

Bladder Microstructural and Biomechanical Modelling: in Vivo, in Vitro and in Silico



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By **Jack Hornsby, Wolfson College**

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Abstract

Lower urinary tract disorders are significant prognostic indicators of institutionalisation and lower quality of life in the elderly and their incidence increases with age. Urodynamics, the gold standard in diagnosis, replicates symptoms to assess functionality through controlled filling and voiding of the bladder but its interpretation is subjective and may be inconclusive; often requiring further testing or leading to inappropriate treatment. Normal filling and voiding biomechanics of the bladder relate directly to the structural composition of the bladder wall. Alterations to tissue composition in aging and pathology have significant impacts on biomechanics but are yet to be fully described.

The aim of this thesis was to gain insight into the individual microstructural components of the bladder wall and how they relate to the gross mechanical response. Additionally, representation of these observations in a mathematical model that can be used to improve our understanding of urodynamic data. This aim was achieved through a combination of in situ mechanical testing and the development of a microstructural constitutive model, which was then included within an overall micturition framework to simulate filling and voiding functions, and evaluated with clinical data.

Coupled systems of multiphoton microscopy and uniaxial, biaxial and inflation testing were used to correlate extra cellular matrix interactions with the mechanical response

of young and aged murine bladder. Wall-layer specific collagen fibre orientation, dispersion and recruitment were quantified and implemented into a novel microstructural constitutive model. The bladder was modelled as a nonlinear elastic, constrained-mixture planar membrane with contribution from smooth muscle and collagen fibres in the detrusor. Collagen recruitment in the detrusor was observed to occur at a finite stretch; correlated with a steep increase in stiffness of the tissue, while collagen of the lamina propria plays a capacitance role. Collagen recruitment was modelled using a triangular probability density function; quantified from sequential microscopy images and fitted to mechanical data. Increased collagen area fraction and changes in dominant fibre orientation were attributed to reduced compliance in aged bladder. This behaviour was captured by the model.

The microstructural model was modified to an isotropic thin-walled spherical membrane for the filling phase of a micturition model framework, consisting of a bladder outlet relation and urethral resistance relation. A contractile smooth muscle element was included in the active response. In the first steps towards clinical application the model was applied to male and female 'normal' patient urodynamic data to observe quality of model fit and estimate baseline parameter values. The model simulated key filling and voiding features seen in normal male and female clinical data.

Mechanobiological modelling combined with clinically relevant micturition modelling has the potential to quantify bladder dysfunction. Moreover, improved understanding of how the microstructure influences macroscopic mechanics will yield improved understanding of how changes to the bladder impair its functionality. We predict that modelling will become a clinically relevant tool in urodynamics; leading to new options for diagnosis and management of patients with bladder dysfunction.

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Author Declaration

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at the University of Oxford or any other educational institution, except where due acknowledgement is made in the thesis.

Any contribution made to this research by others, with whom I have worked at the University of Oxford or elsewhere, is explicitly acknowledged.

Author Dissemination

Peer-reviewed Conference Proceedings

- **Hornsby J.**, Chen H., Thompson M. S. and Watton P. N. A Novel Mathematical Model for the Microstructural Adaption of the Collagen Fabric during Aneurysm Evolution, 7th World Congress of Biomechanics, Boston, USA, 2014.
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- **Hornsby J.**, Daly D., Grundy D., et al. A Multiphoton Microscopy Study of Murine Urinary Bladder Morphology and Quantification of Microstructure during Deformation (*in draft*)

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Acronyms

MPM	Multiphoton microscopy
SHG	Second harmonic generation
TPEF	Two photon excitation fluorescence
LUT	Lower urinary tract
ECM	Extracellular matrix
DU	Detrusor underactivity
DO	Detrusor Overactivity
BOO	Bladder outlet obstruction
PFS	Pressure flow study
PMT	Photon multiplier tube
SD	Standard deviation
PDF	Probability density function
SMC	Smooth muscle cells

Introduction

1.1 Clinical Motivation

The incidence of lower urinary tract symptoms, indicative of bladder outlet obstruction, detrusor disorders and urinary incontinence, increases with age in both men and women. The healthcare implications of this are huge as an estimated 15-30% of people over the age of 60 are affected. These disorders impair the normal function of the bladder, causing significant morbidity, and dramatically lower quality of life for those affected.

Diagnosis of patients presenting with urinary tract related symptoms typically involves an invasive, time consuming and costly procedure called urodynamic testing. Urodynamic studies, or pressure-flow studies, provide a method of quantifying the mechanical functionality of the lower urinary tract by replicating filling and voiding functions. They allow quantification of vesical and abdominal pressures, and a measure of flow both to and from the bladder through the use of a urethral and rectal catheters (See Figure 1.1). An estimate of bladder working function in terms of detrusor contractility, bladder wall compliance, urethral 'resistance', maximum capacity and patient urge to void can be obtained. Often these studies may be combined with electromyography or

x-ray imaging to obtain further information.

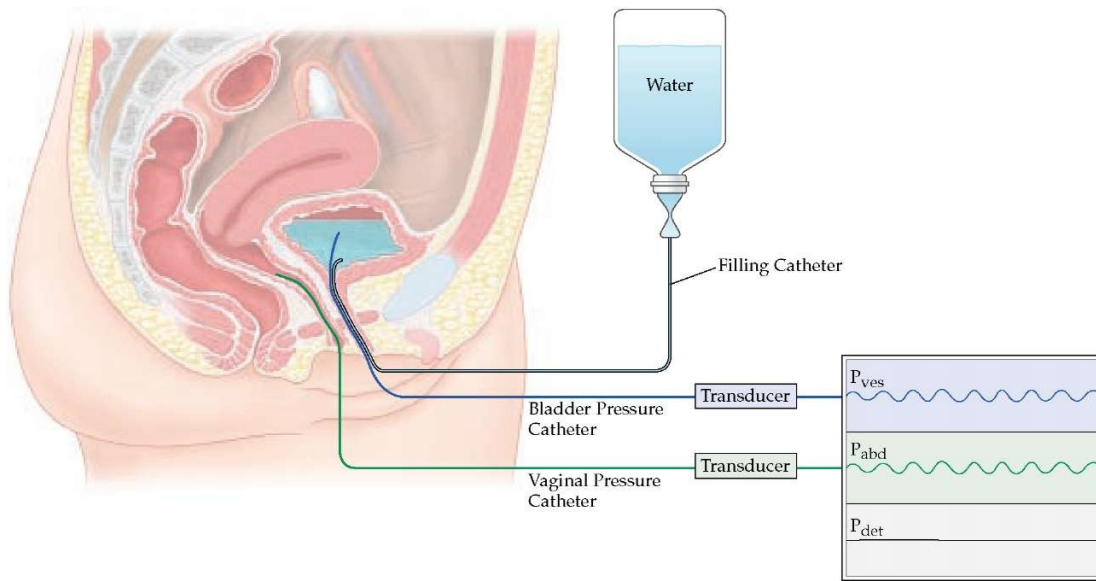


Figure 1.1: Overview of urodynamic study procedure. Image on the left shows catheter insertion into the bladder via the urethral to obtain vesical pressure. A rectal catheter obtains abdominal pressure (not shown). The bladder is filled gradually until patient indicates different levels of fullness and urge to void, where tests such as cough tests may be carried out. Flow and pressure are then measured during voiding, an example trace of which is shown on the right with accompanying x-ray imaging. Sourced from Women's Excellence Ltd (Obstetrics and Gynaecology).

Limitations of Urodynamic Studies

Whilst urodynamics are currently the gold standard of clinical diagnosis, these invasive tests are highly uncomfortable and do not always reveal underlying disorders responsible for patient symptoms, or may not replicate symptoms; resulting in costly follow-up procedures, and/or inefficient treatment. These issues raise the question of whether mathematical modelling can be employed as a predictive tool for lower urinary tract disorders; with the aim of increasing diagnostic success, reducing follow up frequency, or replacing invasive procedures with non-invasive alternatives.

There are two approaches available for mathematical modelling; mechanistic and machine learning; this work will focus on the former. A machine learning type approach

uses historical data to make assumptions about data characteristic. This is applicable when large data sets are available, which is often problematic when dealing with urodynamic data. Often these databases do not exist, or require a huge amount of effort to process data into a usable state. Also, they suffer from the disadvantage of being less patient specific, as previous data will typically be gathered from a group or sub-group, rather than of a sole patient. A mechanistic approach intends to model the different mechanical inputs of the system - in this case the different components of the LUT. This has the advantage of being able to be tailored to a specific patient and their individual parameters and does not require historical data of the patient. These models will however, require quantification of the mechanical constants and parameters to populate the model. These may be difficult to obtain, or require simplification or assumptions to be made; reducing the accuracy of the model.

To date there has been limited effort directed towards the sophistication of constitutive modelling of bladder tissue. For example, models are typically simplistic phenomenological lumped parameter models and lack any detailed information of the tissue structure. Considering that the primary method of clinically diagnosing LUT disorders relies upon mechanically deforming the bladder using urodynamic tests it would be logical that understanding of the material properties would improve interpretation of pressure, flow and volume curves obtained from clinical studies.

1.2 The Bladder

The urinary bladder is a hollow organ composed of smooth muscle and membranous components located in the pelvic cavity and sits upon the pelvic floor muscles. The bladder's main function is the storage of urine that flows from the kidneys via the ureters at low pressure. During normal filling the detrusor relaxes to allow for storage

of urine at low pressures, typically in the region of $20 \text{ cmH}_2\text{O}$ at maximum capacity [1]. At peak capacities of approximately 400 - 500 mL, there is a perceived urgency to void. Following this micturition reflex may be voluntarily initiated or suppressed until suitable voiding conditions are met; allowing emptying of the bladder [2]. A neurologically coordinated contraction of smooth muscle cells (SMC) and relaxation of the urethral sphincter allows voiding of the stored urine via the urethra. Healthy voiding should result in little or no residual urine volume.

The bladder can be divided into three anatomical regions (See Figure 1.2); the apex (dome), the body (mid-region) and the fundus (base) which consists of the trigone and neck [3]. Ureters connect to the adjacent sides of the bladder and supply the bladder with urine from the kidneys. The neck of the bladder opens to the urethra, the opening of which is restricted by a sphincter; preventing leakage in healthy states.

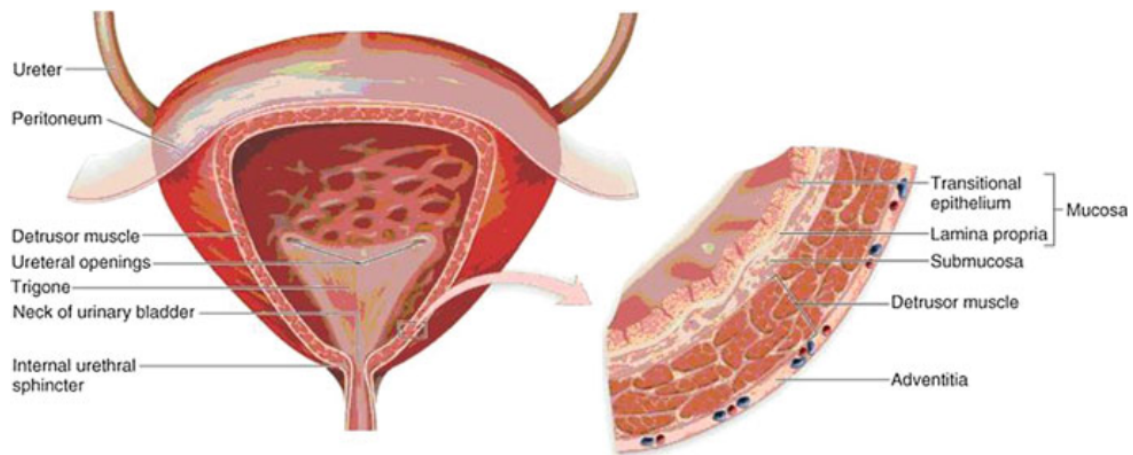


Figure 1.2: Anatomy of the human bladder and the various anatomical layers. The inner layer, the urothelium, provides a protective interface between the urine and rest of the bladder wall. Beneath, the lamina propria in the mucosa forms an undulated structural layer mainly consisting of connective tissue. The detrusor is a thick layer of smooth muscle that forms a majority of the bladder wall and is responsible for active relaxation and contraction during filling and voiding. On the outer surface there is a thin adventitial layer mainly composed of connective tissue.

1.2.1 The Bladder Wall

During filling the bladder must be very compliant in order to store large volumes of urine at a low pressure. The unique composition of the bladder wall constituents permit large deformation and a dramatic thinning during filling due to the highly elastic nature [4]. The urinary bladder wall has four distinguishable layers: the mucous, submucous, detrusor muscle and serosal (or adventitial) layer. The mucosa, consisting of the urothelium, is the innermost, non-permeable lining of the bladder [5]. It consists of a layer of umbrella cells with a deeper layer of cuboid and columnar cells that create a non-permeable barrier to the urine and maintain composition of urine. It has a highly undulated formation (rugae) which allows for large deformation to accommodate large variations in stored urine volume [3]. The hexagonally shaped umbrella cells form the primary interface with the urine and may help prevent bacteria attachment and diffusion of urine contents across the membrane [6]. The ability of umbrella cells to dramatically change their apical surface area allows them to retain a barrier function during filling and voiding [3]. The lamina propria interfaces with the mucosa and is also undulated. This layer is a densely woven network of collagen and elastin fibres with a vascular network. The role of this layer is to limit the extent of distension that the bladder is able to undergo. It has been shown that removal of this layer results in significantly higher distension [4]. Myofibroblasts are the prominent cell type in the lamina propria and play a key role in communication between urothelium and detrusor muscle layer.

The mid-wall muscular layer, muscularis, is the most substantial layer of the bladder wall. It consists of three layers of SMCs in humans (and two layers in rat), the only smooth muscle in human body which allows for voluntary control [7]. This formation of smooth muscle makes up the detrusor muscle, which during relaxation and contraction permits storage and voiding of the urine, respectively [8]. On the outer surface of the

detrusor, the serosal or adventitial layer forms a visceral peritoneum of the urinary bladder on a single side and consists of a dense network of undulated collagen fibre bundles piled in a sheet like array [9].

Collagens contribute to an average 57.4% of the total weight of the bladder and play a large structural role as the main matrix scaffold protein in the bladder wall [10]. Collagen type I is the most abundant structural protein in bladder extra cellular matrix (ECM) and type III, which co-localises with type I, is also distributed throughout the bladder wall [2]. Together types I and III make up approximately 80-99% of the total collagen content. Other collagens present in the urinary bladder include types IV and V, making up approximately 1-2% of the total collagen content [4]. These collagens are biosynthesised from the production of procollagen; a precursor form of collagen. This is modified by hydroxylation of lysine and proline amino acids by specific enzymes and allows formation of the characteristic triple helix structure. The next step involves glycosylation of the amino acids by addition of sugars, such as glucose. Finally, as the procollagen is secreted from the cell the propeptide extensions are cleaved, leaving collagen molecules to be formed into fibres [11].

The main function of collagen fibres is transfer of load borne by the muscle contraction throughout the rest of the bladder wall. The method of collagen load bearing in bladder wall tissue is not yet well understood [4]. Under low loads, i.e. when the bladder is empty, collagen arranged in lamina propria and around the detrusor forms a dense, loose network of coiled fibres with no uniformity in direction. As the bladder begins to fill, collagen fibres gradually unfurl to become fully straightened at maximum physiological distension [2]. The arrangement of collagen in this manner allows the layers of the bladder to undergo large deformations in coordination with the smooth muscle and also protects the organ from over-distension when filling [9]. Elastin is found in small quantities throughout the vasculature of the normal bladder wall, although small

amounts are distributed throughout the lamina propria, serosa and form a loose network around the muscle fascicles. It is these fibres that are thought to contribute to the higher elastic nature and allow shape recovery even after large distension. Elastin fibres found in the serosal layer form a network that lines the peritoneal mesothelium (abdominal lining). These, however are insufficient to contribute mechanically to bladder compliance [9].

1.2.2 Mechanical Properties of the Bladder Wall

The unique ability of the bladder to store large volumes of urine at low pressures is directly related to the structural composition of the bladder wall tissue. These properties also have a significant impact on the functionality of the organ, as well as regulating hypertrophy and remodelling through cell signalling systems [12]. The mechanical response of bladder wall tissue is multi-phasic and characteristic of soft collagenous tissue (See Figure 1.3).

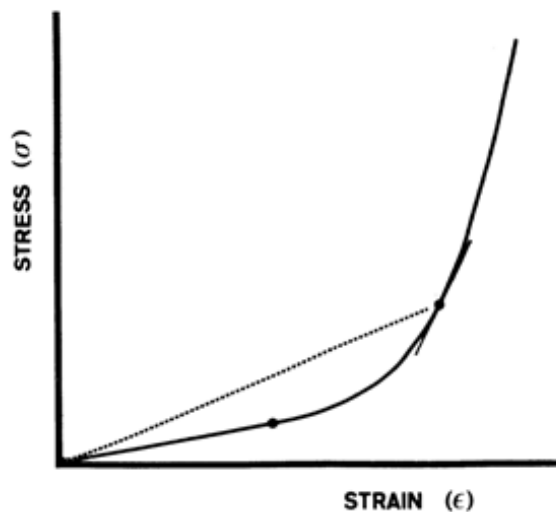


Figure 1.3: Typical stress-strain response for soft collagenous tissues, such as bladder wall tissue.

The structure of the anatomical layers of the bladder wall and their contribution to

loading bearing is critical in understanding the fundamental structural role of the constituents within each layer. A study by [4] investigates the response of the individual bladder wall layers during filling at 0%, 25%, 50%, 75% and 100% of capacity. This study reveals that different anatomical layers will influence the mechanical behaviour at varying levels of stretch. For example, it was shown that the detrusor acts predominantly as a capacitance layer, i.e. allows for large changes in geometry, between 25 and 50 % of capacity as the lamina propria is the main load bearing layer. Towards higher stretches of 50 - 100 % the lamina propria continues to bear tension, however is limited from further distension by the detrusor and collagen bundles creating a girding effect. The result of this is a rapid increase in stress / intravesical pressure towards physiological capacity.

