination or with orcein to quantify elastin fibers, see Fig. 1 (Bancroft and Stevens, 1982).

Virtual images were acquired with a fully automated dotSlide digital microscopy system (Olympus, BX51TF, Aartselaar, Belgium) linked to a Peltier-cooled high-resolution digital color camera (1376 × 1032 pixels) (Olympus, XC10, Aartselaar, Belgium). Digital images of whole tissue sections were recorded at high magnification (100x) producing virtual images with a pixel size of 0.65 μm.

After splitting the original color image to its RGB components, orcein-stained regions were extracted from the red component using the automatic entropy threshold technique (Kapur et al., 1985). The orcein staining percentage was defined as the area stained with orcein divided by the total area of the section.

Image processing and measurements were performed using the image analysis toolbox included in MATLAB (7.9) software (Mathworks, Inc.) (De Landsheere et al., 2013).

2.3. *Mechanical testing*

Tissue samples were frozen in saline solution until the tensile test could be performed according to the protocol defined in Rubod et al. (2007). Tissues were thawed 9 h before

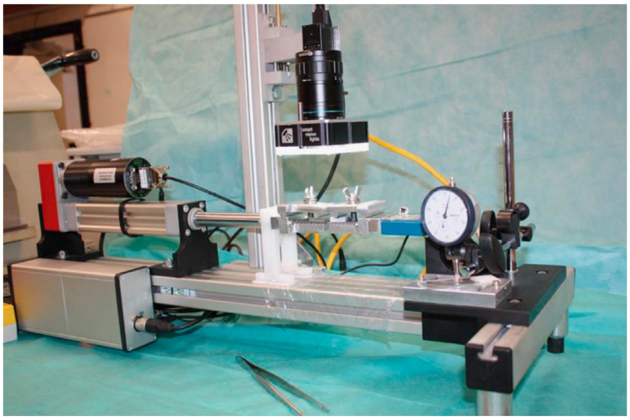

Fig. 2 – Experimental set up for strain measurement.

the tests; uniaxial samples were punched in the same orientation. Because of the size of the sample required (25 mm $\times$ 4 mm) patients were only included in this study if the tissue removed was sufficiently large to provide uniaxial samples and also allow histological analysis. Due to the origin of the tissue and the size of samples, it was not possible to perform biaxial tension tests.

The thickness of each sample was measured using a micrometer (1.8$\pm$0.3 mm). To avoid excessive strain during this measurement, a micrometer with a large contact area was used. The sample was then clamped in a tightening grip and brought into a non-relaxed, non-stretched state where the total force applied was null. The sample was then strained at a constant strain rate (2:10^{-2} s^{-1}) at ambient temperature (21 °C). The test was performed rapidly (less than 45 s); therefore no humidification system was required (Rubod et al., 2007). Monotonic tests were performed using a conventional testing system (Fig. 2). A load cell (200 N) was used to measure force during the test. Strain was measured using a contactless video extensometer.

The stress–strain curves obtained were analyzed to characterize the behavior of the tissues investigated. The mechanical response of the specimen before rupture was then studied. The results presented on graphs do not include rupture of the specimens.

2.4. *Experimental results*

The demographic characteristics of the women included in this study are reported in Table 1. Among the 7 patients who underwent surgery for prolapse, an isolated anterior repair was performed in two patients (28.5%); an isolated posterior repair was performed in three patients (42.8%); and both anterior and posterior repair were performed in 2 patients (28.5%). Tissue samples were only taken from the compartment undergoing POP repair.

2.4.1. *Histological results*

The results of the image analysis to determine the fraction of elastin in each sample are presented in Table 2.

From these results, it appears difficult to correlate the fraction of elastin to parity, age or stage of prolapse.

Table 1 – Clinical history of patients and localization of their resection.

Patient	Age (years)	Parity	Stage of prolapse	Location of resection
Case 1	66	1	4	Ant. walls
Case 2	58	5	3	Ant. and post. walls
Case 3	72	1	2	Post. wall
Case 4	73	0	3	Post. walls
Case 5	64	4	3	Ant. and post. walls
Case 6	59	2	3	Post. wall
Case 7	77	7	4	Ant. walls

Table 2 – Fraction of elastin measured based on image analysis by a morphometric method.

