Journal of Biomechanics 48 (2015) 3088–3096

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Bladder tissue biomechanical behavior: Experimental tests and constitutive formulation

A.N. Natali [a,b,*], A.L. Audenino [c], W. Artibani [d], C.G. Fontanella [a], E.L. Carniel [a,b], E.M. Zanetti [e]

[a] Centre for Mechanics of Biological Materials, University of Padova, Via F. Marzolo 9, I-35131 Padova, Italy
[b] Department of Industrial Engineering, University of Padova, Via F. Marzolo 9, I-35131 Padova, Italy
[c] Department of Mechanical and Aerospatial Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
[d] University of Verona, Department of Urology, Policlinico G.B. Rossi, Ospedale Borgo Roma, P.le L.A. Scuro 10, 37134 Verona, Italy
[e] Department of Engineering, University of Perugia, Via Duranti 65, 06125 Perugia, Italy

ARTICLE INFO

Article history:
Accepted 18 July 2015

Keywords:
Bladder
Soft tissue mechanics
Experimental test
Non-linear mechanics
Computational approach

ABSTRACT

A procedure for the constitutive analysis of bladder tissues mechanical behavior is provided, by using a coupled experimental and computational approach. The first step pertains to the design and development of mechanical tests on specimens from porcine bladders. The bladders have been harvested, and the specimens have been subjected to uniaxial cyclic tests at different strain rates along preferential directions, considering the distribution of tissue fibrous components. Experimental results showed the anisotropic, non-linear and time-dependent stress–strain behavior, due to tissue conformation with fibers distributed along preferential directions and their interaction phenomena with ground substance. In detail, experimental data showed a greater tissue stiffness along transversal direction. Viscous behavior was assessed by strain rate dependence of stress–strain curves and hysteretic phenomena. The second step pertains the development of a specific fiber-reinforced visco-hyperelastic constitutive model, in the light of bladder tissues structural conformation and experimental results. Constitutive parameters have been identified by minimizing the discrepancy between model and experimental data. The agreement between experimental and model results represent a term for evaluating the reliability of the constitutive models by means of the proposed operational procedure.

1. Introduction

Lower urinary tract dysfunction affects about 400 million people worldwide because of mechanical, neurological or idiopathic insult. Different congenital or acquired pathologies determine anatomical and functional alterations also of the bladder-sphinteric apparatus with consequent impaired urinary continence (Korossis et al., 2009, Irwin et al., 2006; Sacco et al., 2006). An engineering approach can provide reliable tools for the treatments of pathologic situations, as to design the most appropriate long-term surgical repair procedures or to investigate and develop materials for bladder reconstruction.

The structural models of specific organs is addressed, by a computational approach, to the investigation of the mechanical response of biological tissue and structures in health and disease, also to analyze interaction phenomena with biomedical devices (Carniel et al., 2013, 2014; Krywonos et al., 2010). The definition of computational models requires both 3D virtual solid models of anatomical region and reliable constitutive formulations of biological tissues. As matter of example, the three dimensional reconstruction of the pelvic district, as virtual solid models, by reverse engineering techniques has proved to be useful for preoperative planning, allowing to analyze the outcomes of different reconstructive solutions or to support diagnostic hypotheses (Chai et al., 2012; Hampel et al. 2004; Marino and Bignardi, 2002; Pel and van Mastrigt, 2007; Vlastelica et al., 2007). The constitutive analysis of soft biological tissues mechanics is usually performed by a coupled experimental and computational approach. Mechanical tests must be accurately designed accounting for the expected tissue properties, considering anisotropic behavior, non-linear effects and time-dependent phenomena, evaluating the tissue structural configuration. The experimental results allows to provide the appropriate constitutive formulation and to identify the associated parameters.

Different authors reported investigations about bladder tissues mechanics, with regard to both experimental and computational activities. Regnier et al. (1983) provided hyperelastic formulation to interpret the non-linear elastic behavior. Salinas et al. (1992) and Nagatomi et al. (2004) developed experimental activities to

* Corresponding author at: Centre for Mechanics of Biological Materials, University of Padova, Via F. Marzolo 9, I - 35131 Padova, Italy. Tel.: +39 049 827 5598; fax +39 049 827 5604.
E-mail address: arturo.natali@unipd.it (A.N. Natali).

http://dx.doi.org/10.1016/j.jbiomech.2015.07.021
0021-9290/

evaluate the viscoelastic properties together with preliminary model formulation. Van mastrigt and Nagtegaal (1981) carried out an investigation of the strain rate dependence of the viscoelastic response of the urinary bladder wall. Korossis et al. (2009) and Chen et al. (2013) investigated the anisotropic and non-linear elastic behavior by experimental activities, considering also the relationship between the tissue histo-architecture and mechanical properties.

The improvement provided by the current study is the combined action of experimental and numerical approach for the analysis of the bladder tissue mechanics, aiming at a reciprocal reliability assessment. Specific experimental tests are developed and, considering histological configuration and experimental results, an appropriate constitutive model is implemented.

Some general notes are reported with regard to the histomorphometric configuration of the bladder tissue, aiming at the biomechanical characterization. The wall is a complex structure made up of mucosa, submucosa, muscolaris and serosa layers as reported by different authors (Bouhout et al., 2013; Zanetti et al., 2012). The mucosa is the innermost tissue and consists of transitional epithelial cells layers, which adapt their shape to the bladder filling. The submucosa is a thick layer of loose connective tissue and it is rich in elastic fibers, nerve, blood and lymphatic vessels. The tunica muscolaris is composed of three smooth muscular layers together with connective elements. Within the inner and the outer layers, muscular fibers are predominately distributed from the apex to the bottom of the bladder, while a circumferential organization characterizes the fibers within the middle layer. The serosa layer is a visceral peritoneum and the mechanical contribution to the wall stiffness and strength in almost negligible. With regard to the overall contribution of connective fibers, elastin provides the recoiling mechanism of the tissues (Korossis et al., 2009), while collagen fibers are preferentially aligned along apex to base direction (Gilbert et al., 2008).