The orientation of smooth muscle and its impact on mechanical behaviour in rat bladder wall tissue is investigated by [13]. Smooth muscle is typically arranged aligned with the longitudinal axis giving a much stiffer response in the longitudinal direction when compared to the circumferential axis. However, upon injury (in this case, 1.5 weeks post spinal cord injury) and subsequent remodelling of the smooth muscle to a more isotropic orientation the stiffness of the bladder becomes almost equal in both directions. The authors conclude that remodelling and reorientation of bladder wall constituents dramatically influences the mechanical response of the tissue. The same authors also note that the stiffness in samples correlated closely with the collagen and elastin content, i.e. samples with decreased compliance exhibited increased collagen / elastin production.

1.2.3 Mechanical Testing

To fully understand the mechanical properties of the bladder wall, the tissue must be isolated and constrained in such a way that all forces acting on the material are known. Studying the bladder wall in vivo has the inherent issue of local organs and connective tissues that may influence the mechanical behaviour of the bladder. Whilst, this provides a more accurate representation of the overall LUT, it lacks the control that in vitro testing provides. Therefore, in vitro mechanical testing using isolated samples, where dimensions and regional thickness can be measured, is a valuable method of obtaining material properties.

Mechanical testing has been utilised to study human [14], rat [15], rabbit [16], and porcine [17] bladder wall tissue. [17] uses uniaxial testing to quantify variations in mechanical behaviour between the various regions and anatomical planes. In all regions tissue loaded in the circumferential direction is reported to be considerably less stiff than when loaded longitudinally. This observed difference in compliance is associated with elastin orientation at lower strains and collagen at higher distensions.

Mechanical testing has limitations in describing the mechanical response of tissue. Gripping of tissue, particularly smaller samples, results in stress concentration local to the gripped edge. Cutting of the tissue strips from the bladder results in fibre reorganisation, particularly at edges and may influence mechanical response to loading direction. Inflation testing somewhat limits these issues. This method has been used as the predominant method of mechanical testing for bladder wall tissue [18] [19] [20], and has the benefit of having direct similarities to clinical cystometrogram. A study by [20] identifies the elastic properties of the urinary bladder in dogs over large stretches and provides a comparison between uniaxial and inflation testing. It shows good correlation between mechanical model constants derived in both testing protocols, although

loading direction and anatomical region are not considered [20].

Biaxial testing has previously been used to test ex-vivo bladder strips [21] [15]. In this method the bladder is subjected to multi-axial loads in-vivo (transmural pressure and in-plane stresses in 2 orthogonal directions). Biaxial testing is able to recreate three dimensional loading of a thin planar membrane when assuming incompressibility; giving advantages over uniaxial testing. The challenge associated with biaxial testing, similar to uniaxial testing, is the gripping method. Various methods have been proposed, including independent sutures and hooks [15] [22] and metal clamps [17]; the advantage of individual hooks / sutures being freedom of movement in lateral directions. However, inserting multiple hooks and tensioning individual sutures is an extremely complex and delicate procedure that if not carried out carefully will cause damage to the tissue. Additionally, the time taken to insert all the hooks will be relatively long, meaning degradation of tissue may occur. Metal grips may alleviate some of the time taken, however they suffer from lack of lateral movement and incorrectly constraining the material, particularly with such small samples. An improved method of clamping would therefore be beneficial to improve results.

1.2.4 Collagen Structure and Mechanical Properties

Collagen plays a fundamental role in tissues as the most abundant structural protein. It has a complex, multi-hierarchical structure over several length scales from nano-scale polypeptide chains to microscopic collagen fibres.

Collagen fibrils have a high stiffness modulus, with values reported between 0.4 - 0.5 GPa at low strain, and do not stretch under normal physiological loading [23], [24]. At higher strains the stiffness modulus approaches 3-12 GPa and is closer to the stiffness of the individual collagen molecules. A potential mechanism for stretch is deforma-

tion of the binding linkages, i.e. crimping and extension of glycosaminoglycan (GAG) crosslinks. D-spacing of the collagen fibrils are observed to increase under extension , however only accounts for approximately 40% of extension [24].

Collagen fibres embedded in soft tissue will accommodate for stretch as the fibres adopt an undulated / crimped state when the tissue is in an unloaded state. This allows for gradual straightening of the fibres as the tissue is stretched. The finite stretch point at which the collagen fibres are straightened, and subsequently recruited into bearing load, is known as the recruitment stretch [25]. Extensibility of various tissues are directly dependent on the crimp amplitude and wavelength of the collagen fibres. It is seen in arteries [26] [27] and tendon [24] that at the point of collagen fibre recruitment there is a dramatic stiffening of the tissue. A similar mechanism is reported to occur in bladder (See Figure 1.4) [2].

1.2.5 Urinary Bladder Smooth Muscle and Innervation

Smooth muscle contains contractile proteins; actin and myosin. These act together to allow contraction and relaxation of the muscle by synchronous shortening and lengthening of sacromeres (unit of contractile apparatus). Actin filaments slide past each other with force generated from myosin cross bridges, thereby contracting the SMC [28]. As the bladder fills bladder wall tension, detrusor pressure and intravesical volume increase. Subsequently, afferent nervous signals sent from the bladder wall and urothelium become increasingly intense. At a certain intensity, the afferent signal will trigger a switch in nervous response as efferent signalling from the spinal cord activates the sacral region [29]. At the point of micturition the nervous system will reduce sympathetic input and revert to a parasympathetic drive. Parasympathetic activity will cause release of transmitters; acetylcholine and adenosine triphosphate, causing rapid transient and

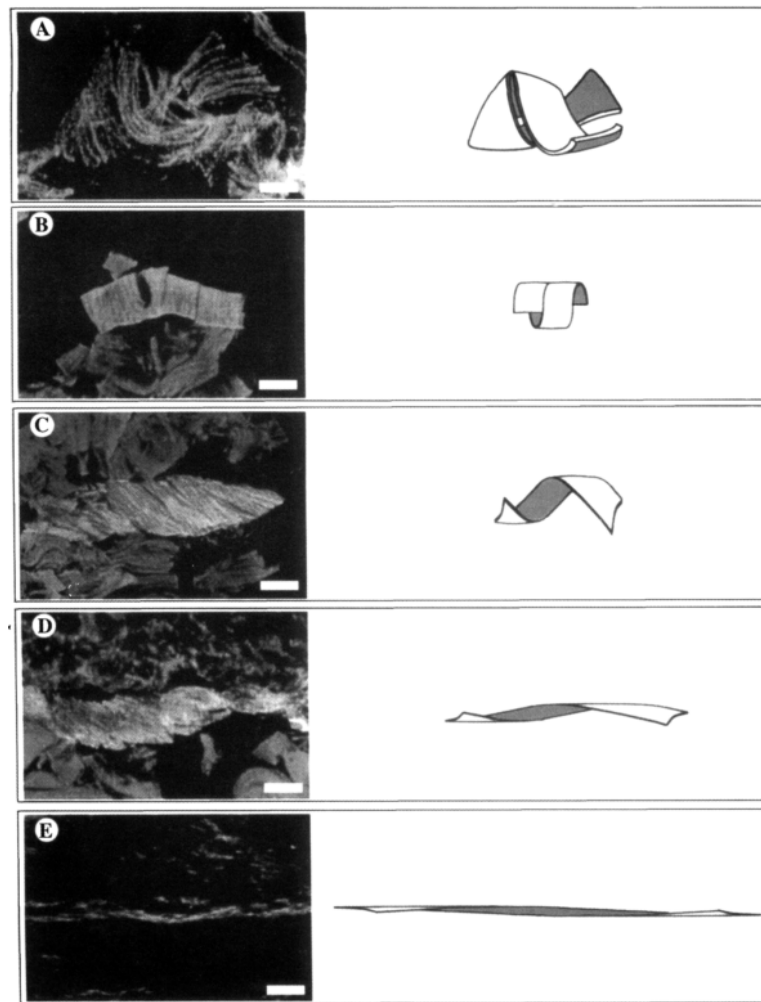


Figure 1.4: Confocal micrographs of collagen fibre straightening in urinary bladder during filling phase, [4]. Reproduced with premission from Taylor & Francis.

prolonged increases in intracellular calcium, respectively. Release of acetylcholine into the dome and body of the bladder causes contraction of the smooth muscle, increasing intravesical pressure. Muscarinic receptors in the neck then allow relaxation in the neck, allowing for opening of the bladder outlet and flow of urine [30]. Until the bladder is emptied afferent signals persist to maintain voiding.

The conscious decision to void is controlled by forebrain processing of afferent signals and other environmental conditions. In the case of most healthy individuals voiding

will typically be suppressed until a suitable location is found and voluntary voiding commences [3].

During the voiding phase the bladder will undergo a large change in volume from full capacity to almost empty within several seconds. Consequently, the mechanical properties of the detrusor muscle change greatly as the geometry of the bladder changes [31]. In 1894 Magnus Blix demonstrated that tension produced by skeletal muscle increased with length up until a certain extent. Above this optimal muscle stretch tension would decrease, giving rise to the well known parabolic active length-tension response. This effect was later attributed to the extent of actin and myosin filament overlap within muscle sarcomeres; a phenomenon also seen in smooth muscle. The function of the detrusor muscle is therefore dependant upon the level of stretch at which the active response is evoked and ultimately affects the voiding function of the bladder.

To date, various studies have examined the force - length relationship of isolated contracting detrusor muscle strips in pigs [31], and rats [32], [33]. However, smooth muscle in the bladder is seldom orientated in a single direction, but rather a mesh of circumferential, longitudinal and oblique layers. Additionally, contractile properties differ according to region of the bladder due to receptor concentration differences along the longitudinal axis of the bladder; limiting the usefulness of uniaxial studies [34].

The urinary bladder receives innervation from the hypogastric nerve and pelvic nerve which provide a dual innervation system of sympathetic (adrenergic) and parasympathetic (cholinergic) fibres, respectively [35]. This system of innervation targets various receptors located with varying densities across the broad anatomical regions of the bladder wall; neck, body and dome. There are three main receptor types; α -adrenoreceptors, β -adrenoreceptors and muscarinic cholinergic receptors.

The filling phase is largely governed by the sympathetic nervous system to maintain a basal tone in the bladder neck and urethra. Smooth muscle in the neck predominantly has α -adrenoreceptors; responsible for contraction during storage to seal the bladder opening. Sympathetic nervous system also allows relaxation in the dome and body of the bladder via β -adrenoreceptors; maintaining a low intravesical pressure as volume increases [10].

1.2.6 Overview of Bladder Disorders

Disorders of the LUT each present a range of symptoms that may not be specific to each disorder. Often, but not exclusively, these disorders are present in elderly people; and may be as a result of LUT deterioration. There exist sex specific differences between the male and female bladder and proximal anatomy. The prostate gland in males, neighbouring the lower urinary tract, has potential for obstruction in disease in the case of prostate hypertrophy. The pelvic floor in women supports the bladder and may become damaged or weakened during childbirth, may give rise to problems such as stress incontinence [36]. A brief overview of the prevalent LUT disorders is given in this section.

Incontinence

Urinary incontinence (UI) typically occurs in elderly persons, although occurrence in younger people is largely caused by alterations to the LUT and its innervation [37]. Two types of UI may present; urge and stress. The main symptom indicative with urge UI is the overwhelming, and often sudden, urgency to void and is often associated with involuntary contractions arising from detrusor overactivity. Impaired sphincter function and increased intra-abdominal pressure are symptoms shown by stress UI. Stress UI is particularly prevalent in middle aged and elderly women, with approximately 13% of

the female population affected with a lifetime risk of surgical treatment for UI around 4% [38].

Bladder Outlet Obstruction

Bladder outlet obstruction (BOO) is a commonly seen disorder in men and women, although underlying etiologies (functional and anatomic) are usually gender specific. Regardless of etiology, BOO increases the resistance to flow or compression of the bladder outlet from anywhere between the bladder neck and meatus of the urethra. Functional obstructions may arise due to sphincter-detrusor dys-synergia, primary bladder neck obstruction, anatomical obstruction due to prostate enlargement (in males) and SUI (in females). Symptoms of an obstructed urinary tract include; hesitancy, low urine flow, sensation of incomplete voiding and residual volume / leakage. Symptoms may also be irritative and may present as nocturia, dysuria, urgency and increased frequency. Additionally, BOO may be symptomless and only become apparent if urinary retention or upper urinary tract decompensation occurs [39].

Morphological growth and remodelling can occur in response to obstruction. Increased pressure is required to overcome the blockage to flow; triggering a rapid hypertrophic response of the detrusor muscle to compensate for the increase power required to generate this pressure to maintain normal voiding function [40]. Untreated obstruction can cause further decompensation of the bladder wall, leading to dysfunction and subsequent loss of contractility and increased post void residual volume; indicative of urinary retention. If the obstruction is removed in a timely fashion, the hypertrophic state may be reversed and normal function restored [41]. Additionally, changes may occur in the ECM as collagen content increases with bladder mass. Prolonged cases may prompt fibrosis of the bladder wall and subsequent loss of compliance [42].

Detrusor Disorders

Disorders of detrusor activity typically present as under or over activity of the muscle and may impact both the voiding and storage functions, respectively. Symptoms of detrusor overactivity (DO), also known as bladder overactivity (BOA), are common in the elderly population and include increased sensation to void at low bladder volumes, and involuntary contractions of the bladder that may cause leakage. However, studies show that positive indicators of overactive bladder used in a clinical setting are approximately 50 %; particularly as detrusor overactivity is common in elderly urodynamic studies [43]. Urodynamic studies can be used to identify the underlying remedial causation of some detrusor activity disorders; such as BOO and SUI. Treatment of these may result in a secondary solution of the detrusor overactivity.

Detrusor underactivity (DU) is also prevalent in elderly people and is defined by the bladder producing insufficient contractile force or contraction duration to complete voiding [44]. Symptoms of reduced contractility include loss of flow rate, increased post-void residual volume; symptoms also seen in BOO. As a result it may be difficult to distinguish between DU and BOO without the need for invasive urodynamic studies. Due to the lack of insight into DU and its underlying etiology; it lacks detailed literature, clinical definitions and accepted clinical diagnostic criteria; other than low pressure during voiding with post-void residual volume [45]. The pathological conditions required to cause DU are not yet fully realised, however it is said to require a broad deterioration of the SMCs and nervous function in the bladder wall [46].

1.2.7 Clinical Evaluation of Bladder Function

The gold standard clinical assessment of bladder health is urodynamic studies. The purpose of these studies is to replicate urinary symptoms presented by the patient and to objectively distinguish the underlying disorder responsible, and subsequently select

the most appropriate form of treatment. Typically, urodynamic studies are used to differentiate between normal bladder function and bladder outlet obstruction, detrusor underactivity, urinary stress incontinence and detrusor overactivity.

One method of evaluation is a cystometrogram. This is an invasive test to measure the intravesical pressure (measure of abdominal vs. detrusor pressure) as a function of volume during filling. A double-lumen catheter is inserted into the bladder via the urethra through which the bladder is filled. Mechanical compliance and capacity of the bladder are compared to a standard to identify non-compliance as a result of various maladies [47]. Replication of voiding function disorders is clinically assessed using pressure-flow studies (PFS). Patients are asked to void under controlled conditions following a cystometrogram.

There are also non-invasive urodynamic techniques, such as external condom and penile cuff methods, which measure isovolumetric bladder pressure through free flow rate of urine upon voiding. Variation in urine flow can be distinguished and associated with obstructive and detrusor abnormalities; although diagnosis using these methods are often insufficient to identify specific disorders [48].

In conjunction with urodynamics there are various nomograms that have been proposed as a tool to categorise patients with varying degrees of BOO and detrusor contractility (See Figure 1.5) [49]. These nomograms use relationships between pressure and flow, often the gradient between the two (or a conjugation of the two), to distinguish level of obstruction. The underlying assumption here is the detrusor is a limited power source and the urethra 'splits' this work between pressure and flow; governed by the urethral resistance, or outlet size. For example, a small outlet (or high resistance) results in high pressure with low flow; and vice versa for a larger outlet. Nomograms such as the Schafer nomogram [50] looks to categorise strength of detrusor contraction in addition to grade of obstruction.