Patient	Anterior wall (%)	Posterior wall (%)
Case 1	15.5	NS
Case 2	11.4	7.5
Case 3	NS	7.9
Case 4	NS	17.3
Case 5	5.0	3.1
Case 6	NS	8.8
Case 7	11.2	NS

NS=No Sample.

2.4.2. *Mechanical results*

The results of tension tests are reported in Fig. 3.

From the results in Fig. 3, it can be noted for each patient that the tissue can be extensively stretched at a large strain. The stress–strain response appeared to be non-linear, confirming that vaginal tissue is a hyper-elastic material (Rubod et al., 2008). Furthermore, considerable dispersion is noted between patients. Previous experimental studies (Gabriel et al., 2011; Jean-Charles et al., 2010) failed to explain these differences between patients. Introducing a model with a physical description of the tissue, rather than using a phenomenological approach, might be more relevant when seeking an explanation for the differences. However, this type of histology-based model could explain the differences between patients only if a reduced number of parameters is used to facilitate comparison and comprehension.

As with the histology results, it seems impossible to correlate the mechanical behavior of the vaginal soft connective tissue to the patient's history since the difference between patient was, at the very beginning, already different. However, the mechanical behavior could correlate with the histological analysis. When the results in Fig. 3 are compared to those in Table 2, it appears that the stress level in response to a given strain correlates with the volume fraction of elastin. Thus, the more the elastin, the lower the stress, as previously described by Tong et al. (2013). To illustrate this point, we compared the elastin levels determined by both methods and the stress at four different strains for three patients (Table 3).

The patients with the highest and lowest volumic fractions of elastin, and one patient in the middle of the range were chosen for this comparison. It can be noted that a higher volumic fraction of elastin is linked to a lower stress at each chosen strain.

This observation suggests that elastin might be a physical parameter that helps to characterize the mechanical properties of this tissue.

Based on these observations, we will now propose a model with a reduced number of parameters based on a histological description of the soft connective tissues.

3. Histology-based mechanical model of hyper-elasticity

In previous studies, vaginal tissue was shown to display visco-hyperelasticity (Rubod et al., 2007). As a first approach, it is common to neglect the contribution of viscosity when modeling hyper-elasticity (Diani et al., 2006). Among the various models of hyper-elastic behavior for polymers available in the literature (Rivlin, 1948; Mooney, 1940; Ogden, 1984; Treloar, 1975), some are purely phenomenological and some are based on a physical description of the macromolecules making up the polymer, and the links between the physics of these macromolecules and their mechanical properties. For the purposes of this study, which aims to link the physical and histological properties to the mechanical properties of tissues, we have chosen a macromolecular approach.

3.1. *Macromolecular model*

The macromolecular model for non-linear elasticity was introduced in the '50s (Treloar, 1975, 1954). To introduce the distribution of the macromolecule in all the directions of space a spatial distribution of the macromolecular chain and the contributions of each direction were proposed (Treloar and Riding, 1979; Treloar, 1954). These full-network models required integration over the surface of a sphere, which resulted in long computation times. In an attempt to simplify the calculations, a finite number of directions can be introduced (e.g. 3-chain Wang and Guth (1952), 8-chain Arruda and Boyce (1993)) to model the contribution of the different macromolecular chains. In these latter models, the actual network is represented by a finite number of directions (3 or 8), which follow the principal directions of the right stretch tensor. Such approaches have been improved to take into account anisotropy, such as orthotropy (Bischoff et al., 2001, 2002), of the material under investigation. More recently, direction-based constitutive models, still based on macromolecular approaches, were proposed (Gillibert et al., 2010; Diani et al., 2006; Goktepe and Miehe, 2005). These approaches did not follow the principal directions of the right stretch tensor but involved material-specific directions. For instance, the set of directions could be chosen according to the initial isotropy or anisotropy of the material. The strain-energy function, W, which characterizes the non-linear elastic behavior, depending on the deformation gradient, F, in the case of directional models can thus be defined by:

$$W(\underline{\underline{F}}) = \frac{1}{V}\int_S w\left(\theta,\ \varphi,\ \underline{\underline{F}}.\underline{u}\right)dv = \sum_{i=1}^{M}\omega^i w^i\left(\underline{\underline{F}}.\underline{u}^i\right) \tag{1}$$

where w is the elementary strain-energy function of the macromolecules defined on the unit-sphere defining the elastic behavior of the materials considered and $\underline{u}$ the unitary orientation vector of direction θ, φ. In the directional model, the integral expression is approximated by a summation over M directions in space. ω^i is the weight of direction i giving a good approximation of the unit-sphere expression over a limited number of directions. ω^i also makes it possible to introduce anisotropy between directions. w^i is the elementary strain-energy function of the macromolecules in direction i, u^i the unitary direction vector of direction i, defined by Bazant and Ah (1986).

