ARTICLE IN PRESS

Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Location-dependent correlation between tissue structure and the mechanical behaviour of the urinary bladder

Enrique Morales-Orcajo [a], Tobias Siebert [b], Markus Böl [a,*]

[a] Institute of Solid Mechanics, Technische Universität Braunschweig, Braunschweig D-38106, Germany
[b] Institute of Sport and Motion Science, University of Stuttgart, Stuttgart D-70569, Germany

ARTICLE INFO

Article history:
Received 5 January 2018
Received in revised form 4 May 2018
Accepted 9 May 2018
Available online xxxx

Keywords:
Urinary bladder wall
Biaxial tension testing
Tissue composition
Microstructure
Smooth muscle
Tissue staining

ABSTRACT

The mechanical properties of the urinary bladder wall are important to understand its filling-voiding cycle in health and disease. However, much remains unknown about its mechanical properties, especially regarding regional heterogeneities and wall microstructure. The present study aimed to assess the regional differences in the mechanical properties and microstructure of the urinary bladder wall. Ninety ($n = 90$) samples of porcine urinary bladder wall (ten samples from nine different locations) were mechanically and histologically analysed. Half of the samples ($n = 45$) were equibiaxially tested within physiological conditions, and the other half, matching the sample location of the mechanical tests, was frozen, cryosectioned, and stained with Picro-Sirius red to differentiate smooth muscle cells, extracellular matrix, and fat. The bladder wall shows a non-linear stress-stretch relationship with hysteresis and softening effects. Regional differences were found in the mechanical response and in the microstructure. The trigone region presents higher peak stresses and thinner muscularis layer compared to the rest of the bladder. Furthermore, the ventral side of the bladder presents anisotropic characteristics, whereas the dorsal side features perfect isotropic behaviour. This response matches the smooth muscle fibre bundle orientation within the tunica muscularis. This layer, comprising approximately 78% of the wall thickness, is composed of two fibre bundle arrangements that are cross-oriented, one with respect to the other, varying the angle between them across the organ. That is, the ventral side presents a 60°/120° cross-orientation structure, while the muscle bundles were oriented perpendicular in the dorsal side.

Statement of Significance

In the present study, we demonstrate that the mechanical properties and the microstructure of the urinary bladder wall are heterogeneous across the organ. The mechanical properties and the microstructure of the urinary bladder wall within nine specific locations matching explicitly the mechanical and structural variations have been examined. On the one hand, the results of this study contribute to the understanding of bladder mechanics and thus to their functional understanding of bladder filling and voiding. On the other hand, they are relevant to the fields of constitutive formulation of bladder tissue, whole bladder mechanics, and bladder-derived scaffolds i.e., tissue-engineering grafts.

1. Introduction

The urinary bladder (UB), as a musculomembranous hollow organ, is accountable for two main cyclic functions: to temporally store urine at a low-pressure level (passive phase) and to control micturition (active phase). During the passive phase of filling, the bladder is exposed to enormous deformations, thereby keeping the internal pressure nearly constant until reaching its maximal capacity [66]. Dependent on the species, the process of bladder filling takes several hours, whereas micturition occurs within a few seconds. Consequently, the passive filling phase is frequently assumed to take place under quasi-static conditions. Interestingly, even if the bladder bears in its maximum loaded configuration enormous deformations, its shape returns completely back to its reference state. This capability is due to the microstructure of the UB wall, consisting of several layers (from the inside out): tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. For more details on the microstructure, the interested reader is referred to Seydewitz et al. [58].

* Corresponding author.
E-mail address: m.boel@tu-bs.de (M. Böl).

https://doi.org/10.1016/j.actbio.2018.05.014
1742-7061/

Please cite this article in press as: E. Morales-Orcajo et al., Location-dependent correlation between tissue structure and the mechanical behaviour of the urinary bladder, Acta Biomater. (2018), https://doi.org/10.1016/j.actbio.2018.05.014

To understand the load transfer mechanisms inside the UB wall during the passive phase, mechanical experiments in combination with detailed information on its microstructural constituents, i.e elastin, collagen, and smooth muscle (SM) cells, as well as their distributions are essential. Furthermore, these data are suitable for three-dimensional continuum mechanical-based models [58], which in turn could help in understanding the load transfer mechanisms inside the UB wall, as some scenarios are feasibly computational but cannot be realised experimentally.

Two types of mechanical experiments can be found in the literature. The first type addresses the characterisation of the mechanical behaviour of whole bladders, and the second considers dissected UB wall tissue strips, to be tested uniaxially or biaxially, to determine appropriate material characteristics. To study the mechanical functions of the bladder, observations on whole bladders during the filling and micturition cycles are indispensable. Consequently, cystometry, a urodynamic testing technique, is the preferred method [2,65,68,55,19,38,49,63,7,34,54]. Experimental studies in this field range from analyses using classical cystometry to more advanced methods where the cystometrical setup is combined, e.g. with imaging techniques, such as ultrasonography. The main advantage of this testing technique is the fact that it can be realised *in vivo* and non-invasively. With this technique, it is possible to study the mechanical functions of healthy and diseased UBs, which enables cystometry to be applied daily in clinical practice.