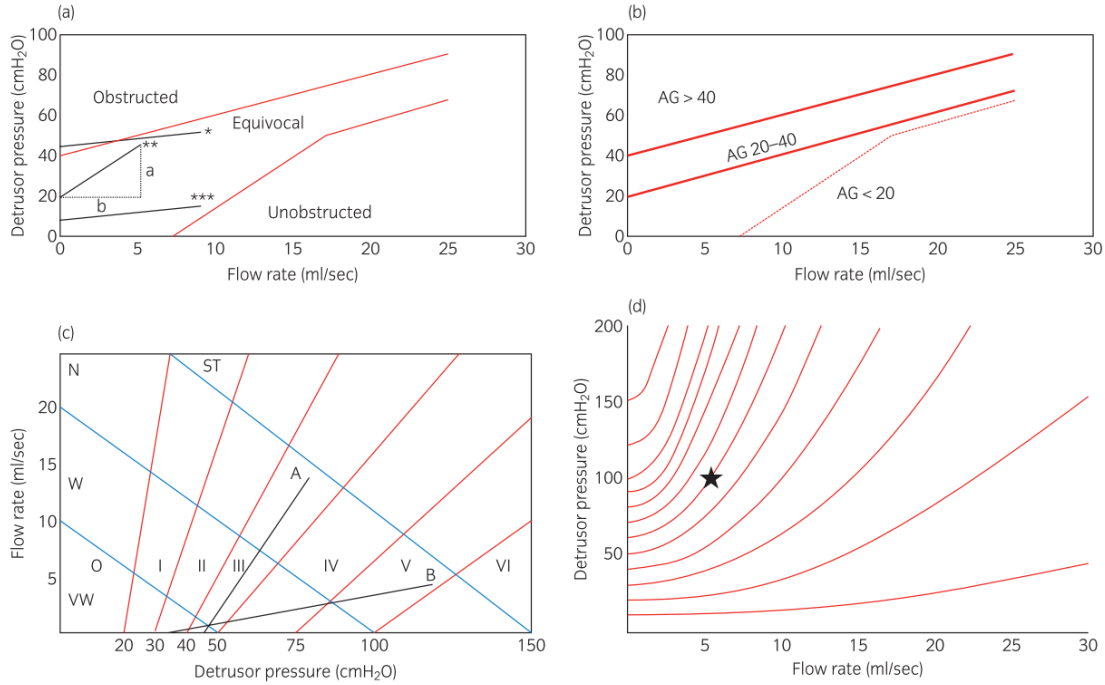


Figure 1.5: Overview of existing nomograms used to categorise patients. a) Abrams-Griffiths (AG) nomogram used to distinguish between obstructed, equivocal and normal outlet by calculating gradient of pressure vs. flow. Slope of line a/b where a is $P_{det@Q_{max}} - p_{op}$ (detrusor pressure at max flow and opening pressure of urethra) and b is Q_{max} (maximum flow). Obstruction present is $p_{op} > 40\text{cmH}_2\text{O}$ or gradient > 2 b) ICS nomogram is a modified AG nomogram with the addition of the line dividing equivocal and unobstructed c) Schafer nomogram grades level of obstruction into 7 categories and compares with strength of detrusor contraction d) URA nomogram takes a moment during voiding and gives a value of urethral resistance at that time as a measure of obstruction. *Modified from [51].* Reproduced with permissions from John Wiley and Sons.

1.2.8 Urodynamics and Clinical Parameters

Urodynamic studies are an invasive procedure used to diagnose LUT disorders by measurement of physiological parameters. Urodynamics aims to elucidate the specific LUT disorder responsible for a patient's symptoms by reproducing the symptoms under controlled conditions. Catheters inserted into the bladder, via the urethra, and into the rectum record pressure and flow measurements. Detrusor pressure is calculated as the difference between intravesical pressure, recorded by the bladder catheter, and the ab-

dominal pressure, as recorded by the rectal catheter. Once catheters are inserted, the bladder is filled with warm saline solution at a controlled rate. Patients indicate when they have feelings of urgency and a need to void. Once capacity is reached, cough tests will typically be performed to evaluate leakage before the patient voids. Flow, volume and pressure measurements will be recorded during voiding. Diagnosis of various disorders may be based upon evaluation of physiological parameters obtained from various urodynamic studies. The types of invasive studies performed are discussed here.

Filling Cystometry

Cystometry is used to assess storage of the bladder and measures the detrusor pressure during controlled filling. The patient will indicate at any time when feelings of urgency (first and strong desires to void) occur and whether symptoms are reproduced. Clinical parameters that are evaluated with cystometry include bladder capacity (V_{max}), compliance, patient urgency and signs of detrusor overactivity [43]. Additional tests, such as cough tests, straining or listening to water may also be conducted to check for involuntary leakage.

Detrusor underactivity is a disorder which can be distinguished by cystometry as voiding or leakage during filling may indicate the detrusor muscle is contracting involuntary. Incontinence is also described by involuntary leakage, however has characteristic high abdominal pressure in the absence of detrusor contraction.

Pressure-Flow Studies

Voiding function of the bladder is assessed using PFS by measuring detrusor pressure (P_{det}) and rate of urine flow (Q) during micturition. PFS are typically utilised to distinguish between the underlying cause of poor urine flow, whether that be detrusor underactivity or BOO. The parameter, $P_{det}@Q_{max}$ is the most important parameter when characterising flow issues. For example, BOO is characterised by low flow rate

and high detrusor pressure, whereas DU displays low detrusor pressure with similarly low urine flow rate [45].

Urethral Pressure Profile

A further examination of the urethra and its closing function can be evaluated by urethral pressure profiles (UPP). A pressure sensor catheter is drawn along the length of the urethra to obtain a pressure profile. The closure function of the urethra can be approximated by the maximum urethral closure pressure (*MUCP*); calculated as the difference between maximum detrusor pressure and urethral pressure.

Urodynamic studies are a valuable tool in evaluating LUT function and diagnosing disorders. However, the procedure faces challenges when distinguishing disorder characteristics across a range of ages and genders [52] [53]. For example, parameters used for assessment in certain populations are unlikely to be universally applicable across all genders and ages; meaning there is often ambiguity when making diagnoses. Diagnosis of BOO in women is particularly controversial as symptoms will not always show on pressure-flow traces; meaning BOO is often underestimated. For example, classic indications of BOO are low maximum flow rate and high voiding detrusor pressure. However, thresholds for these are not clear-cut, potentially leading to follow up examinations or an incomplete diagnosis [52].

Invasive urodynamic studies are time consuming and expensive. In addition they are uncomfortable and often embarrassing for patients. Despite this, approximately 74 to 95 % of patients would repeat a urodynamic study if it were necessary for medical diagnosis [43]. With these issues in mind, it is beneficial to reduce the number of follow up procedures a patient may have to undergo. Therefore, the motivation behind progression of mathematical modelling becomes clear.

1.2.9 Overview of Active Model Components

Micturition is the result of multiple systems acting in a coordinated fashion, each with a specific role. Additionally, the LUT is a complex system to model subject to many external factors, such as coughing / sneezing, cold temperatures, nervousness and many more [36].

The LUT can be divided into the external sphincter, urinary bladder, urethra and a sympathetic / parasympathetic nervous system. As such, the various components of the LUT will be broken down and discussed in the following sections.

The Bladder and Urethra

The bladder is a hollow organ, approximately spherical in shape at low volumes. During filling it will begin to stretch longitudinally and assume a more ovoid geometry. However, for modelling purposes an assumption of a thin membrane, incompressible spherical wall can provide a good description of the bladder [30]. Relaxation of the detrusor muscle during filling allows intravesical pressure to remain low (approximately 5-10 cmH_2O) to avoid damage to the upper urinary tract. To accommodate higher volumes the detrusor muscle has multiple functions involving relaxation, elongation and reorganisation over large length scales [54].

Towards higher physiological strains the relaxation ability of the bladder reduces and intravesical pressure may rise [14]. In the voiding phase the detrusor then acts as a pump to generate pressure inside the bladder and forces the urine out through the urethra. As the urethral sphincter opens and flow rate increases, the pressure that the detrusor is able to generate decreases until voiding is complete.

The urethra acts as an energy converter in that the energy provided by the detrusor is split into pressure and flow; the two parameters measured by pressure-flow studies.

Energy loss in the urethra is largely due to resistance, which in males is as much as 70% in males compared to 50% in females [49]. This is due to anatomical differences in the lower urinary tract, as the male urethra is considerably longer and more tortuous than in females. Mathematically, the urethral function can be characterised using its relation between the pressure generated by the detrusor and flow rate through the urethra. Initially, the pressure generated by the bladder must exceed the resistance caused by the collapsed urethra to force the initial flow of urine through (approximately 25-50 cmH_2O in males) [50]. Once opened, the flow generally increases to a maximum flow, characterised by the parameter Q_{max} . Once flow has plateaued, the pressure generated by the detrusor decreases and ceases flow. Further leakage after complete voiding is prevented by urethral sphincter contraction and collapse of the tube.

Nervous Control

Bladder function relies on both excitatory and inhibitory bladder-bladder, bladder-urethra, urethra-bladder and urethra-urethra reflexes. In the case of filling, these reflexes are inhibited at many levels to promote storage and prevent involuntary voiding (known as guarding reflex) [55]. In the brain many neuron populations are recruited to control the bladder. The reflex (bladder-bladder micturition reflex) responsible for release of urine is located in the rostral pontine tegmentum (also known as the pontine micturition centre, PMC, or '*L-region*'). The PMC fires electrical signals eliciting a micturition response by relaxing the bladder neck and urethral sphincter via the PMC's efferent pathway [35]. This activity is modulated, in part, by a voluntary control located in the cerebral cortex and allows switching between storage and voiding. Activation of these reflex pathways is elicited by bladder-afferent activity and conveyed by various nerves organised by the spinal cord [30].

The LUT is regulated by three sets of nerves, (somatic, sympathetic and parasympathetic) originating in the sacral and thoracolumbar regions of the spinal cord [55].

Sympathetic innervation, originating in the upper lumbar and lower thoracic portions of the spinal cord outflow, is active during bladder filling. This system is mediated by α -adrenoreceptors and primarily responsible for contraction of the bladder neck and internal sphincter to ensure urine is held without leaks. To accommodate increasing volume during filling noradrenaline is released from the sympathetic system via postganglionic fibres in the hypogastric and pelvic nerves respond to relax the detrusor muscle (See Figure 1.6). This distension, mediated by β -adrenoreceptors and α -adrenoreceptors, also acts to inhibit parasympathetic activity which would otherwise contract the detrusor and relax the internal sphincter (subsequently voiding the bladder) [35].

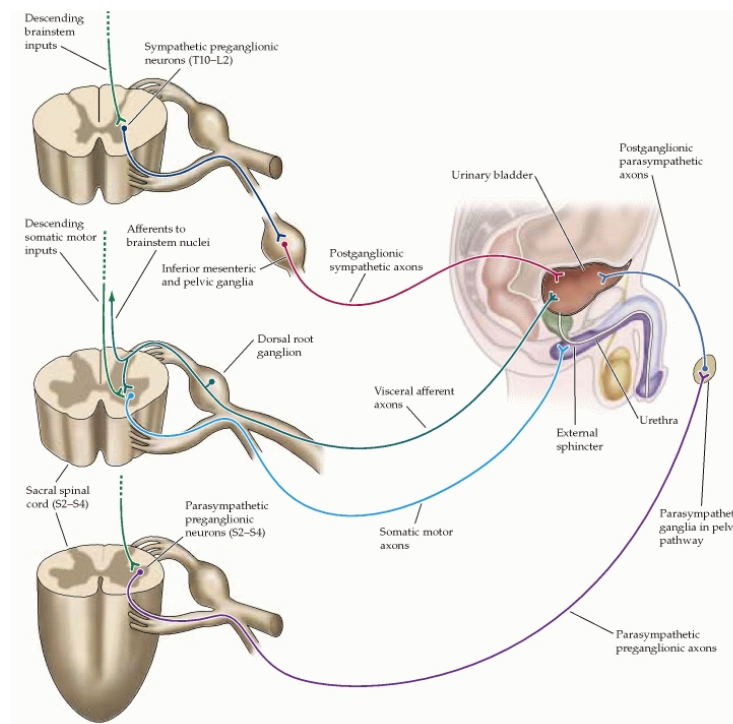


Figure 1.6: Schematic of the afferent and efferent innervation forming the urinary bladder autonomic control. Reproduced from [56]

In most people volumes of 250 mL (in humans) will trigger the first feelings of urgency, however micturition can be inhibited voluntarily. At higher volumes of 450 - 500 mL

urgency dramatically increases as vesical capacity nears the typical physiological limit. Stretch or tension receptors in the bladder wall are stimulated and excite parasympathetic nervous fibres located in the serosa and detrusor; relaying signals to the sacral region of the spine [35]. Release of cholinergic (acetylcholine) and non-adrenergic receptors then excites parasympathetic motor neurons, contracting the detrusor muscle and increasing intravesical pressure. Simultaneously, the external sphincter somatic motor neurons are inhibited via stimulation of muscarinic acetylcholine receptors, allowing the sphincter to open and urine to flow through the urethra [55].

Voluntary and involuntary control over voiding is influenced by a complex interaction between somatic and autonomic efferent pathways; mediated by pudendal nerves, and sympathetic and parasympathetic nerves, respectively. Additionally, regions of the brain are responsible for the many reflexes evoked during micturition and storage. For example, the pontine region that regulates micturition has as many as 12 reflexes that contribute to LUT function. This vast complexity necessitates simplification of models to a few regulatory loops [55]. A broad modelling assumption that greatly reduces complexity is the notion of the LUT being controlled by two mutually inhibitive systems; one controlling the urethra and sphincter, the other controlling the detrusor. This approach requires that when the detrusor is active, the sphincter and urethra are inactive; and vice versa.

1.2.10 Bladder Aging

Bladder voiding dysfunctions occur more frequently than cardiovascular disorders or rheumatoid arthritis as an estimated 15 - 30% of the elderly over 60 suffer from incontinence or post-void residual urine [1]. Aging triggers a series of anatomical changes that may be associated with a gradual decline in bladder physiological capacity and normal

functionality during storage and voiding. These symptoms are linked to prevalence of LUT symptoms; indicative of bladder outlet obstruction (BOO), urinary incontinence, overactive and under-active detrusor. These disorders are rarely isolated to a specific physiological change and will often have interaction from various factors; necessitating the need for investigation into morphological, neurological and anatomical changes that occur during ageing [43].

As the integrity of the LUT deteriorates due to aging, the bladder and urethra will undergo functional changes; some consistent across genders. It is well accepted that bladder capacity decreases with age, increasing the frequency of urination and reducing voided volume. In addition, morphological changes that may occur in the autonomic nervous system and smooth muscle of the bladder wall lead to over or under activity of the detrusor. Detrusor muscle disorders may cause spontaneous contractions or impaired detrusor contractility; decreasing the ability to control urination, increasing post-void residual urine and in cases, may cause nocturia [57]. Further, observations of the urethelium lining of the bladder wall often show signs of damage, i.e. detachment and degeneration, which may lead to loss of barrier functionality, with subsequent increased permeability [58]. Damage of this kind may result in prevalence of urinary infection and inflammation with resulting symptoms.

The urethra will undergo a similar deterioration in function with aging, although dissimilar between genders due to differences in the LUT. It is known that the urethra loses the ability to prevent leakage, as the closure pressure of the external sphincter may decrease. Control of stored urine therefore becomes difficult as resistance to out-flow is lost. In males, the prostate influences the function of the urethra. With age, it is seen that the prostate will enlarge (as much as 3 % volume increase per year) in approximately 70% of men over the age of 45; potentially causing obstruction to urine flow through the urethra [59]. In women, changes in the urethra appear as a result of

oestrogen level drop after menopause. This fall in oestrogen has been implicated to cause a series of alterations that reduces muscular thickness of the urethra, decreasing closure pressure and increasing the likelihood stress incontinence. Child birth also plays a major role in post-delivery functioning of the LUT as the pelvis floor muscles can often become weak or damaged. A host of urinary related disorders may arise in this case, for which surgical treatment may be necessary to retain urinary function [60].

It is important that functional changes of the LUT are related to the underlying changes in morphology and microstructure of the tissue. Collagen is a primary load bearing protein in soft tissues and greatly influences the mechanical behaviour of these tissues. Increased collagen synthesis is observed in the bladder and urethra with age, although rates of synthesis vary between genders. For example, collagen content in the bladder in females is reported to be approximately 10% higher than males, with the gender specific difference in content increasing proportionately with age [61]. Additionally, it is observed the ratio of collagen to smooth muscle in the detrusor significantly increases with age in mice [58]. Raised deposition of collagen can affect the mechanical compliance and contractile properties of the bladder wall and severe cases lead to fibrosis and subsequent loss of voiding function [62].

Smooth muscle forms the large majority of the bladder wall. Deterioration of the myogenic and neural components of the detrusor has great impact on the functionality of the LUT and is the underlying cause in certain pathologies, i.e. detrusor under and over activity [7]. It is known prevalence of these disorders are linked to aging of the LUT [63]. At a structural level the detrusor is observed to thin whilst the collagen-to-muscle ratio increases with age in mice [58]. The effect of this is loss of contractility, although studies are unresolved as to whether contractility increases or decreases with age. For example there are examples of reduced [34] [64], increased [65] and indifferent [66] muscle contractile ability between young and aged rat bladder

(muscarinic response). Additionally, contractile response varies in strength according to region stimulated [34].

Similarly, electrical stimulation studies in rats remain inconsistent in determining whether contractility diminishes in age or not [67]. Human studies have shown that nervous response of the detrusor tends to change with age as elderly persons show denervation across the bladder wall in some studies [58]. However, despite this, bladder contractility may not necessarily deteriorate, although instability becomes more prevalent. Studies in women show a common trait of reduced flow rate, with maintained isometric vesical pressure; suggesting diminished contraction velocity as opposed to contraction strength [67].

It is usually the case that once elastin has been synthesised, laid down and cross linked, the turnover rate is very low; approximately 1% in most tissues [68]. It is speculated that increased deposition and cross-linking of elastin with age or in response to injury or pathology is a factor in voiding dysfunction [69]. It is also observed that vesicle elastin content increases considerably in BOO, with deposits becoming more significant with age [57].

1.2.11 Multiphoton Microscopy

Multiphoton microscopy (MPM) is a recently developed non-linear optical method of imaging that exploits second harmonic generation (SHG) and two photon excitation fluorescence (TPEF) to distinguish between the various constituents of soft tissues. It allows increased depth penetration over traditional confocal microscopy, reduced photo-bleaching and removes the need for exogenous staining. MPM allows high resolution imaging of collagenous structures in fresh tissue, such as arteries or bladder wall tissue, with no requirement for addition of exogenous stains. MPM has advantages over

traditional confocal microscopy, including; greater sample penetration, higher axial resolution and reduced photo-bleaching of dyes used for staining. The use of multiphoton microscopy for biological applications was infrequent until recently, despite having been first demonstrated in 1961 [70].

The process of multiphoton excitation arises from absorption of two or more photons simultaneously in a single event [71]. The probability of multiple photon absorption is significantly lower than a single photon. This is due to absorption probability increasing as the square of the photon intensity, meaning imaging is restricted to the focal plane of the objective where the intensity of the beam is at its maximum (reducing background signal). Additionally, a highly powerful laser is required. This can be achieved by focusing pulsed (mode-locked) lasers, whereby power during the pulse can generate sufficient flux of excitation photons [72].