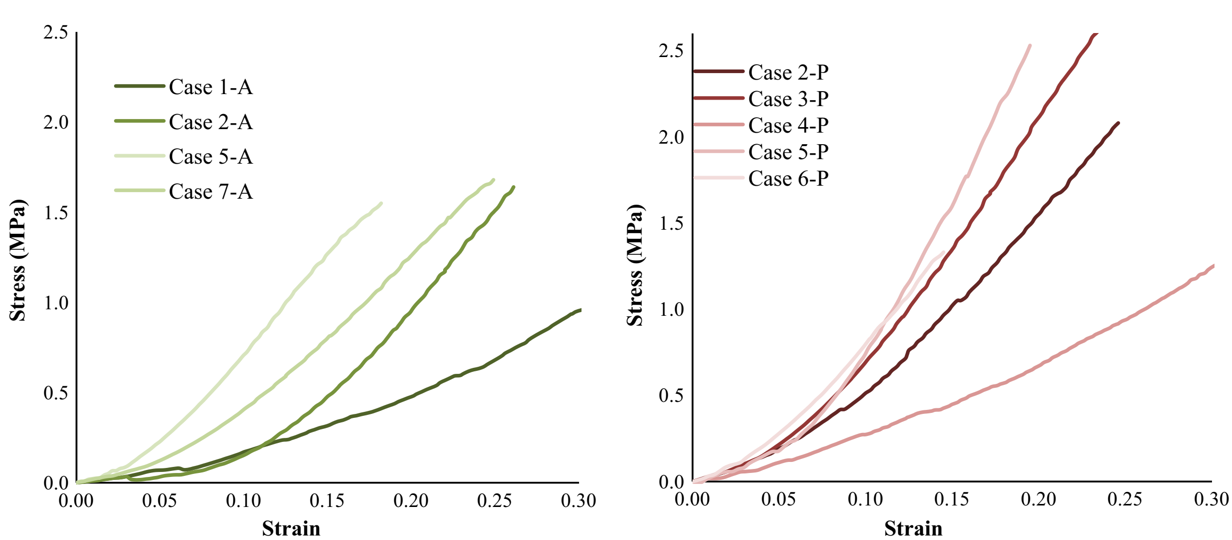

Fig. 3 – Stress–strain curves obtained for uniaxial tension. Left panel: anterior wall; Right panel: posterior wall tissues.

Table 3 – Fraction of elastin and stress at four different strains for three patients.

	1-A	7-A	5-A
	Morphometric elastin content analysis		
	15.5%	11.2%	5.0%
	Stress (MPa)		
Strain			
0.05	0.07	0.13	0.23
0.09	0.14	0.34	0.60
0.13	0.25	0.63	1.06
0.18	0.41	1.06	1.53

Assuming isotropy of the material, the elementary strain-energy function can thus be defined (Kuhn and Grün, 1942) as:

$$w^i\left(\underline{\underline{F}}.\underline{u}^i\right) = \frac{C}{\sqrt{N}}\left(L^{-1}(\nu^i)\nu^i + \ln\left(\frac{L^{-1}(\nu^i)}{\sin h(L^{-1}(\nu^i))}\right)\right) \quad \text{with } \nu^i = \frac{\sqrt{\left(\underline{\underline{F}}.\underline{u}^i\right)^t\cdot\left(\underline{\underline{F}}.\underline{u}^i\right)}}{\sqrt{N}} \tag{2}$$

where C corresponds to the rigidity of the macromolecular chains in direction i, N corresponds to the average length of the macromolecules in the polymer considered and ν^i is the stretch in direction u^i. The function L^{-1} is the inverse of Langevin's function $L(x) = \coth(x) - 1/x$. For analytical convenience, the inverse function is generally approximated by a function which was first introduced by Cohen (1991).