However, besides the aforementioned advantage of cystometry, its disadvantage is that questions concerning location-dependent mechanisms of structural characteristics remain unanswered. For the determination of these location-dependent characteristics, experiments on dissected tissue strips are of high interest. Two types of experiments on tissue strips can be found. While uniaxial tension experiments have been frequently realised (e.g. [23,55,18,11,37,41,72,5,48,42,58]), the number of biaxial tension studies is significantly smaller [6,28,45,47,40,26,64,69,14]. Generally, uniaxial experiments can be realised much more easily than biaxial tension experiments. In doing so, a rectangular tissue strip is fixed into two mounting devices that are pulled apart from each other. Meanwhile, an optical system records the distance changes in markers applied on top of the specimen. Depending on the mounting device, axial or uniaxial deformation can be achieved. However, when determining the mechanical characteristics of anisotropic materials, single uniaxial tests are insufficient, and several, orientation-dependent uniaxial tests are needed to identify the anisotropic behaviour. Even if this seems logical from a mechanical point of view, only five of the aforementioned uniaxial studies realised orientation-dependent experiments [37,41,72,48,58]. Furthermore, for a more comprehensive understanding of bladder mechanics, especially in relation to its function, knowledge of location-dependent characteristics is of particular importance. Within the studies found on uniaxial experiments, only one focused on location dependency with respect to mechanical characteristics [37], detecting significant regional differences. In contrast to uniaxial experiments, biaxial tests need only one quadratic tissue strip to detect anisotropic material characteristics, supporting comprehensive tissue characterisation in case less tissue is available. Further, biaxial testing is a more meaningful deformation state as it mimics the physiological loading state of UBs better than uniaxial tests. Besides an early study by Baskin et al. [6] that applied inflation tests on bovine UB tissue and a recently published work by Cheng et al. [14] realising biaxial experiments on a rat bladder wall, to the best of the authors' knowledge, only one group around M. Sacks has broadly addressed experimental characterisation in terms of biaxial testing of UB tissue [28,45,47,40,26,64,69]. The authors applied biaxial tension experiments on tissues of different animals (rat, mice, pig) to determine the mechanical characteristics in healthy and diseased (spinal cord injury) bladders. However, even after an intensive literature research, location-dependent biaxial tests on bladder tissues are not available.

For a better understanding of UB mechanics, and also as a database for three-dimensional continuum mechanical-based models, as recently proposed by Seydewitz et al. [58], microstructural information, such as fibre orientations/distributions and the proportions of the tissue constituents (elastin, collagen, SM cells) are of high interest. In particular, the combination of mechanical and microstructural data allows for the discussion of load transfer mechanisms inside the UB wall and thus enhances the understanding of UB mechanics. However, studies that focus on the mechanical characteristics as well as on the determination of all aforementioned microstructural information could be not found. In fact, only single studies that address only parts of the aforementioned issues are available [45,46,26,64,37]. Studies combining mechanical and microstructural information to enhance the functional understanding of the bladder or to create compressive input data for mechanical modelling concepts could not be found. In this regard, it is worth mentioning the recent study of Cheng et al. [14] that combined biaxial mechanical testing with multiphoton microscopy, where they were able to match the percentage of collagen fibres straightened with the stress-strain curve of rat bladder wall specimens.

Within the present investigation, we examined the location-dependent mechanical and microstructural behaviour of porcine UB. To this end, equibiaxal tension experiments were performed on three regions (apex, body, and trigone, see Fig. 1 (c)) of the UB. Within each of these regions, the tissue was tested at three different locations. To interpret the resulting mechanical responses, microstructural information was additionally determined at the identical locations. Consequently, histological analyses were conducted, and the main constituents of the UB were determined to be SM cells, extracellular matrix (ECM, including collagen and elastin), and fat as well as the location-dependent smooth muscle fibre bundle orientations.

2. Materials and methods

2.1. Urinary bladder and sample dissection

In this study, twenty-eight ($n = 28$) urinary bladders of domestic pigs (*Sus scrofa domestica*), that were 3 to 5 months old and about 90 kg in weight, were obtained from a slaughterhouse immediately after animal sacrifice and transported within 20–30 min to the laboratory. During the transport, preparation, and handling, the organs/tissues samples were directly stored in calcium-free Krebs solution ([50]: 113 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 5.9 mM dextrose, 3.3 $CaCl_2$, 1 mM EGTA) at 4 °C. The calcium-free solution was used to prevent spontaneous contractions, especially during biaxial testing, as they would distort the results.

Prior to mechanical testing, the UBs were measured and weighed in their deflated, unstretched state, cp. Fig. 1(c). The average sizes were 99.9 ± 10.1 mm in projected length (L_{long}) and 66.8 ± 6.2 mm in projected width (L_{circ}), and the mean weight was found to be 51.9 ± 9.9 g. Within the animal body, the UB is mounted on the *Ligamentum vesicae laterale* and *Ligamentum vesicae medianum* proceeds along the longitudinal axis of the bladder [8,29], see also Fig. 1(a) and (b). Along these ligaments, the bladder was sliced, dividing the bladder into three tissue parts indicated by D (dorsal) and V (ventral), cp. (d). From each part, three square samples (20×20 mm) were dissected, matching their orientation with the longitudinal and circumferential axes of the bladder and labelled as D_i and V_j, respectively. Overall, ninety ($n = 90$) samples were used during this study, one-half for mechanical (Section 2.2) and the other half for histological (Section 2.4) analyses. For the

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