Typically, the objective will be used for excitation and collection of signal photons. Emissions are detected by photon multiplier tubes (PMT) powered at high voltage, maintained in a vacuum. PMT consist of several electrodes; a photocathode to allow detection, dynodes to amplify the signal and an anode to output signal. Electrons collected are amplified with gains between 10^6 - 10^8 , depending upon the sensitivity and material of the photocathode. Signals are separated by the use of wavelength sensitive dichroic filters (See Figure 1.7) [73].

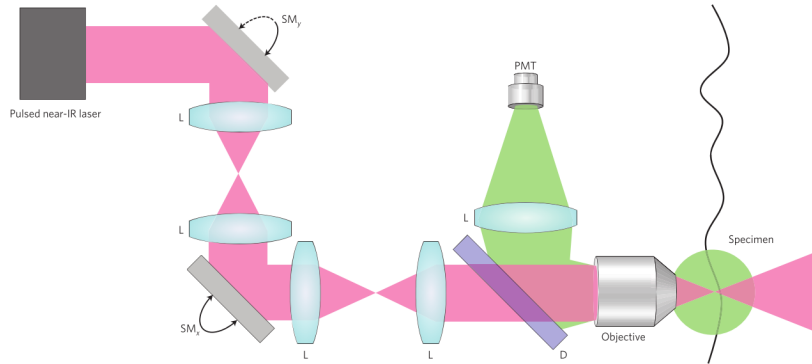


Figure 1.7: Typical arrangement of a near IR laser fed MPM. In this example a dichroic (D) is utilised to separate excitation light from the two-photon fluorescence. L denotes the arrangement of lenses, and SM the scanning mirrors. Sourced from [73]. Reproduced with permissions from Nature Publishing Group.

SHG imaging uses near-infrared pulsed femto-second lasers to excite a molecule from its ground state to a higher energy state. Contrast is obtained from variation in individual constituent's ability to produce second harmonic light. The highly crystalline formation and intrinsic triple helix structure of collagen makes it particularly effective at generation of second harmonic incidences [74]. Another variant of MPM is TPEF and uses infrared light to excite fluorophore molecules in certain tissue constituents or fluorescent stains from a ground state to an excited state. Two low energy photons are absorbed in a simultaneous event; resulting in a fluorescence emission that is dependant upon the fluorophore, typically of a higher energy than the excitation photon.

1.3 Thesis Overview and Research Aim

The current limitations of urodynamic studies and diagnosis of LUT disorders poses the question of whether mathematical modelling can be used to improve the clinical pipeline. The overall aim of this work was to examine this question from a ground-up approach, where experimental work played a large role in model hypotheses and

development and eventual progression towards a micturition framework. Further, to allow future progression of micturition modelling to be used as either a method to reduce subjectivity of diagnoses, improve diagnostic parameters or criteria, or provide the 'missing information' required to improve viability of non-invasive procedures in place of invasive methods for specific disorders.

The chapters in this thesis are largely multidisciplinary with experimental, modelling and clinical contributions. The use of mechanical testing and MPM imaging studies aimed to elucidate the complex mechanisms of various layers and microstructure of the bladder wall during deformation so that information may be incorporated into microstructural models of the bladder wall with the aim of developing these with a micturition model framework. Further, patient specific cases of normal voiding will be used to establish baseline values for model parameters, so that dysfunctional urodynamic traces may be differentiated from normal into the future. More specifically, the aims of this work include:

- Provide comprehensive MPM imaging studies of the bladder wall and identify key structural components in young and aged murine bladder.
- Through the use of coupled MPM and uniaxial, biaxial and inflation mechanical testing gain insight into the microstructural response, relative to the mechanical stress, of the bladder wall subject to deformation,
- Identify mechanical and microstructural differences that may exist between young and aged murine bladder wall,
- Develop a novel microstructurally motivated, bladder specific constitutive model to describe the bladder wall with physically relevant parameters to describe collagen and smooth muscle constituents,
- Develop a micturition framework to simulate normal male and female patient

urodynamic studies.

The bladder structure is examined on a macroscopic and microscopic scale using coupled MPM and uniaxial / biaxial mechanical testing. It is known that the overall mechanical properties of a tissue are governed directly by the materials and layers it is composed. Therefore, the aim of experimental studies was to attribute individual microstructural components of the bladder wall to macroscopic mechanical response characteristics. Based on these observations a microstructural model was developed to provide a more sophisticated representation of the bladder wall with structural aspects of collagen and smooth muscle playing a role. Microstructural data obtained from imaging studies was implemented and fitting of the model performed using biaxial mechanical test data obtained from coupled MPM / biaxial studies on murine bladder. Further, clinical patient data was examined and a micturition framework including novel active and passive constitutive models developed to simulate normal male and female voiding patterns. Baseline parameters were obtained from clinical literature, urodynamic observations and x-ray imaging. A simplified nervous system model was implemented and fitted models were compared with clinical urodynamic studies. Details of the work can be found in the following chapters:

Chapter 1 - The first chapter overviews the current understanding and literature in related fields of the bladder and provides background into disorders and aging of the lower urinary tract.

Chapter 2 - The second chapter provides background into MPM imaging and some of the current literature. MPM was used to image bladder wall from young and aged mice from various orientations. Custom automated scripts were used to measure various microstructural attributes of the visible bladder wall from 2D imaging stacks and quantify differences between young and aged bladder wall tissue.

Chapter 3 - In this chapter MPM imaging was coupled with various means of deforming bladder wall tissue through uniaxial, biaxial and inflation methods. Murine bladder wall was imaged under incremental deformation and the microstructure observed over a range of stretches. Anatomical wall layer specific collagen recruitment, orientation and dispersion were quantified and correlated with macroscopic mechanical behaviour.

Chapter 4 - A novel bladder specific microstructural model including representation of smooth muscle and collagen fibre orientation, dispersion and recruitment was developed. Microstructural data from MPM / biaxial tests were implemented into the model and fit to mechanical test data. Microstructural parameters and mechanical tests of young and aged murine bladder were compared.

Chapter 5 - Urodynamic pressure-flow curves were obtained from clinical patients. An existing micturition framework based on bladder outlet relation and urethral resistance relation was modified and microstructural description of passive material properties was implemented into the framework. An active smooth muscle model allowed control of the range of stretches over which active force generation is possible by the detrusor. Relaxation of the urethra and contraction of the bladder is controlled by an exponential neural control model. Clinical urodynamic data was obtained from University College London Hospital and male and female patient voiding was simulated.

Chapter 6 - An overall discussion sums up the main findings and main limitations of the study. The key contributions of this research are summarised and several potential future directions for the research proposed.

Multiphoton Microscopy: Structural Role of Collagen in the Bladder

2.1 Introduction

The macroscopic mechanical behaviour of a soft tissue such as the bladder is directly related to the properties of the constituents of each anatomical layer, and their distribution and orientation. Remodelling in response to disease or disorders can impact the arrangement of wall layers, and subsequently alter the mechanical functionality. It is difficult to identify these changes in vivo through mechanical urodynamic studies alone. Imaging studies and quantification of structure arrangement may allow better insight into local differential effects across the wall thickness and allow the health state of the tissue to be better understood. Collagen fibres arranged in soft tissue are hypothesised to be organised with a preferential orientation corresponding to loading direction [75]. Quantification of these structures through visualisation techniques such as MPM allows microstructural based constitutive models to be implemented. Quantification of tissue-specific parameters, such as collagen recruitment, orientation, distribution and area fraction can provide a better description of the macroscopic mechanical properties in terms of the interaction of the microstructure.

Use of multiphoton microscopy to visualise bladder tissue constituents and morphology has received limited attention to date [76] [58], although it has recently been investigated for application in bladder carcinoma identification [77], [78]. The morphology in the various physiological layers, including nerves and vasculature, of the healthy bladder is investigated by [76]. Authors conclude MPM is able to accurately image through 300 μ m thickness of murine bladder wall and given its ability to image nerves, has benefits over traditional histological examination. An extended study by the same author, looks at quantification of components of young and aged bladder dome, including; collagen and muscle layer thickness and various nerve parameters [58]. The study reveals significant differences between all measured parameters when comparing between 23-25 week old and 85-90 week old mice. However, the relationship of these parameters to the structural behaviour is not commented on, and imaging studies were limited to the bladder dome.

These parameters are particularly valuable for identification of direction specific changes to mechanical properties and functionality. Considering the variation of the mechanical behaviour between regions [17], qualitative and quantitative characterisation of the structurally relevant morphology in the bladder is beneficial; particularly for application in structurally motivated constitutive models where analysis of structure is critical.

Onset of certain LUT disorders, or spinal cord injury has been shown to cause remodelling of wall layers and orientation of structures. Rapid reorientation of smooth muscle in spinal cord injury, for example, has significant impact on material behaviour and direction dependant mechanical response in as little as 10 days [79]. Changes to mechanical properties due to spinal cord injury [79], aging [36] [34], detrusor disorders [80] and obstructive disorders [81] suggest remodelling mechanisms occur continuously in the LUT. However, structural remodelling that may occur in aging and the potential

impact on mechanical functionality has yet to be observed and quantified by imaging studies.

It is currently very challenging to accurately measure local structural and mechanical properties of the bladder wall in vivo, however, imaging modalities such as MPM can be readily used to observe and quantify layer specific morphology. In the long term this would be expected to aid development of microstructurally motivated constitutive models to be used in computer simulations and used to predict mechanical functionality and aid assessment of the health state of the tissue. Collagen, elastin and smooth muscle are organised throughout the bladder with structural and functional roles specific to individual wall layers.

Clinically, urodynamic studies involve mechanically stretching the bladder. The pressure-flow results obtained are directly related to the underlying microstructure and layer composition. Better understanding of the morphology and relating this to the function of the bladder will ultimately improve our understanding of what goes wrong during disorder development or aging. MPM has practical uses for identification of how constituents and wall layers are formed in healthy states and re-configured in diseased or aging states. Key morphological features or mechanisms that trigger changes to morphology or functionality during disease and aging and how they impact the mechanics of the organ may identified through imaging modalities such as MPM. Therefore, the aim of this study was to use MPM to provide insight into wall layer arrangement of murine bladder and identify differences in wall structure between young and aged mice. More specifically, differences in collagen, elastin and smooth muscle morphology, density and orientation were observed and quantified. Hypotheses relating the microstructure and deformation mechanisms to bladder wall compliance and tissue layer function were made.

2.2 Methods

2.2.1 Tissue Preparation

Urinary bladders from 3 to 4 month-old young adult (referred to as young) and 23 to 24 month-old (referred to as aged) were extracted from 16 male C57/BL6 mice (8 of each type) for mechanical testing. 5 of each were used for planar imaging studies and 3 of each type used for cross sectioning. Mice were sacrificed by cervical dislocation following isoflurane (sacrifice of animals followed good laboratory practice in compliance with UK laws and regulations). Intact bladders were placed in phosphate buffer saline and kept frozen at -20 °C in excess of 24 hours. All experiments were carried out under an approved UK protocol and project license and in accordance with the University of Sheffield Animal Care Committee.

Prior to imaging and mechanical testing samples were removed from the freezer and allowed to warm to ambient temperature. Full length strips were cut to measure approximately 12 x 4 mm. Thickness and width dimensions were measured three times with a CCD optical micrometer and values averaged.

Microtome

Circumferential 'hoops' of mouse bladder were dissected for microtome sectioning (similar to cryotome tissue protocol). Tissue was flushed in 10% formalin for approximately a week to ensure tissue was adequately infiltrated. Tissue was then washed. Following an overnight wash, tissue samples were embedded in paraffin wax on a mold and left to set for 24 hours. Microtome sections were cut at a thickness of 10 μm and applied to adhesive slides before baking at 60 °C for 30 minutes and 30 °C for 60 minutes.

Tissue Staining

To identify the cellular components of the bladder wall DAPI was applied to tissue prior to imaging. Briefly, DAPI is a fluorescent (counter) stain that binds to A-T regions of DNA. Its high selectivity and ability to pass through cell membranes makes it ideal for cell nucleus staining. Primary cells of interest include SMCs, fibroblasts and myofibroblasts. DAPI stock solution (Fisher Scientific) was diluted 1:1000 in PBS. All solutions were stored at - 20°C. Tissue samples were incubated in DAPI working solution for 10 minutes in a dark room at ambient temperature then washed several times with PBS.

2.3 Imaging Protocol and Acquisition

A Zeiss LSM 710 inverted confocal microscope (Zeiss LSM710, Oberkochen, Germany) fitted with a pulsed femto-second Chameleon laser operating at 860 nm was utilized for imaging urinary bladder tissue. Collagen and elastin constituents were imaged at wavelengths of 430 nm (SHG) and 520 nm (TPEF) respectively with spectral bins of ± 20 nm at magnifications of 20x (air objective). All images were processed using custom written Matlab scripts (described in Section 2.4) to quantify collagen fibre orientation and recruitment using the methods describe in subsequent sections.

Planar images, with a resolution of 1024 x 1024 pixels and averaged 4 times, were acquired over a 5 x 12 tile regime. Tissue strips imaged in a resting configuration were allowed to assume a natural state on a microscope cover slip and submerged in PBS. A second cover slip was placed over the sample to secure the tissue and prevent movement and folding of tissue. Samples were scanned in a tile format from both mucosal and adventitial perspectives across the entire length and width of strip. Additionally, planar images were taken across the wall depth of the bladder with a z-resolution ranging between 2-4 μm (based on imaging time and signal penetration depth).

Cross sections prepared using a microtome or cryotome on a cover slide were cut as 10 μm thick sections. Images were obtained over 4 averaged images with a resolution of 1024 x 1024. Number of tiles was tailored to individual sample size to capture the entire section. Images were taken in a single plane, corresponding to the approximate mid-point of the tissue.

Measurements

Measurements of wall and layer thickness made on MPM images were obtained by taking 5 measurements using ImageJ. Measurements of interest were averaged to obtain final dimensions and standard deviation (SD) calculated for each dimension. Significance was tested for using Student T-test with built in Matlab function *Ttest2*.

2.4 Collagen Orientation Analysis

A custom-written Matlab script to extract feature orientation (in this case fibres) obtained from MPM imaging studies is described in the next section.

2.4.1 Fourier Transform and Orientation Discretisation

Collagen orientation was measured across MPM SHG z-stack images with the detrusor side facing the objective using a custom-written algorithm in Matlab (Mathworks, Natick, MA). Orientation magnitudes from 0 to 180° were obtained for 1024 x 1024 images using a 2D fast Fourier transform method, which converts spatial patterns into frequency components with a directional dependency [82]. A raised cosine window was applied to minimise frequency domain errors due to periodic discontinuities caused by image boundaries (this function reduces pixel values in edge regions to zero, while keeping central pixels at a similar magnitude).

$$W = \cos(\omega_i) \quad (2.1)$$

Where $\omega \in [-\pi/2, \pi/2]$ with i corresponding to image resolution. For a 2D case the product of windows for both x and y directions was obtained. The greyscale image with applied cosine window can be represented by a distribution function $g(x, y)$ where x and y define indices of the greyscale image. Points, (u, v) in the Fourier space are obtained by,

$$\mathcal{F}(g(x, y)) = G(u, v) \quad (2.2)$$

A polar shift in the Fourier space was then applied using the Matlab function, *fftshift*. This transforms the lowest spatial frequency points to the centre origin, where spatial frequency increases with distance from the origin. The power spectrum, P , was yielded by multiplication of the transform by its complex conjugate, G^* (See Figure 2.1).

$$P(u, v) = G(u, v) \cdot G^*(u, v) \quad (2.3)$$

To account for the 90° shift due to the Fourier transformation, the power spectrum was rotated back to its original alignment with the greyscale image. Aligned objects can be characterised in the power spectrum by amplitude $I(\theta)$, clustered along the direction of alignment, θ . Aligned objects in this case collagen fibres, can be distinguished quantitatively by orientation and spatial frequency.

A wedge-like binning method was used to extract fibre orientation and magnitude from the power spectrum. Using the centre of the power spectrum as a rotation point, 180 individual arcs spanning 1° are created (See Figure 2.1). All values within each arc are

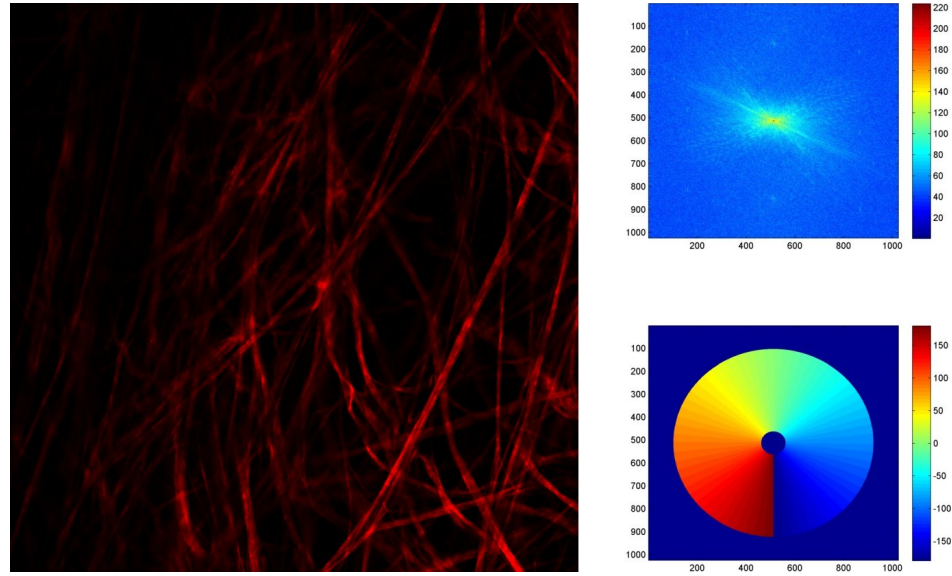


Figure 2.1: Steps involved in orientation analysis. (Left) Example Multiphoton image of 2D planar collagen used in this processing example. (top right) Power spectrum of 2D Fast Fourier Transform from MPM image of collagen fibres (converted to polar coordinates, i.e. highest intensities in center region). Note, the power spectrum was offset by 90 degrees in the process and was later rotated 90 degrees about the center axis to obtain orientation data. (bottom right) Plot of how 2D array was separated into 1° bins for summing intensities.

summed individually to obtain relative magnitude of orientation within that specific segment (corresponding to a specific orientation). This resulted in a 1×180 array with relative strength of orientation for each angle. This vector was smoothed using Matlab built in function '*smooth*' with a filter size of 7.