Due to the amount of water contained in soft tissues – close to 70% – it can be assumed that they are incompressible. Furthermore, according to Jean-Charles et al. (2010), soft connective tissue from pelvic organs can be assumed to be isotropic. Thus, if the tissues considered are incompressible isotropic materials, the first Piola Kirchhoff stress tensor, $\underline{\underline{\sigma}}$, (i.e., the nominal stress) can be written as:

$$\underline{\underline{\sigma}} = \frac{\partial W}{\partial \underline{\underline{F}}} - p\underline{\underline{F}}^{-t} \tag{3}$$

where p is the hydrostatic pressure induced by incompressibility.

3.2. *Two-phase model*

As already mentioned, the mechanical properties of soft pelvic tissues are conferred by the extracellular matrix (ECM) making up the tissues. This ECM is mainly composed of macromolecules of biopolymers (Albert et al., 2012): mainly collagen and elastin (Badiou et al., 2008).

Compared to elastin fibers, collagen fibers (1–12 μm length) are rigid and poorly deformable (Fung, 1973; Elliott, 1967; Shen et al., 2008). Different types of collagens exist, but they are all more rigid than elastin. Compared to collagen fibers, elastin fibers (0.1–1 μm length) have a low stiffness and a high capacity to stretch without breaking (Gartner and Hiatt, 2014). As a first approach, all the collagen might be assumed to be only one type of macromolecule and elastin fibers might be assumed to be another macromolecule. Both fibers, collagen and elastin, are organized in a three-dimensional cross-linked macromolecular chain network. Even though the mechanical properties of collagen types and subtypes may vary, the average rigidity of collagen fibers is always much higher than the rigidity of elastin, and collagen is therefore assumed to be a unique material with constant mechanical properties, with a greater stiffness than elastin. Thus, for our purposes, the soft tissue is composed of two different materials: collagen fibers and elastin fibers. The behavior of each of these materials can be modeled using Eqs. (1) and (3). Furthermore, we assume that both components can be modeled as a Langevin-chain type whose elementary strain-energy function is defined by Eq. (2). The behavior of each can be described by its own strain-energy density W^c and W^e respectively for collagen and elastin. W^c and W^e are defined with respect to Eq. (3) introducing N_c and C_c the average length and strength of collagen macromolecules and N_e and C_e are the equivalents for elastin.

The mechanical response of the different samples could be analyzed as a contribution of these two different phases. However, such strain-energy does not take into account the volume fraction of each phase. Thus, a homogenization technique is used to take volume fraction into account. The homogenization techniques in the case of non-linear elasticity can be based on two different approaches: periodic approaches (Brieu and Devries, 1999; Lahellec et al., 2004) which assume that phases are periodically distributed in space, and random approaches (Ponte Castaneda and Tiberio, 2000; Bouchard et al., 2008) which assume that phases are randomly spread throughout the volume.

To produce a very simple model, we chose to use a homogenization technique, making no assumption on the microstructure architecture. The second-order homogenization technique described by Ponte Castaneda and Tiberio (2000) is dedicated to non-linear elasticity in the case of random approaches. However, Bouchard et al. (2008) indicate that this type of approach is quite difficult to use and results in long computation times. To avoid this extension to the computation time, we chose to apply a second-order approach (Ponte Castaneda and Tiberio, 2000) in its simplest expression, which is equivalent to a Voigt approach. We defined the strain-energy density of the two-phase (collagen and elastin) composite material, $\tilde{W}$, as:

$$\tilde{W}(\underline{\underline{F}}) = (1-\phi) * W^c(\underline{\underline{F}}) + \phi * W(\underline{\underline{F}}) \tag{4}$$

where ϕ is the volume fraction of elastin in the tissue, $1-f$ being the volume fraction of collagen.

Finally, the stress–strain relationship of the composite material, made up of elastin and collagen, is given by Eq. (4) and the behavior can be defined by a total of five parameters: ϕ, C_c, N_c, C_e and N_e.

3.3. *Identification process*

Having selected our model, we next aimed to identify the parameters to be determined. Looking at Eq. (4), it is obvious that the model depends on five parameters. However, according to the results of Madhavan et al. (2010), Young's modulus for collagen can be set to 150 kPa. This modulus corresponds to the tangent modulus of the collagen in the vicinity of the stress-free state. It might easily be demonstrated, with a Taylor-expansion of Eq. (3) combined with Eq. (2) in the case of uni-axial tension, that, close to the stress-free state, the proposed strain-energy density (Eq. (2)) induces a tangent modulus, E, such that:

$$E = 2.\frac{C}{N}\sum_{i=1}^{M}\omega^{i}.\left(\underline{u}^{i}\right)^{2} \tag{5}$$

This relation reduces the number of parameters for the proposed behavior law to four.