Such a complex configuration, with particular regard to fibrous components, entails the anisotropic and non-linear behavior. Fibrous structures, as muscular and connective ones, undergo stiffening phenomena with stretch, leading to non-linear elastic properties. Micro-structural rearrangement phenomena, as fibers uncrimping and interaction with liquid components, lead to viscous effects (Bouhout et al., 2013; Korossis et al., 2009; Zanetti et al., 2012). Aiming at the mechanical characterization, tensile tests on tissue specimens are developed along different directions and at different strain rates. A constitutive formulations is developed in the framework of a theory that accounts for anisotropic behavior, coupled geometrical and material non-linearity and time-dependent phenomena. The identification of constitutive parameters is performed comparing model results and data from mechanical tests by fitting procedures. The reliability of constitutive formulation and parameters obtained is assessed by comparison of computational and experimental results with reference to additional experimental tests.

2. Materials and methods

2.1. Mechanical tests

The experimental tests have been performed on tissue specimens from pig bladder, because of the extensively supported similarity of pig and human tissues mechanics. Different authors (Dahms et al., 1998; Korossis et al., 2009) investigated bladder of different species, finding that pig and human bladder tissues show similar histomorphometric conformation and mechanical behavior. The intact bladders of three Large White pigs, that were 11–13 months old and about 160 kg in weight, were collected from a local abattoir and transferred to the laboratory. The bladders were cleaned for luminal contents, adjacent tissues were dissected and the organs were frozen at −20 °C. Twenty-four hours before testing, bladders were put into the fridge and completely defrosted (Dahms et al., 1998). They were dissected along the apex-to-base line, and samples were harvested from the lateral region of the wall. In detail, specimens were isolated using an I-shaped die cutter along apex-to-base (AB) direction and along transverse (*T*) direction (Fig. 1). This shape prevented sample from over-stressing next to the grips, as witnessed by frequent failure in this area in the case of constant section specimens. Specimens were measured by means of photogrammetry to identify the central region and to evaluate width and thickness profiles using the image analysis software ImageJ (Zanetti et al., 2012). The number of specimens and their average width and thickness are reported in Table 1, while the length of each specimen in the central region of the I-shape was 12 mm. The length of the specimen has been chosen in relation to the limits of the loading machine and in agreement with the efficacy of the experimental tests performed (Bose Electroforce® 3200; 225 N maximum force; 12 mm stroke; static to 300 Hz frequency response), considering a 100% strain was to be reached. After their isolation, specimens were stored in the fridge within glass tubes containing PBS for less than 10 h. During tests, samples were continuously dampened with the solution to keep them wet.

A Bose Electroforce® equipment was used to perform tensile tests on the tissue specimens. Because of preliminary evaluation of samples strength, a load cell with capacity of 200 N with accuracy of ± 0.1% was adopted. Clamping of the specimens was performed by grips (5 mm length and 20 mm wide), according to an intensity of about 100 kPa, adjusted to avoid the slippage and the damage of the specimens. The slippage has been checked during tests looking at the overall trend in the force/displacement curve. At the end of tests, it was verified that the length of the specimen clamped remained unchanged, and that there were no marks of longitudinal slips. Force and displacement signals were synchronized by means of a

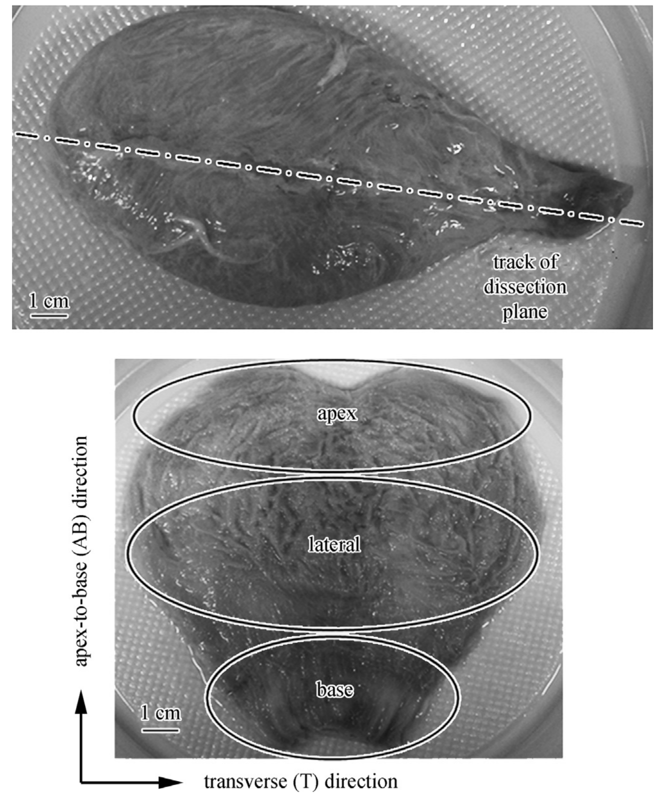

Fig. 1. Bladder dissection and region definition: (a) bladder in the anterior–posterior view with indication of the track of dissection plane; (b) cut-opened bladder showing the apex, the lateral and the base region of the bladder.

Table 1
Number of specimen and their average width and thickness.

	No. of specimen	Width mean ± standard deviation (mm)	Thickness mean ± standard deviation (mm)
Apex-to-base direction	9	4.95 ± 0.56	4.37 ± 1.00
Transverse direction	16	4.49 ± 0.78	3.77 ± 1.03