Validation

The method proposed was validated in a controlled setting using binary images with known orientation of straight, black lines. The orientation analysis algorithm was run on each image and results compared with input orientations (See Figure 2.2).

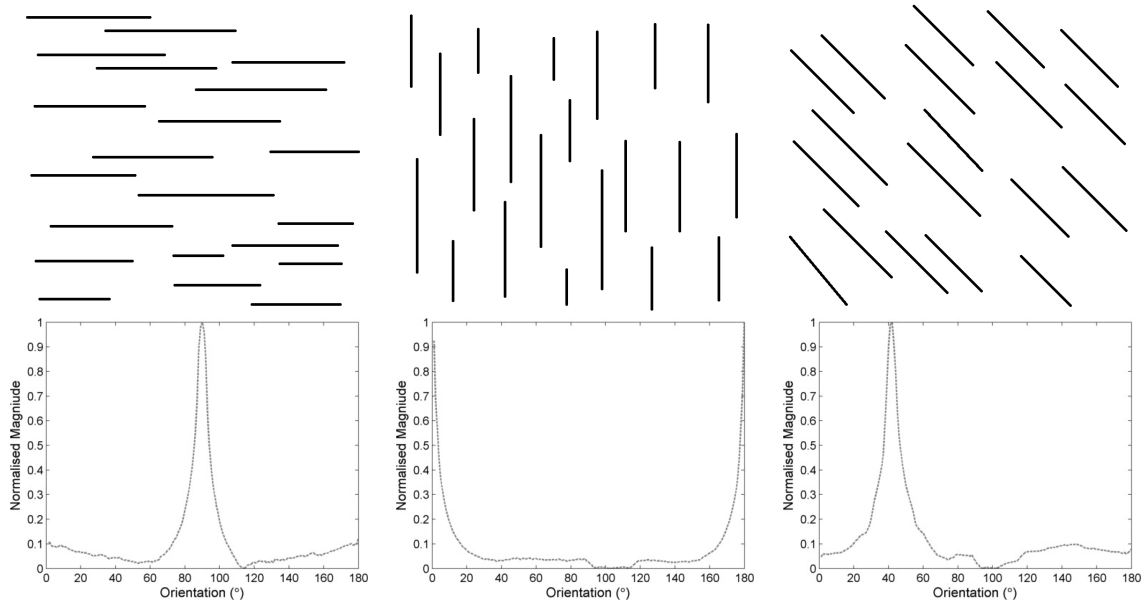


Figure 2.2: Plots of orientation algorithm validation with single families of fibres at 90° , 0° and 45° .

Test images show the algorithm was very capable of identifying the dominant orientation of image characteristics orientated at 0, 45 and 90° . Small fluctuations around the minimal magnitudes occur due to slight variation in pixel quantities used to sum the pixel values within each arc. The impact of this can be minimised by increasing image resolution. Here, images were 668×590 ; an arbitrary rectangular resolution to test algorithm robustness on uneven image dimensions. These plots show the ability to capture a single family of 'fibres'; Figure 2.3 shows validation of the algorithm with multiple known fibre orientations.

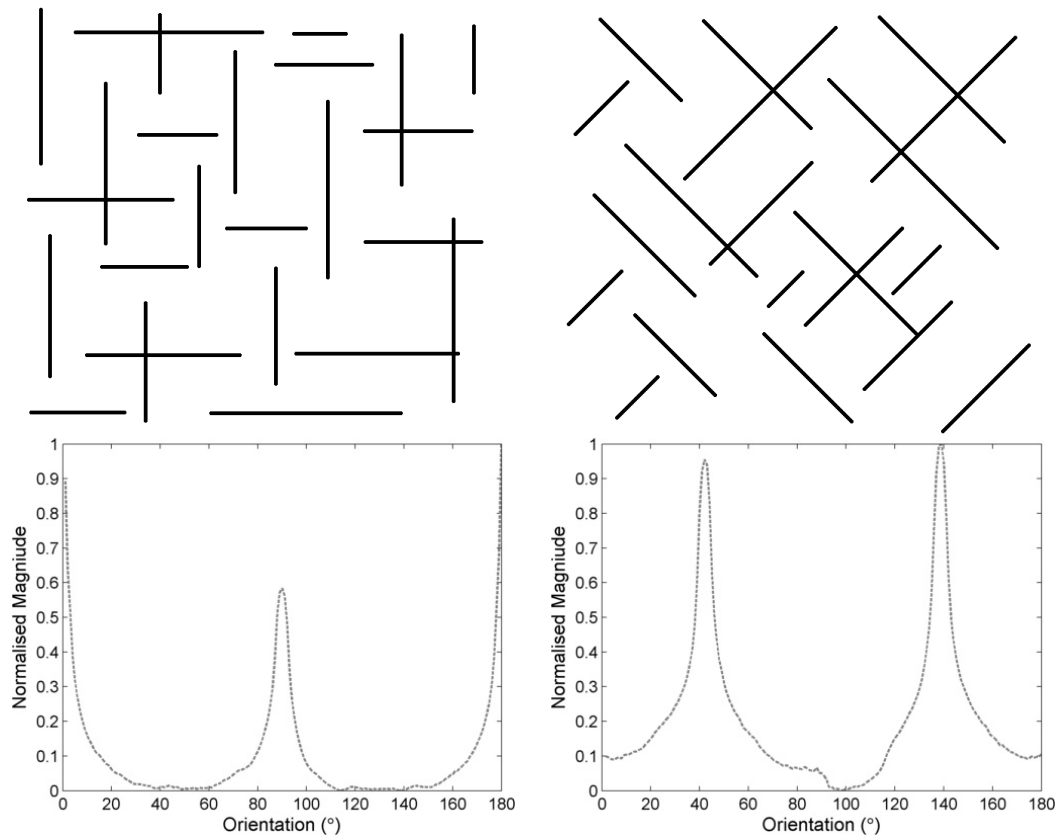


Figure 2.3: Plots of orientation algorithm validation with two families of fibres at 90° and 0° , and 45° and 135° .

As before, the algorithm was able to capture both families of fibres. Interestingly, this method also identifies the stronger magnitude of fibre direction and can be attributed to line or 'fibre' length and quantity in that particular direction.

2.5 Results

The structural morphology of the collagen, elastin and SMCs are examined in this following section. The anatomical layers and constituents that compose these layers were identified using MPM as a basis for modelling assumptions and hypotheses.

2.5.1 Imaging of Planar Sections

Tiled MPM images of collagen in the entire bladder wall are seen from inner and outer wall perspectives in Figure 2.4 and Figure 2.5, respectively. Forming the stroma of the lamina propria is a fine, densely interwoven network of collagen. Folds in the rugae are clearly visible as dark regions as they fold out of plane (note, rugae have mostly been flattened against the cover slide). A majority of the rugae folds follow the longitudinal axis with very few folds across the circumferential plane. Closer detail is shown Figure 2.5 (top-left). Collagen density was significantly higher than the detrusor and fibre arrangement was, by comparison, isotropic with no obvious preferred direction. Magnified collagen fibres in the lamina propria are shown in Figure 2.4 (bottom-left). Fibres are larger relative to the stroma and are loosely orientated with a highly coiled structure.

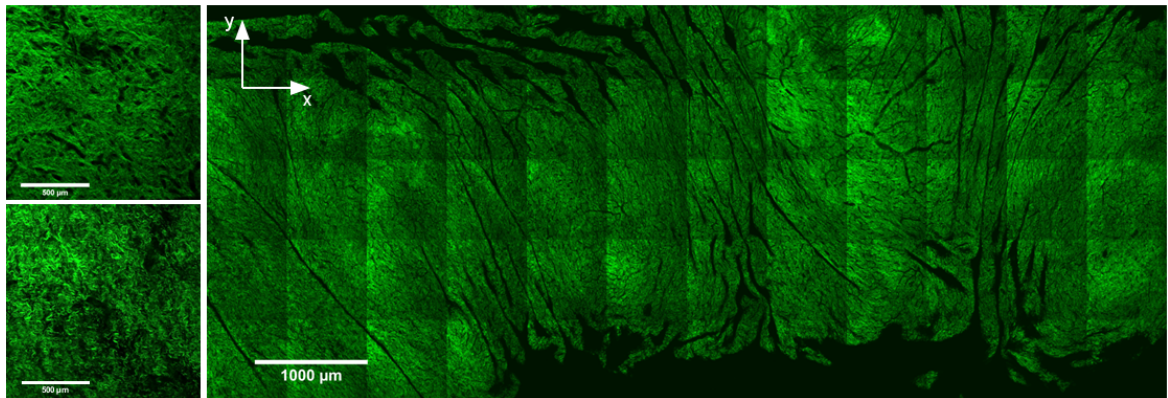


Figure 2.4: MPM images at 10x magnification of collagen in complete body of the inner (right) murine bladder wall and detailed views of different collagen morphology in stroma and lamina propria (top-left and bottom-left, respectively). Axes x and y correspond to circumferential and longitudinal orientation, respectively.

Highly aligned discrete 'bundles' of small collagen fibres are seen in the detrusor layer with an emphasis of orientation towards circumferential (horizontal) and longitudinal (vertical) axes. Abrupt changes in collagen fibre orientation between perpendicular bundles of collagen suggest these bundles may overlay each other throughout the depth.

Additionally, a highly tortuous blood vessel lies across the longitudinal axis of the bladder wall body in the detrusor layer. Closer detail of detrusor collagen in Figure 2.5 show straight fibres within discrete aligned bundles. Overlying bundles are perpendicular in the longitudinal direction. Small regions of fibres are relatively coiled; this type of fibre typically appears between bundles.

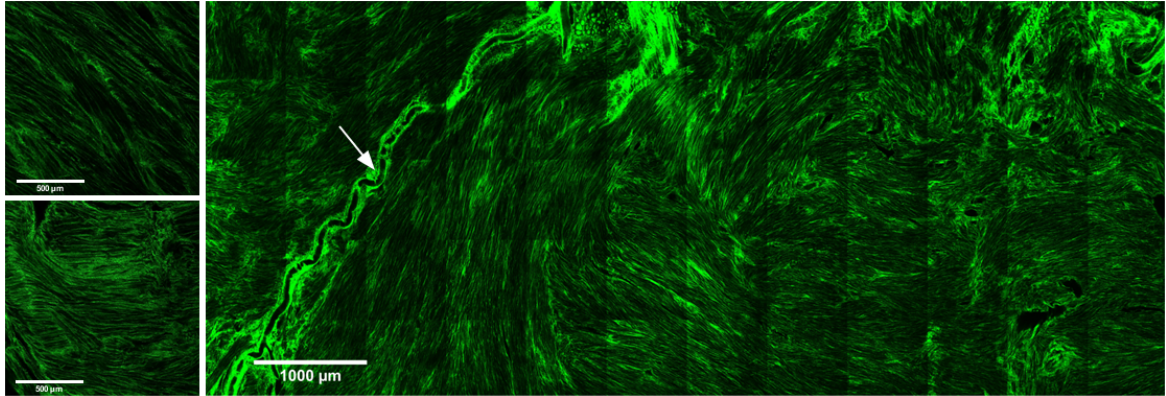


Figure 2.5: MPM images of collagen in the detrusor across the body of the bladder wall at 10x magnification (right). Detailed views of highly aligned collagen 'bundles' overlapping in the detrusor (left). Arrow indicates blood vessel in the wall.

Imaging of detrusor stained by DAPI is shown in Figure 2.6. Smooth muscle cell nuclei are elongated and highly aligned in the direction of the smooth muscle bundle; closely correlated with direction of collagen fibres surround individual bundles. Overlapping bundles are evident in all three images as orientation of cell nuclei abruptly shifts. Figure 2.6c shows high resolution close up structure of the SMCs. Nuclei are mostly elongated, however more circular nuclei are seen at boundaries of SMC bundles; indicating their direction may be perpendicular to the circumference of the bladder wall; or another cell type was present in this particular region.

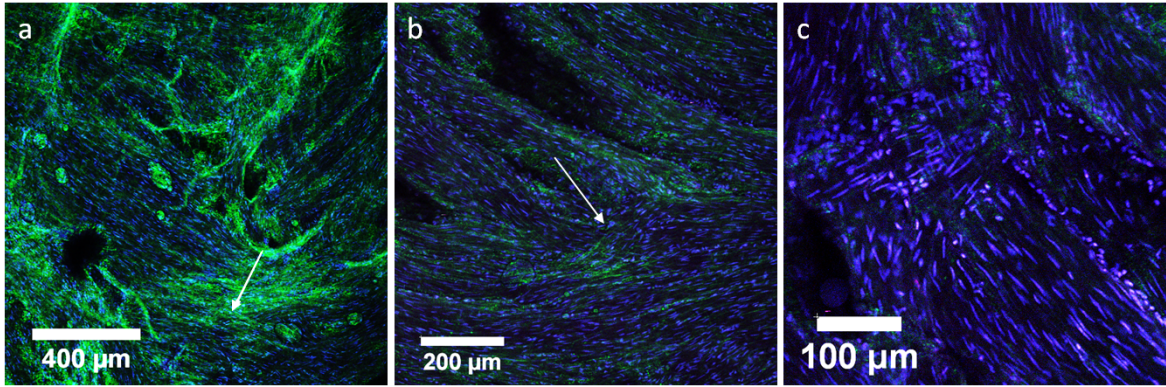


Figure 2.6: MPM images of DAPI stained detrusor of mouse bladder. (Green) Collagen fibres and (Blue) cell nuclei shown in each image at 10x magnification. Arrows highlight regions of smooth muscle over lapping layers as direction of nuclei are seen to alternate across the regions shown.

MPM used in this study was able to image to depths of approximately $200\ \mu\text{m}$ from the outer surface. Figure 2.7 shows collagen and cell content in the detrusor imaged from the outer surface at increasing depth.

Figure 2.7a shows that the surface was primarily a dense collagen layer with very few cells. Deeper at $45\ \mu\text{m}$ the first layer of muscle was seen with highly aligned fibres and elongated SMC nuclei. $90\ \mu\text{m}$ from surface shows a transition between muscle layers as wavy, sparse collagen interweaves between muscle bands. The deepest region captured at $180\ \mu\text{m}$ shows the second band of muscle, lying approximately perpendicular to the outer muscle layer.

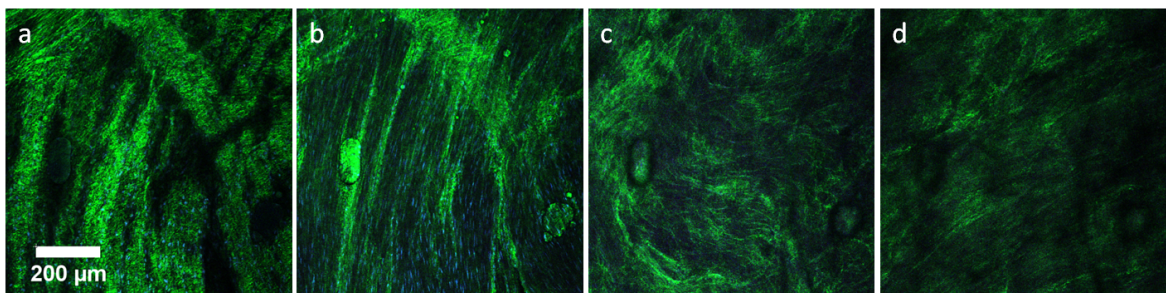


Figure 2.7: MPM images of collagen (green) and cell nuclei (blue) at different depths of the mouse bladder detrusor. (a) $0\ \mu\text{m}$, (b) $45\ \mu\text{m}$, (c) $90\ \mu\text{m}$, (d) $180\ \mu\text{m}$ from the outer surface.

Figure 2.8 shows a similar thin, dense collagen adventitia on the surface. Deeper images show a sparse, aligned collagen network, and SMC that lie between bundles of fibrils. At depths of $90\ \mu m$ contrast was greatly reduced, however some wavier collagen was seen in the transition between muscle layers (second muscle bundle is not seen here). Orientation of collagen and smooth muscle was approximately 45° , and consistent across the observed wall depth.

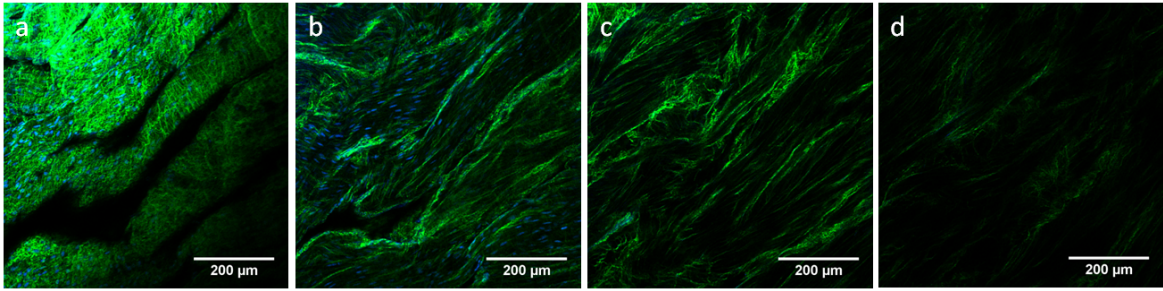


Figure 2.8: MPM images of DAPI stained bladder wall imaged at $18\ \mu m$, $30\ \mu m$, $60\ \mu m$ and $90\ \mu m$ depth from outer surface.