However, such approach still introduces 4 independent parameters: 3 for the two macromolecular models, and one for the volume fraction of elastin or collagen. This remains a large number of parameters to determine. To further reduce the number of parameters, we finally, assume that the macromolecular characteristics of elastin and collagen (N_c, C_c, N_e, C_e) will remain constant from one patient to another. This hypothesis simply assumes that collagen and elastin are both chemical molecules present in a living organism and that their properties have no particular reason to vary from one organism to another. Consequently, all five parameters may not vary between patients. Indeed, we assume that the difference between two samples will only be linked to variations in the volume fraction of elastin.

According to Gillibert et al. (2010), the directional network used for a macromolecular model must be chosen carefully to achieve isotropy in the model. We therefore chose the 66-direction network first introduced by Bazant and Ah (1986).

In order to identify the macromolecular characteristics of elastin and collagen, we randomly selected five of the mechanical tests available to us and determined (N_c, C_c, N_e, C_e) – which would then become constants in future mechanical tests – and the volume fraction, ϕ_i, which would vary for each mechanical test. All the different parameters were identified on five different tests to identify the four material parameters (C_c, N_c, C_e, N_e) and the 5 volume ratios. The process for parameter optimization was performed by a Levenberg–Marquart method (Marquardt, 1963). By this procedure, the parameters were given the following values: $C_c=4747$ kPa; $N_c=21.1$; $C_e=853\times10^{-3}$ kPa; $N_e=2.3$. These values resulted in a tangent modulus with mild strain of $E_c=150$ kPa and $E_e=0.24$ kPa.

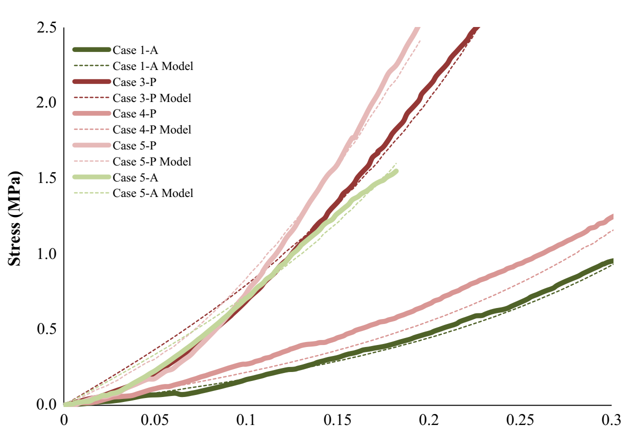

Fig. 4 – Comparison of the model to experimental data.

The results of application of these values to the model are presented in Fig. 4.

As can be seen in Fig. 4, the proposed model gives a good approximation of the mechanical response of soft tissues.

According to the literature (Fung, 1973; Elliott, 1967; Shen et al., 2008; Gartner and Hiatt, 2014), collagen is much stiffer than elastin, and collagen fibers are between 10 and 100 times longer than elastin fibers. The results obtained using the proposed model are in agreement with this information.

3.4. *Validation and discussion*

To validate our approach, we set the values of the macromolecular parameters for elastin and collagen (C_c, N_c, C_e, N_e) to the constant values determined above. We thus assumed that the mechanical properties of elastin and collagen remain the same for all individuals and that only the volume fraction of elastin varies between patients.

For the three remaining cases, we only had to determine one parameter: the volume fraction of elastin, ϕ. Results are presented in Fig. 5.

Fig. 5 compares the experimental results to the model determined. The apparent volume ratios of elastin identified by the proposed model are reported in Table 4 for the different patients.

As observed in Fig. 5, the experimental results are fairly well fitted by the model. Thus, the model proposed provides a good approximation of the behavior of vaginal tissues for this group of patients. The apparent volume fraction of elastin in the tissue determined by the proposed model shows the same trends as the fraction of elastin determined by morphometric analysis on histological tissue samples (Table 4). These results appear very different to those reported by Jackson et al. (1996), who indicated that the mass ratio of elastin in dried prolapsed vaginal tissue was close to 2%. However, our results confirm that the model proposed makes it possible to establish a link between the volumic fraction of elastin and the elasticity of soft connective tissues.