2.5.2 Collagen Orientation in the Lamina Propria and Detrusor: Young vs. Aged

Tiled MPM images of collagen in complete aged urinary bladder strips viewed from adventitial and mucosal perspectives are shown in Figure 2.9.

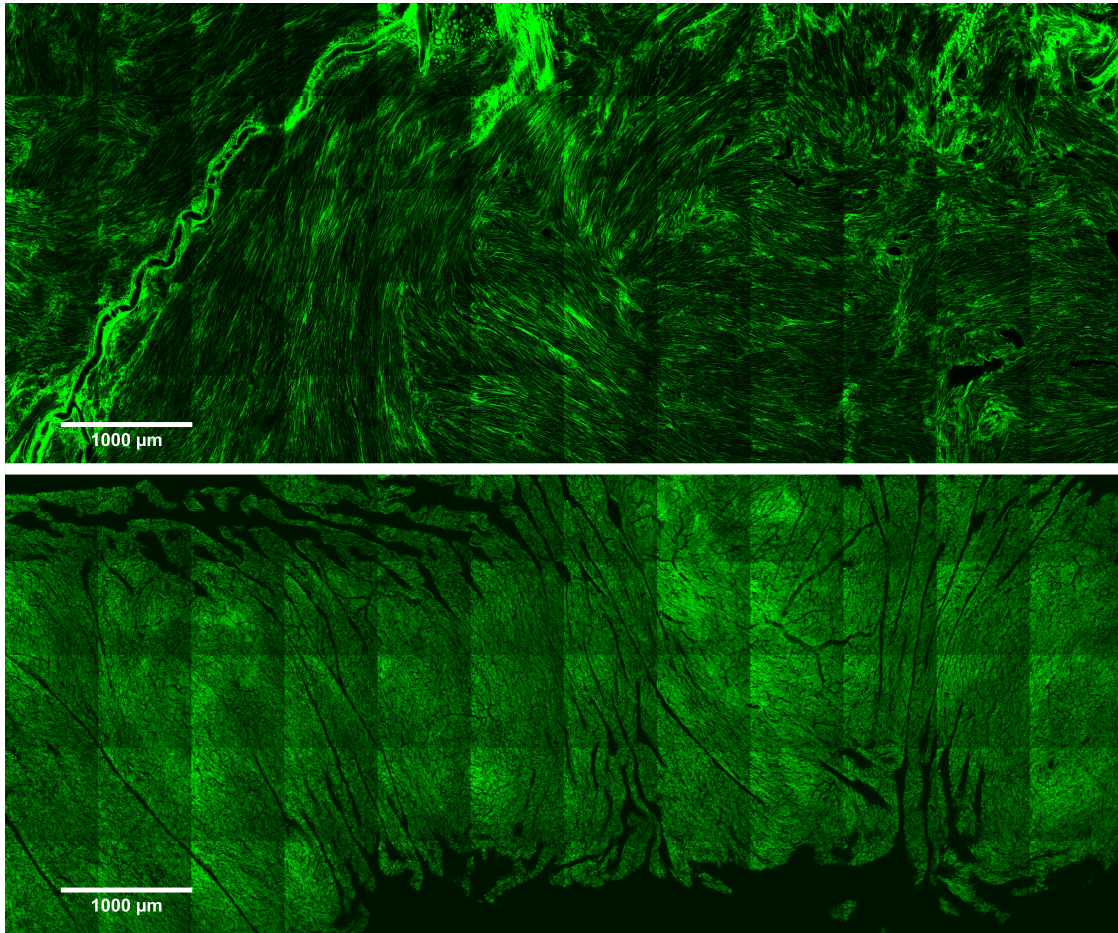


Figure 2.9: Planar tiled MPM imaging of collagen in entire aged mouse urinary bladder strips at 10x magnification. (Above) Detrusor layer. (Below) Lamina Propria

Collagen in the detrusor muscle appears to have localised alignment of bundles of collagen fibres, typically orientated circumferential or longitudinal axes. Outer detrusor layers follow a circumferential alignment, whilst a second deeper layer primarily aligns longitudinally. Collagen arranged in the lamina propria was considerably more isotropic both locally and generally throughout the strips. Inner layers display clear rugae undulations and are visible as the tissue collagen mass folds out of the imaging plane (seen as dark areas).

Collagen fibre bundle density in the outer layers was relatively sparse when compared to the denser regions of collagen of the innermost tissue wall. Collagen in the outer

layers was markedly straighter in a resting state when compared to the highly crimped collagen in the inner layer and appears to have strong localised alignment relative to muscle bundles. These typically run along the circumferential and longitudinal axes of the bladder strips. Figure 2.10 also reveals significant differences in collagen formation between the anatomical wall layers; the outer layer containing sparser wavy collagen fibres when compared to the highly dense, crimped fibres arranged in the lamina propria.

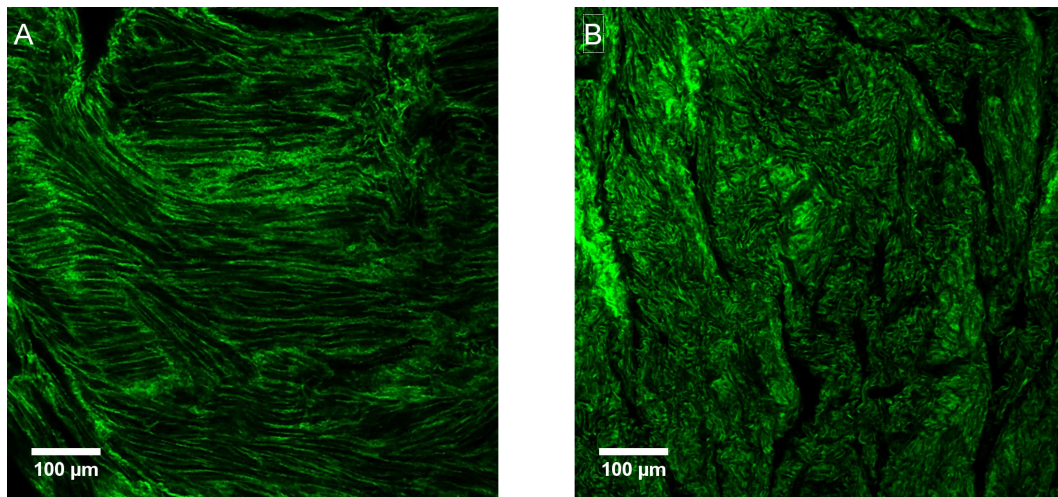


Figure 2.10: Single MPM projection of collagen in aged mouse urine bladder at 20x magnification. A) Detrusor muscle. Fibres seen to run circumferentially locally in bundles with some overlapping longitudinal muscle strips B) Lamina Propria. In between rugae collagen fibres appear to have highly coiled state with no preferential alignment.

Estimation using the FFT method to determine orientation of fibres in the lamina propria reveals collagen to assume an isotropic formation with no dominant alignment (Figure 2.12 left). The coiled network of fibres seen in this layer appear to follow rugae out of plane as dark regions show these obvious folding features. The abundance of rugae and their alignment may have significant influence on mechanical behaviour due to unfolding during loading. Rugae have a clear folding along the circumferential axis of the bladder, whilst minimal folding appears longitudinally. This architecture may contribute to anisotropic mechanical properties, i.e. unfolding of rugae during stretch

of circumferential axis provides minimal resistance.

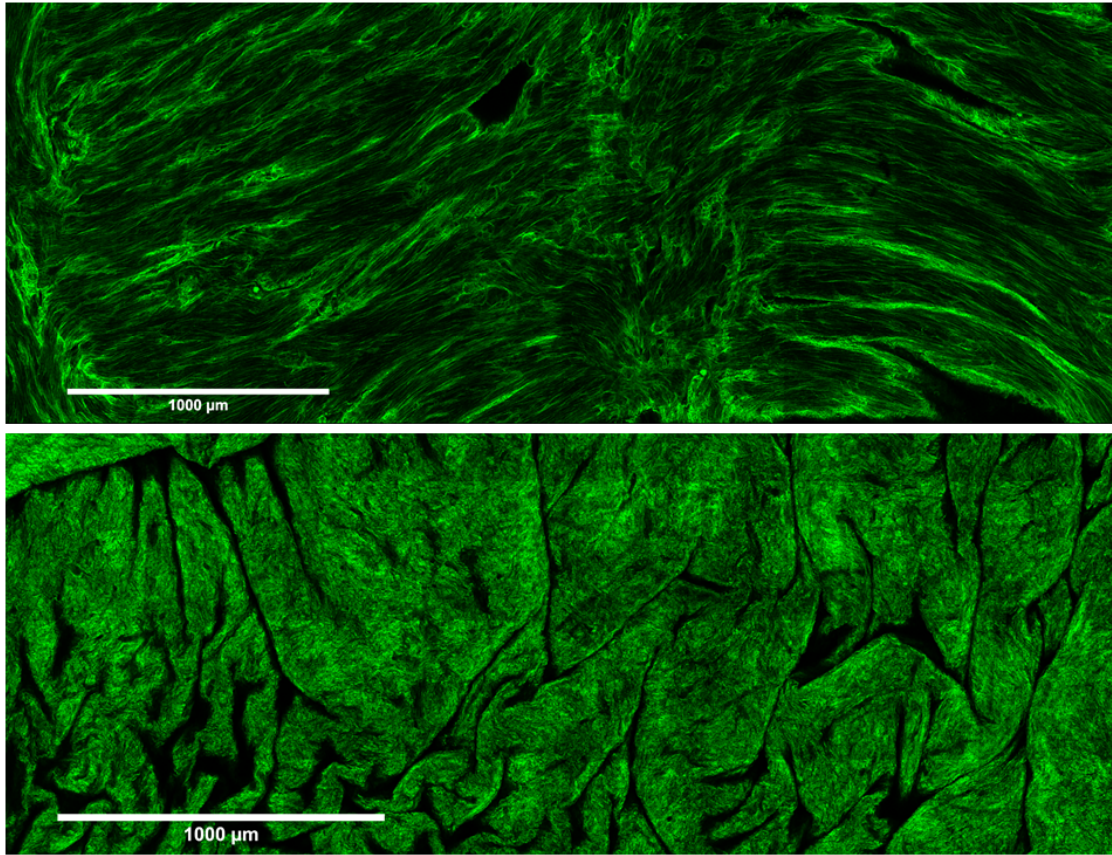


Figure 2.11: Planar tiled MPM imaging of collagen in entire aged mouse urinary bladder strip at 20x magnification. (Above) Detrusor layer, (Below) Lamina Propria.

Orientation of collagen fibres was obtained across entire strips of young and aged urinary bladders in an unloaded state from mucosal and adventitial perspectives (see Figure 2.12). Observations of collagen in the detrusor muscle shows both young and aged samples to have 2 families of fibres with preferential alignment towards the longitudinal and circumferential axes of the bladder. Mean fibre family direction of the aged bladder were 2° and 89° , and for young; 168° and 82° in longitudinal and circumferential directions, respectively. Strength of orientation in the circumferential axis appears to diminish with aged bladder specimen, although deviation about the mean fibre angle was lower.

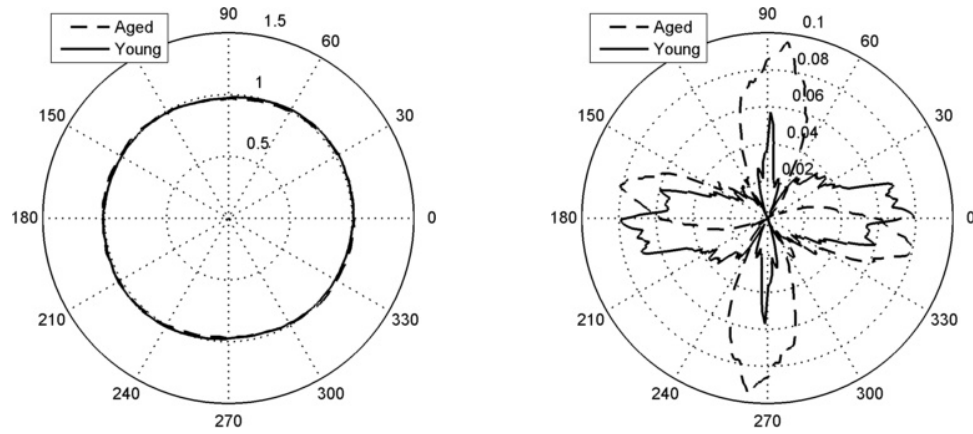


Figure 2.12: (Left) Normalised measured collagen fibre orientation in lamina propria layer for $n = 5$ young and aged samples. (Right) Polar plot of collagen orientation in the detrusor. Normalised measured collagen fibre orientation in detrusor layers for $n = 5$ young and aged samples. 90° lies on circumferential axis, 0° & 180° lies on longitudinal axis.

Upon closer inspection of local regions of the detrusor, it was apparent that collagen has a highly locally aligned, anisotropic network. These fibres alternate between circumferential and longitudinal alignment across the width and depth of the body of the bladder. Typically the transition was abrupt as overlapping layers are seen. Fibres are also seen to curve gradually from longitudinal to circumferential and vice versa, suggesting a single layer of muscle that occupies different orientations depending upon region. Absolute fibre angle was measured as the mean fibre angle calculated from dispersion plots (note, the absolute fibre angle does not consider dispersion of fibres). Mean orientation of fibre families are shown in Table 2.1 for young and aged detrusor.

Area fraction of collagen in the detrusor and lamina propria was measured using a intensity thresholding method. A threshold of 5 % of the maximum intensity was used for results shown in Figure 2.13. Other fractions compared were 2 % and 10 %. 5% was chosen as a mid value and was kept consistent across all samples.

Table 2.1: Table of all measured collagen fibre family orientation in $n = 4$ aged and young bladder samples in the detrusor.

Sample	Family 1 ($^{\circ}$)	Family 2 ($^{\circ}$)
Young 1	29	159
Young 2	24	92
Young 3	45	139
Young 4	32	89
Aged 1	15	110
Aged 2	43	178
Aged 3	28	109
Aged 4	41	123

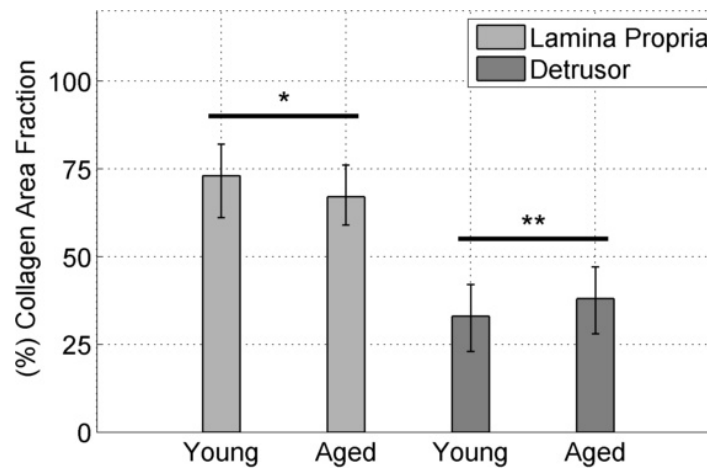


Figure 2.13: Area fraction of collagen quantified in lamina propria and detrusor layers from MPM images of $n = 5$ young and aged mouse bladder. Error bars show standard deviation. $p < 0.3(*)$, $p < 0.2(**)$.

Collagen Orientation Across Wall Depth

Orientation of collagen fibres and SMCs stained with DAPI was obtained across wall thickness of urinary bladders in an unloaded state from adventitial perspectives (see Figure 2.14). Observations of collagen in the detrusor muscle shows 2 families of fibres with preferential alignment approximately towards the longitudinal and circumferential

axes of the bladder. Closer detail of local regions of the detrusor shows collagen has a highly locally aligned, anisotropic network. A thin adventitial layer was seen on the surface and consists of loose, wavy fibres.

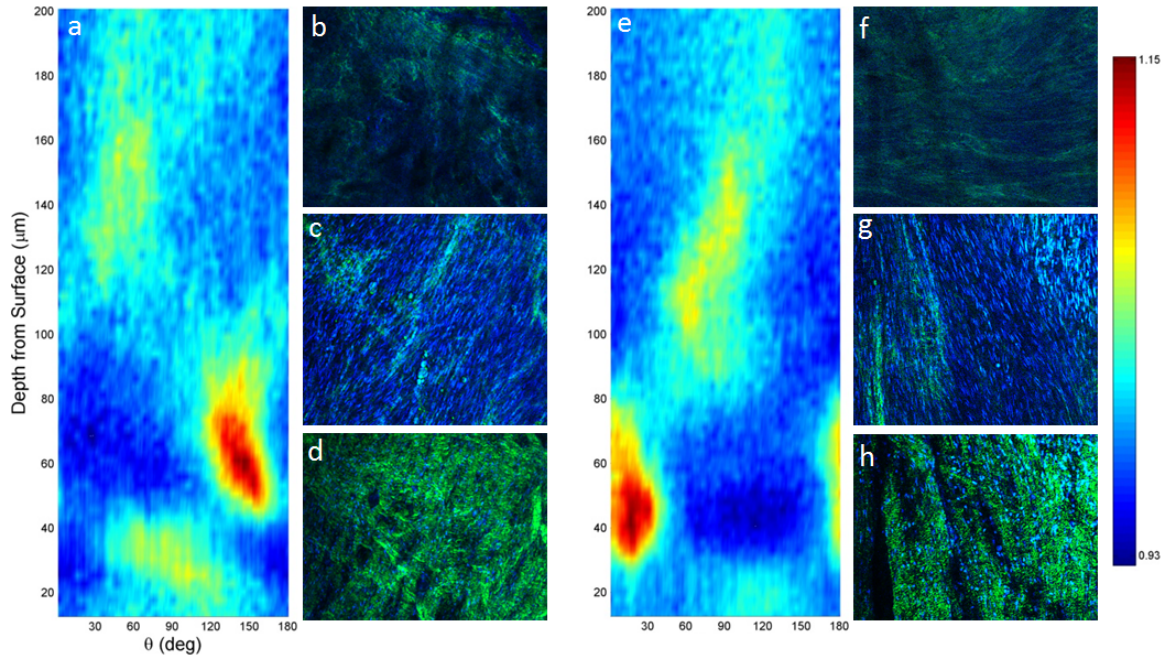


Figure 2.14: Normalised orientation magnitude plots and corresponding images of combined collagen (green) and DAPI stained cells (blue) across the wall depth imaged from the surface of the detrusor in 2 bladder walls (Orientations of 90° correspond to circumferential axis, 0° and 180° correspond to the longitudinal axis). Red regions indicate strong alignment and blue indicates relative isotropy.

Smooth muscle cell orientation and location was identified using DAPI. Nuclei align with orientation of collagen fibrils throughout the depth of the detrusor. Following a thin adventitial layer (Figure 2.14d and Figure 2.14h), consisting largely of loose collagen fibril bundles, the first layer of smooth muscle was observed 20-40m from the outer surface (Figure Figure 2.14c and Figure 2.14g). The initial muscle layer occupies approximately 50-90 m of the wall, offset from the circumferential axis by $32 \pm 9.6^\circ$. Deeper to this, bundle orientation changes abruptly through approximately 90° ; defining a thicker longitudinal muscle layer seen in Figure Figure 2.14b and Figure 2.14f.

2.5.3 Imaging of Cross Sections

Representative bladder cross sections with detailed views are shown in Figure 2.15. A fine, dense collagen layer was seen on the inner surface of the bladder wall approximately $8\text{-}18\mu\text{m}$ thick, depending upon region and sample. This was underlaid by larger, undulated fibres sparsely localised throughout the lamina propria. Rugae on the inner wall give the lamina propria a highly variable extent of thickness. Deeper in the wall a clear transition in collagen content and structure to increased elastic fibres can be distinguished between the lamina propria and detrusor.

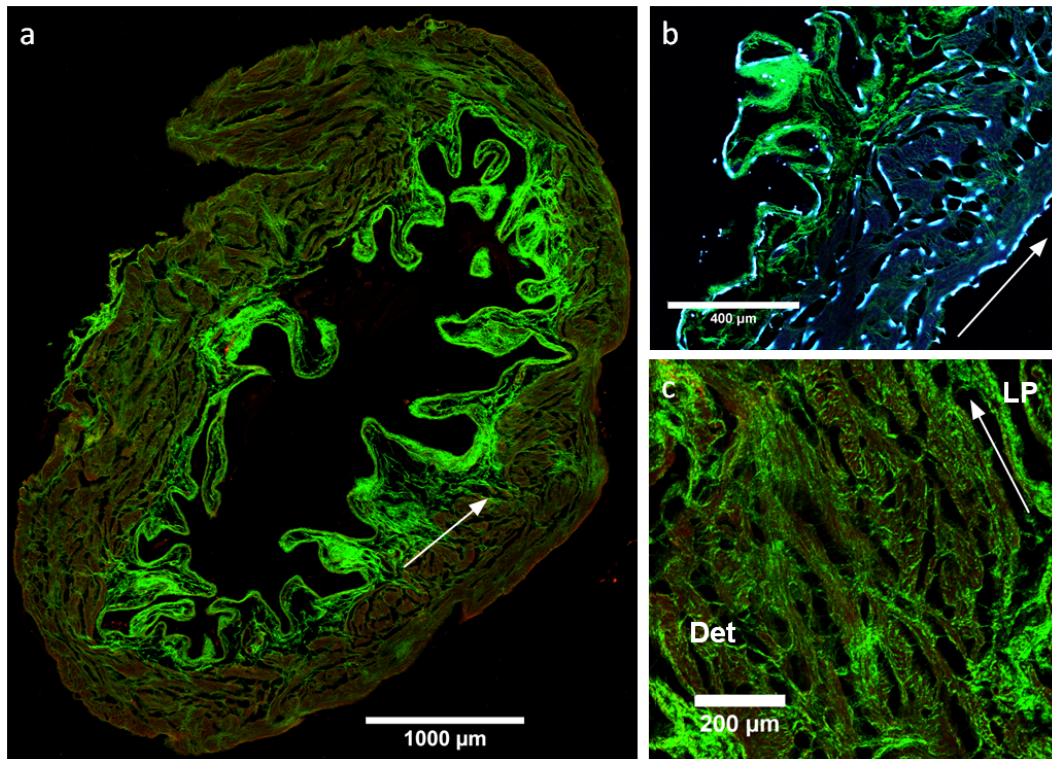


Figure 2.15: MPM images of murine bladder wall cross sections at 10x magnification with collagen (green), cell nuclei (blue) and enhanced contrast elastin (red). Arrows indicate direction of bladder wall circumference. (a) Entire bladder wall, (b) and (c) show detailed view of detrusor layers. (b) enhanced elastin contrast, (c) enhanced collagen contrast. **LP** and **Det** indicate orientation of lamina propria and detrusor, respectively.

The detrusor forms the majority of the bladder wall, with thickness between 480 -

770 μ m. Elastin localises sparsely in the detrusor layer and serosa, conforming to the orientation of smooth muscle bundles. Smaller collagen fibres are seen to interweave throughout and around smooth muscle bundles both longitudinally and circumferentially in Figure 2.15c. Closer detail of the detrusor in Figure 2.15b shows 2 larger layers of muscle consisting of several smaller aligned bundles; the outer most with a degree of undulation. Frequently between muscle bundles empty regions form circumferential and longitudinal 'pockets'. Tortuous and sparse collagen fibres occupy pockets between muscle bundles.

Figure 2.16 shows representative MPM images of DAPI stained bladder wall cross sections. The innermost layer of collagen was lined with a thin layers of cells; making up the urothelium. The lamina propria has sparse population of cells; possibly fibroblasts or myofibroblasts. Location, frequency and orientation of these cells were inconsistent across samples tested; suggesting these may only be present in response to certain pathologies [83]. Transition to the detrusor was delineated by a large SMC population.

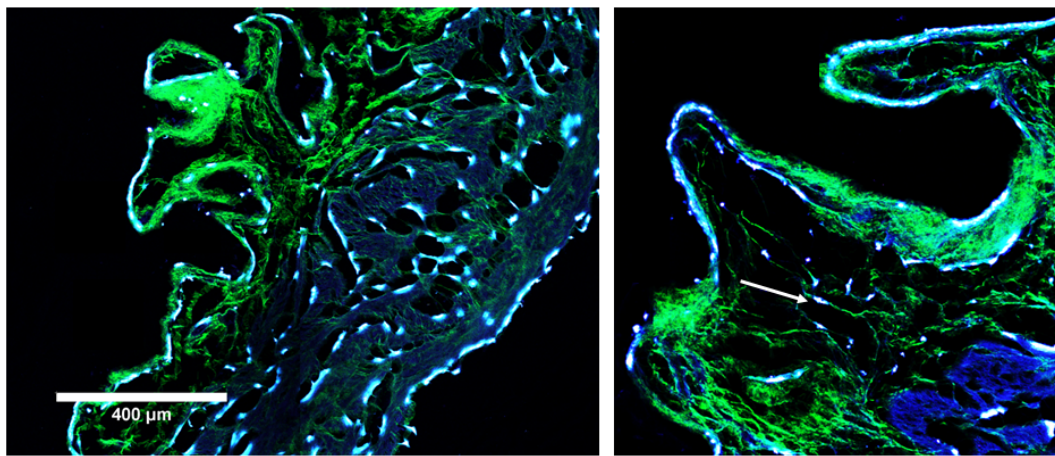


Figure 2.16: MPM images of DAPI stained cross sections of mouse bladder wall. Collagen shown in green and cell nuclei in blue. SMC are identifiable by elongated shape and arrange local to the detrusor. Arrows show sparse cell population in the lamina propria.

2.6 Discussion

Detailed morphology of murine bladder was examined using MPM and orientation and collagen area fraction quantified in young and aged mice. DAPI was used to stain tissue and observe cell content of the individual wall layers in cross sectioned and planar tissue strips. For the first time, layer specific structure orientation of the tissue was quantified and compared between young and age and hypotheses into individual constituent roles made.

Differences in young and aged tissue were quantified by orientation and area density fraction in both detrusor and the surface of the lamina propria. It is known there is correlation between the aging and reduction in mechanical function of the LUT. Loss of compliance is one such impact observed [67]. It was hypothesised that increased deposition of collagen, amongst other changes, is one of the mechanisms responsible for stiffening and geriatric voiding impairment in the elderly. Increased collagen in the detrusor is also characteristic of fibrosis; commonly seen in LUT of the elderly [58]. Contrarily, MPM in the present study reveals a decrease in area fraction of collagen in the lamina propria by approximately 5 % in aged tissue. [64] find increased total collagen content in aged rat when compared to young, however layer specific content is not measured.

[84] report significantly decreased collagen content in both lamina propria and detrusor in aged rats. Collagen content in the detrusor in this present study is found to increase marginally, agreeing with observations made by [64]. Human urinary bladder studies suggest that collagen deposition may be sex specific, as one study finds an increase of 10 % and a decrease of 3 % in females and males, respectively [85]. Reasons for the differences between study findings are unknown, however may be reflective of strain / species-specific variations or development of age related changes in

microstructure.

The bladder is capable of significant remodelling in a very short period during disease. Whilst it is known mechanical changes to the tissue may occur during aging, the underlying structural changes specific to wall layers have not been identified. Measurement of orientation of collagen in the lamina propria shows minor differences between young and aged tissue, although the layer is largely isotropic. Orientation in the lamina propria has not been previously quantified, however a scanning electron studies reveals collagen fibrils to have a random dispersion along various directions [9]. This arrangement remains until the transition to the detrusor where collagen becomes highly aligned in organised local bundles. Here, two main families of fibres in the detrusor are identified in young and aged tissue with mean fibre angles of each family orientated nearly perpendicular to each other.

Measurement of structure orientation reveals two fibre families present in the detrusor. Observations of two fibre families are reported in several studies and also correlates with similarly aligned smooth muscle orientation [79]. Stronger alignment of fibres in the longitudinal axis of young murine bladder was observed; a characteristic supported by [79]. A stiffer longitudinal mechanical response would be expected as a result of this fibre alignment, particularly at higher strain (in the collagen phase). Conversely, aged tissue would be expected to have a relatively similar response in both axes. Representative biaxial testing by [13], [15] and [17] demonstrates a stiffer longitudinal plane in rat and porcine bladder, supporting this hypothesis [13] [15] [17]. The cause of this remodelling in aging has not been identified, although changes to a tissue's structure are typically subject to the mechanical environment it is required to deal with. For example, in spinal cord injury rats the inability to void for several days causes high intravesical pressure which triggers a series of compensatory mechanisms in the wall tissue, including loss of compliance [79]. Throughout aging it is likely the LUT will

have to remodel specifically to an individual's mechanical environment to function in the most efficient way and may not be consistent across the population.

Collagen in individual layers of the bladder were observed from cross sectional and planar orientations using MPM in stained and unstained tissue. The surface of the lamina propria, i.e. directly below the urothelium (mucosa), has highly undulated rugae of varying height and geometry. Covering the rugae is a dense mesh of loosely woven collagen which is observed to have two configurations depending upon depth. Initially, collagen has an organised woven mesh with perpendicular overlapping fibres and small pockets where no collagen is seen. Similar observations are made using MPM by [86] and demonstrated that this layer interfaces with the urothelium. Deeper into the mucosa a dense stroma is seen and collagen in this region lies in a loose, wavy state. Bundles of fibrils observed in the stroma are relatively small compared with fibril bundles deeper in the lamina propria. Collagen retains this appearance further into the lamina propria, although fibril bundles increase in size and density decreases.

Cross sectioned bladder wall allowed the deeper lamina propria to be observed, where signal would be hindered in planar sections. A clear transition is seen between the urothelium boundary layer as collagen becomes significantly more sparse. Fibril bundles are large and loosely woven. Thickness of the bulk of the lamina propria varied significantly due to the large undulations of the rugae. It is likely that stretching the tissue slightly would allow for the rugae to flatten enough to obtain a more uniform thickness of rugae for quantification. When unloaded, rugae are primarily orientated so that the folds run along the longitudinal axis; circumferential folds were seldom observed. The folds are known to flatten during loading of the bladder wall; giving the bladder a highly extensible structure that can undergo large deformations [3].

Collagen, elastin and smooth muscle in the detrusor was imaged from the outer surface and throughout wall depth in cross sectioned strips. Immediately below the lamina pro-

pria an abrupt change in collagen morphology is observed at the transition to the detrusor. Loose and wavy collagen in the lamina propria changes to locally aligned straighter bundles of fibrils. Planar images clearly show bundles of fibrils grouped together following a similar alignment; closely related to the orientation of the surrounding smooth muscle seen in other imaging channels and DAPI stained tissue. Cross sections reveal smooth muscle and collagen are closely aligned and lie in two perpendicular layers. Closer to the lamina propria the smooth muscle and collagen layer runs longitudinally, compared to the circumferential outer layer. This configuration of smooth muscle and collagen varies according to animal / human tissues. For example, [87] show rat bladder detrusor has two perpendicular layers similar to mouse, [88] reports guinea pigs to have three poorly defined layers (inner and outer longitudinal, and intermediate circumferential). Large, highly tortuous blood vessels were observed on the outer surface of the detrusor. These vessels typically spanned the entire longitudinal height of the bladder and were identified with high collagen and elastin content. Smaller blood vessels were seen in the deeper lamina propria, directly on top of the detrusor in cross sections.

Cell population throughout the wall depth in wall cross sections and planar strips of the bladder was visualised by DAPI staining. Cross sections of the bladder reveal a majority of the cellular content is located in the detrusor muscle. Elongated SMC nuclei are seen throughout the layers of the detrusor, alternating orientation in perpendicular directions between muscle layers. Bundles of collagen fibrils are closely located to SMC nuclei; acting as a sheath to the muscle bundle, providing structural support [3]. Cell population in the adventitia may indicate the presence of nerve cells that provide innervation to the detrusor. Additionally, the presence of macrophages and fibroblasts is observed in the adventitia by [76]. The lamina propria was found to have sparse cell density. The cells located in the lamina propria are typically composed of fibroblasts, myofibroblasts and interstitial cells; however, further imaging is required to identify

the individual cell populations [89]. The mucosal surface of the lamina propria has a large cell density confined within the thin layer. It would be beneficial in future studies to differentiate cell populations in individual anatomical layers; potentially identifying changes to cell phenotypes and location based on age, pathology or injury.

This imaging study has limitations. Ability to capture microstructural information at deeper regions in the tissue was limited to approximately 200 μm from the outer surface and further reduced when imaging from the lumen surface due to the density of collagen at the mucosal surface. This was overcome to an extent by imaging from both surfaces of the bladder wall and performing additional cross sectional imaging studies that allowed observations of the entire wall thickness to be made. Further studies may look to improve imaging penetration depth through optical clearing of tissue and would allow better quantification of through wall collagen area fraction density and orientation. Additionally, description of fibre angles is challenging. The current method uses a single metric to describe the absolute fibre angle, which works well if all fibres are narrowly distributed along a single orientation. It becomes more challenging to use a single metric when fibres are more dispersed. Dispersion may be measured by fitting a Gaussian, or similar, distribution and integrating to obtain area under the curve (i.e. the smaller the area, the narrower the distribution of fibre orientation). For modelling purposes, described in later Chapters, an alternative method is outlined to more accurately quantify and compare fibre orientation between specimen.

Another constraining factor of this study is the lack of human tissue; as availability of cadaver tissue was limited and biopsies are rare. Murine bladder tissue was available for this study. Porcine bladder would provide improved relevance, however the lack of a disease or aging model made the murine model a more viable option. To the best of the author's knowledge a comparative study between porcine / murine and human bladder structure does not exist. Apart from size differences, the most notable structural

difference between murine and human is the detrusor layers. Human bladder tends to have three distinct layers; two orientated longitudinally and one circumferentially; each surrounded by collagen rich bundles. In smaller animals; such as rabbit and murine the collagen bundles and muscle arrangements are less complex and typically only 2 layers (longitudinal and circumferential) are observed [30]. Differences in layer arrangement is likely to alter direction specific behaviour.

A comparative study between the electrical properties of the detrusor in porcine and human shows similarities between the two species [90]. Several characteristic electrical properties are shared by pig and human; although coupling between detrusor smooth muscle cells is less extensive in porcine. Differences in contractile strength in murine and human and shown as contraction potential at optimal muscle length has been reported as 200 mN/mm^2 in humans and 60 mN/mm^2 in mouse detrusor [30]. Porcine bladder has also received attention as a replacement scaffolding material due to its similar mechanical properties to human [91]. Similar to murine tissue porcine bladder is highly heterogeneous across different anatomical regions with varying levels of anisotropy, depending upon elastic fibre orientations [17]. Whilst this study provides detailed microscopic information of murine urinary bladder structure and function, further tests involving porcine or human tissues would be beneficial in providing more relevant data for clinical use. Additionally, despite structural, mechanical and size variations between human and murine bladder, the availability of healthy and aged / diseased murine models make them a useful tool for extracting functional and mechanical behaviour and changes of the LUT and also testing hypotheses.

In depth insight into murine bladder wall morphology was obtained and quantified using MPM imaging studies. 2D and 3D images reveal the complex microstructure of individual anatomical wall layers. Insight into the microstructural mechanisms that allow the bladder to undergo large deformations during filling and voiding is obtained,

allowing speculation into the load bearing roles of individual layers and microstructural constituents. Quantified information such as orientation and collagen area fraction may allow differentiation between healthy and diseased tissue in future studies, improving understanding of how pathology may influence growth and remodelling of the tissue. Better understanding of the individual layers of the bladder and the microstructure, and how these change with age or pathology provides insight into the mechanical behaviour and functionality at a tissue and organ level. Information gained from quantification of microstructure can be used to aid constitutive modelling of soft tissues and hence model the mechanical behaviour of the organ.