Thus, the proposed model succeeds in modeling the non-linear elastic behavior of soft connective tissue while only requiring a single parameter to be identified: the volume

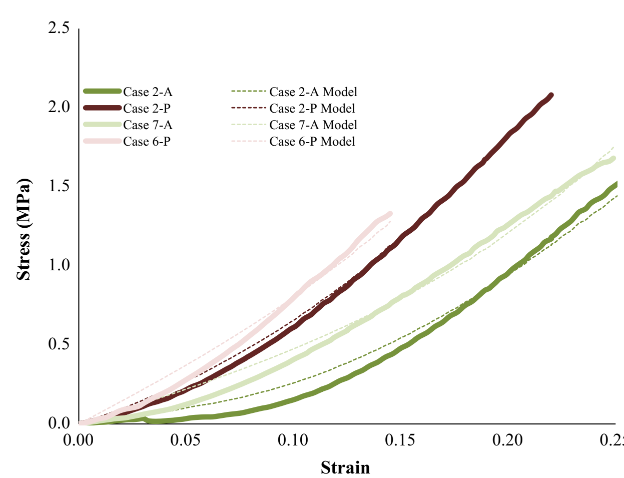

Fig. 5 – Validation of the model by comparison with experimental data for uniaxial tension.

Table 4 – Comparison between the morphometric analyses performed based on histology of soft tissue and apparent elastin volume fraction determined using the proposed model.

Patient	Anterior wall (%)		Posterior wall (%)	
	Morphometric analysis	Model identification	Morphometric analysis	Model identification
Case 1	15.5	9.5	NS	
Case 2	11.4	7.3	7.5	9.5
Case 3	NS		7.9	4.3
Case 4	NS		17.3	10.3
Case 5	5.0	1.5	3.1	1.9
Case 6	NS		8.8	4.3
Case 7	11.2	8.3	NS	

fraction of elastin. This new model thus makes it possible to extrapolate for aging. This is of interest as it has been suggested that the stiffness of pelvic soft connective tissue increases with age (Chantereau et al., 2014), while other studies report that the types and amounts of collagen and elastin vary with age (Braverman and Fonferko, 1982; Cotta-Pereira et al., 1976; Diridollou et al., 2001; Gogly et al., 1997; Imayama and Braverman, 1989; Ma and Cowdry, 1950). This phenomenon is in line with results obtained from our single-parameter model for non-linear elasticity, indicating that stiffness will increase as the volume fraction of elastin decreases. The proposed model can also help us to better understand how pelvic organ prolapse occurs. As yet, the literature has not reached a consensus on whether prolapse induces or is induced by a decrease in the volume fraction of elastin in the connective tissue. According to our model, a decrease in the volume fraction of elastin would lead to stiffening of the tissue and loss of elasticity, which has previously been noted on older and prolapsed tissues (Jean-Charles et al., 2010; Chantereau et al., 2014).

4. Conclusions

This study proposes a model for the non-linear elastic behavior of soft connective tissues, such as vaginal fascia tissue. The model takes into account the heterogeneity of tissues composed of collagen and elastin, and the polymeric macromolecular nature of each component. Experimental tests on vaginal fascia tissues were performed to determine the stress–strain response of this type of tissue. The experimental results revealed non-linear elastic behavior and a large dispersion between samples collected from different patients. The results also revealed a correlation between the volume fraction of elastin within the connective tissue and its stiffness.

The non-linear elastic behavior of these tissues was modeled by a macromolecular approach combined with a homogenization technique to take into account both the polymeric macromolecular nature of elastin and collagen, and the respective volume fraction of each polymer.

The approach proposed assumes that the properties of collagen and elastin remain the same across all samples. Thus, the differences between the tissues studied are assumed to be only due to variations in the volume fraction of elastin in the tissue. This assumption makes it possible to characterize the non-linear elastic properties of soft tissues using a single parameter to produce very satisfactory results.

The model presented offers extensive opportunities for better modeling and understanding of the behavior of soft tissue, such as vaginal fascia tissue. It may help researchers to better understand how tissue aging is related to variations in the volume fraction of elastin. It also offers opportunities for a better understanding of the pathophysiology of pelvic floor disorders which are linked to alterations to connective tissues. Finally, this model will allow numerical approaches to be developed with the aim of identifying the single parameter, φ of the model using an inverse method comparing numerical simulations to MRI strain-field displacement. Through this approach, development of patient-specific simulations becomes possible thanks to more feasible non-destructive characterization methods (Lecomte-Grosbras et al., 2015).

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