Correlation of Microstructure and Macroscopic Mechanical Behaviour in Adult and Aged Murine Urinary Bladder

3.1 Introduction

Distension of the bladder is used in urodynamic studies to characterize the functionality of the LUT by assessing mechanical function and is the primary clinical method for the diagnosis of bladder disorders leading to lower urinary tract symptoms. Whilst there is good understanding of functional compliance measures in the clinical context, the contribution and role of the various bladder layers and constituent contribution to overall compliance is not well understood.

For simplicity, a majority of experimental studies investigating the constitutive material behaviour of planar soft tissue use uniaxial or biaxial testing [22], [92], [93], [91]. Uniaxial testing is limited by only applying deformation in a single direction; typically not relevant in a physiological setting, i.e. tissue will be subject to deformation across a broad range of orientations. This is a particular challenge in fibre reinforced materials as fibres will typically reorientate preferentially to loading [92]. Biaxial deformation

lessens these limitations significantly and provides closer insight to physiological deformation.

To date, there have been various studies examining the mechanical properties of the bladder using uniaxial [14], biaxial [94] [95] and pressure-inflation [96] [20] studies. However, the contribution of individual wall layers or constituents has never been attributed to characteristics of the macroscopic mechanical response.

The identification of the role of individual bladder wall constituents and structures serves to improve our understanding of the deformation mechanisms that allow the bladder to function, consequently aiding interpretation of clinical functional testing. Similarly, identification of the key microstructural components that may be altered or damaged during disease or aging may allow us to predict growth and remodelling or better predict intervention outcomes. The aim was to gain improved insight into the role of wall constituents and layers during wall deformation in murine bladder. Using a combination of MPM and uniaxial, biaxial and inflation testing a comprehensive overview of anatomical wall layer and constituent behaviour to loading was observed and quantified. Additionally, changes to the morphology and mechanical behaviour that occur with aging were identified.

3.2 Collagen Recruitment

As one of the key structural proteins, collagen significantly influences the mechanical behaviour of soft tissues. When unloaded collagen fibres adopt a coiled state, and do not contribute to tension bearing. Only when the tissue has undergone sufficient stretch fibres will straighten and subsequently bear load [15] [2]. This stretch is known as the recruitment stretch and plays a vital role in the mechanical response of a soft tissue. The general concept can be seen in Figure 3.1.

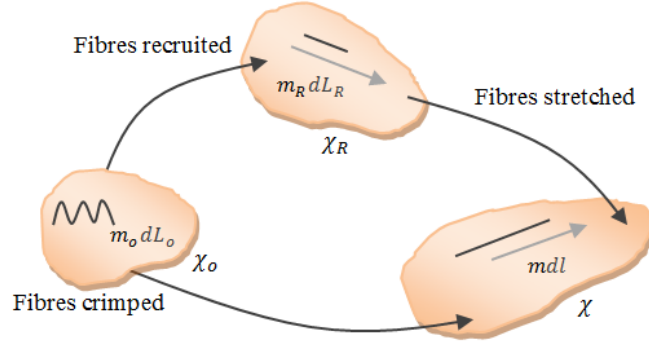


Figure 3.1: General schematic of collagen recruitment concept with considered configurations later discussed in modelling sections. Initially fibres are crimped until pulled by a finite tissue stretch where they eventually become taut. These straightened fibres are now able to bear tension and contribute to stress with further stretch. Typically both initial waviness and fibre orientation relative to loading direction will influence the stretch at which the fibres are recruited.

A key aspect of structural constitutive models is quantification of fibre recruitment stretch, which can be directly imaged and measured from tissue subjected to uniaxial, biaxial or inflation stretching. Multiphoton microscopy is an ideal imaging modality for this type of investigation as it does not require destructive fixing or staining, meaning the same sample can be tested at different loading states [97]. MPM has been previously shown as a tool for investigation of collagen fibre waviness and fibre recruitment in artery [92], [98]. Both studies show fibres straightening at increasing tissue stretch, both under uniaxial and inflation conditions; however, there are substantial differences in fibre recruitment fraction distribution. [98] shows abrupt fibre recruitment at low pressures of approximately 40 mmHg (peak systolic pressure usually 120 mmHg), whilst [92] shows fibre recruitment to initiate at higher stretches of over 1.5 (peak physiological stretch typically 1.3–1.4). Differences shown between studies may be the result of differences in reference configuration.

The challenge associated with correctly determining fibre recruitment is based on various aspects in loading, imaging, processing and analysis of data. Firstly, the choice

of straightness value whereby fibres are considered to have been recruited. There is currently a lack of literature that examines and optimises this key parameter; it is currently subjective. For example, [92] use a value of 0.95 compared with [99] who use 0.998. Differences between values used will be subject to imaging parameters, such as resolution and intensity, and fibre tracing technique. Secondly, the loading methodology will greatly influence fibre interaction, as shown between [92] and [98]. These factors influence observations; the extent of which have not been investigated to date and would provide an improvement to the experimental information implemented into constituent based models.

To date there has been effort to quantify recruitment stretch in arterial tissue [92], [99] and skin [100]. However, whilst studies such as [2] image the collagen uncrimping process in bladder tissue, there is a lack of quantitative studies specific to the urinary bladder. A common assumption is that the collagen fibres are continually being deposited and configured to the matrix in a way that is reflective of its role and the load it undergoes. Hence, understanding of how collagen fibres are configured may give insight into physiological changes due to pathology or aging. As an example, this is evident during bladder outlet obstruction, as collagen synthesis increases with severe obstruction [101]. Increased collagen deposition is also seen as a result of spinal cord injury, greatly reducing compliance of the bladder [13]. To date there is little information into how the collagen is configured, both in healthy bladder and diseased states, and how configuration influences bladder mechanics, and ultimately its functionality. Further insight into the relationship between collagen configuration and mechanical behaviour will improve interpretation of clinical pressure-flow studies and aid understanding of pathology progression.

The aim of this study was to visualise and quantify the detailed microstructure of wall layers of healthy mouse and rat bladder. Further, for the first time a comprehensive

insight into bladder wall interaction during loading was visualised using coupled MPM and in-situ uniaxial, biaxial and inflation mechanical testing. Interaction of collagen in the lamina propria and detrusor was quantified in terms of recruitment, orientation and dispersion during bladder wall loading giving insight into the relationship between collagen microstructure and mechanical response.

3.2.1 Methods

Collagen recruitment was measured directly from MPM images taken during both uniaxial and biaxial testing protocols (See Sections 3.3 and 3.4). After mean curve tests were complete the uniaxial and biaxial testing machines were fitted into their respective MPM platforms. Uniaxial testing was used to image the samples from a cross sectional perspective and planar perspectives, and biaxial imaged only from a planar view (See Figure).

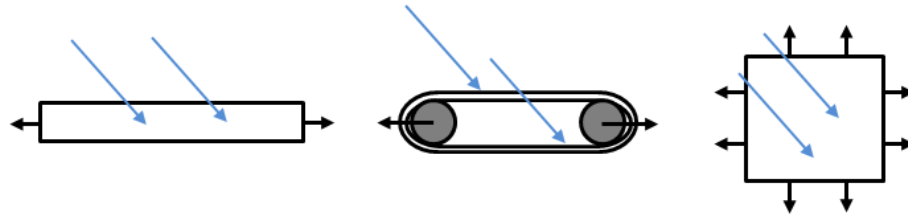


Figure 3.2: Array of various imaging perspectives taken during mechanical testing. (Left) Uniaxial planar, (Middle) Uniaxial cross section 'hoop', (Right) Biaxial planar. Black arrows indicate direction of loading during imaging.

Here, a brief overview of methods used to combine mechanical testing and MPM to obtain fibre recruitment data is given. Further details are given in the individual sections discussed later.

Recruitment: Uniaxial Protocol

After average curve testing was complete, the uniaxial testing machine was inserted

into the microscope stage with the sample clamped. All samples were attached with a 3 mm gap between clamp edges in planar cases and for cross section loops. The reference stretch ($\lambda = 1$) was obtained from the same reference state as the uniaxial tests (See Section 3.3 for protocols). After imaging in the reference configuration, the tissue was stretched in $\lambda = 0.2$ intervals, pausing at each increment to image the tissue until $\lambda = 2$. At each increment the stretched tissue was imaged from a single surface (either luminal or adventitial perspective in the case of planar tests). Imaging depth was limited by signal penetration of the tissue. Z-axis resolution was chosen specific to the individual tissue cases (subject to signal penetration, collagen density and tissue stretch) and ranged from 1 μm to 4 μm .

Recruitment: Biaxial Protocol

All biaxial testing was performed at the University of Pittsburgh, Mechanical Engineering & Materials Science, Bioengineering Department in collaboration with Prof. Anne Robertson and F. Cheng. Equibiaxial stretching was initially performed at stretch increments of 0.1 from the initial reference configuration ($\lambda = 1 : 0.1 : 2$). After several tests it was discovered that collagen would not become taught until stretches exceeding at least 1.6. To optimise experimental time and quality of recruitment distributions, the first recorded imaging sequence was at equibiaxial stretches of 1.6 (1.8 in some cases) with a finer stretch increment of 0.05 until a stretch at which the tissue began to fail (ranged from $\lambda = 2$ to 2.2). This approach allowed better insight into the physiological stretch range of collagen mechanical function and increased information for model fitting. At each stretch increment a single surface was imaged with maximum obtainable tissue penetration depth. Resolution of the z-axis was dependant upon the individual tissue case and ranged from 2 μm to 4 μm (Due to practical time constraints or image penetration depth).

Fibre Tracing and Recruitment

After a sequence of images was obtained, the fibres were traced in the first layer of detrusor muscle at each level of stretch using *NeuronJ*, an *ImageJ* plugin (for details see [102]). This semi-automated method was used for all 2D images. 30 fibres were traced in at each stretch increment; giving fibre length and end-to-end point distance (See Figure 3.3. Of these 30 fibres, 20 were randomly selected by a Matlab program to reduce user bias. A relative measure of straightness of each fibre was then obtained as a straightness ratio (denoted as τ) between the end-to-end distance, l , and total fibre length, L , i.e. $\tau = l/L$. Straightness values therefore range from 0 to 1, where fibres with $\tau = 1$ are completely straight and fibres near 0 highly crimped.

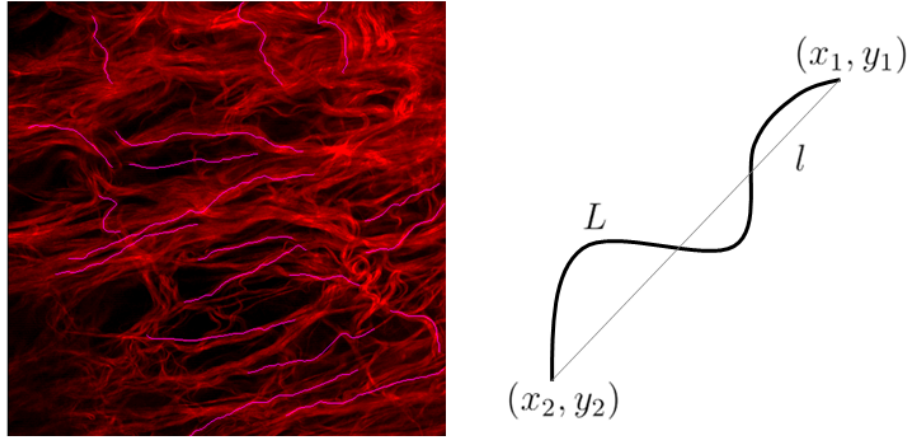


Figure 3.3: (Left) Selection of collagen fibres chosen for tracing on 2D planar MPM image. Traced fibres seen in magenta. (Right) Method of calculating straightness ratio of traced fibres, where (x_i, y_i) denote end points of the fibre.

Fibres traced were identified as either 'recruited' or 'non-recruited' based on a straightness ratio threshold value using a custom Matlab script. This method was used as a fibre will never be 100 % straight due to imaging and tracing inaccuracies. As such the fibre threshold must be set < 1 . Values of this threshold have been given previously as 0.98 [92] and 0.998 [99]; however these values will depend upon specific imaging system

and tracing methods. Based on parameter studies a value of 0.95 was chosen. After thresholding a percentage of fibres recruited was obtained at each level of stretch.

To obtain fibre recruitment as a function of stretch in fibre direction, fibres were mapped to the reference configuration using current orientation, θ and principle in-plane stretches λ_1 and λ_2 ,

$$\tan \theta = \frac{\lambda_1}{\lambda_2} \tan \theta_0 \quad (3.1)$$

Initial orientation, θ_0 , was then used to obtain stretch in the fibre direction from the reference configuration. All recruited and non-recruited fibres with their associated fibre stretches were separated in 0.05 stretch bins to obtain the final recruitment distribution.

Section Overview

The following sections of this chapter are separated into uniaxial, biaxial and inflation testing - in this order. Imaging and loading protocols are contained within the individual section headings for each testing method.

3.3 Uniaxial Testing and MPM Imaging

An *in-situ* system allowed MPM and uniaxial mechanical testing to be used to capture stress-stretch response with imaging of collagen in the bladder wall under incremental stretch. Young adult and aged murine bladder wall was used in this study to compare collagen microstructure and mechanical response of each tissue type. Observations of collagen in the lamina propria and detrusor muscle are made and correlated with finite points along the stress-stretch curve.

3.3.1 Methods

Tissue samples

Urinary bladders from 3 to 4 month-old young adult (referred to as young) and 23 to 24 month-old (referred to as aged) were extracted from 16 male C57/BL6 mice (8 of each type) for uniaxial mechanical testing, 10 mice for cross section mechanical testing (5 of each), and an additional 10 (5 of each) mice for imaging studies. Mice were sacrificed by cervical dislocation following isoflurane (sacrifice of animals followed good laboratory practice in compliance with UK laws and regulations). Intact bladders were placed in phosphate buffer saline and kept frozen at -20°C in excess of 24 hours. The absence of calcium in the storage medium ensured that the bladders were kept in an inactive state as to eliminate influences of spontaneous smooth muscle contraction. All experiments were carried out under an approved UK protocol and project license and in accordance with the University of Sheffield Animal Care Committee.

Prior to imaging and mechanical testing samples were removed from the freezer and allowed to warm to ambient temperature. For mechanical testing rectangular strips were cut along the circumferential axis, removing the apex and base regions. Full length strips were cut to measure approximately 12 x 4 mm. Thickness and width dimensions were measured three times with a CCD optical micrometer and values averaged.

Uniaxial Testing

Mechanical testing data was obtained using a custom-built tensile device (linear actuator Zaber T-NA08A25, Vancouver, Canada; precision $<1\ \mu\text{m}$, 20N load cell, Interface, Scottsdale, USA; precision 10 mN) designed to fit onto the MPM table. Tissue strips were loaded in the device with circumferential axis aligned to extension plane and set

to a 3 mm separation between clamp edges. Clamps were submerged in a bath of phosphate buffer saline (PBS) at ambient temperature. The system was calibrated both before and after tissue was clamped into place to ensure the tissue was not subjected to pre-strain and minimise slack length. This allowed $\lambda = 1$ to be defined as the stretch at which the tissue was unstressed, i.e. passive components are bearing no load.

Prior to recording the tissue was subjected to three preconditioning cycles to a stretch of 1.5 and allowed to rest for 5 minutes between each test. Uniaxial stretch was applied according to a triangular ramp function at a strain rates of $1 \% s^{-1}$ up to a maximum stretch limit of 2. Loading was recorded three times and values averaged. Tissue was allowed to rest for 5 minutes between each extension.

Imaging Protocol and Acquisition

A Zeiss LSM 710 inverted confocal microscope (Zeiss LSM710, Oberkochen, Germany) fitted with a pulsed femto-second Chameleon laser operating at 860 nm was utilized for imaging urinary bladder tissue. Collagen and elastin constituents were imaged at wavelengths of 430 nm and 520 nm respectively with spectral bins of ± 20 nm at magnifications of 20x (air objective). All dimension measurements from images were made using *ImageJ*; taken three times and averaged. SD was calculated for dimensions at each stretch interval.

3.3.2 Results

Mechanical Response: Age vs. Young

Uniaxial testing was performed on $n = 8$ young and $n = 8$ aged mouse bladders and mechanical response recorded. Figure 3.4 shows the collective mechanical responses of

all tests at continuous strain rate of $1 \% s^{-1}$ to a maximum stretch of 2. Aged mouse bladder shows a shorter toe region compared with young bladder; indicating an earlier progression into the collagen load bearing phase. The range of stretches over which the toe region occurs was also much greater in aged material; between approximately 1.3 and 1.7. The toe region remains until stretches of approximately 1.6 to 1.8 in young bladder.

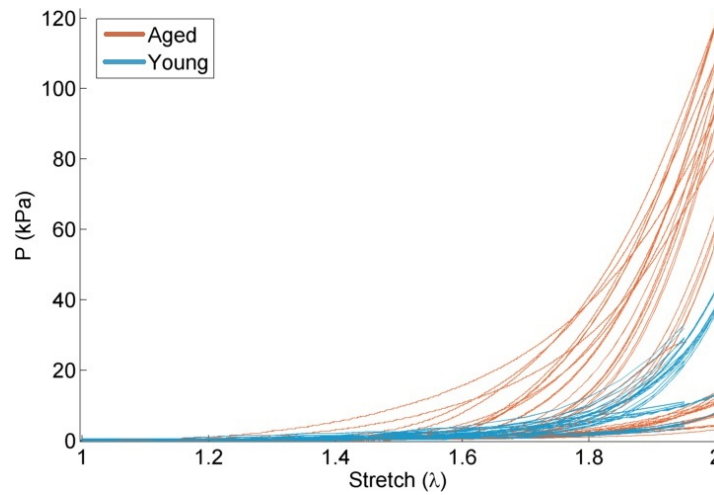


Figure 3.4: Plot of 1st P-K stress versus stretch along the circumferential axis of 16 mouse bladders (8 young, 8 aged) under uniaxial extension.

The non-linear stiffening of the bladder occurs following the heel region of the curve in both bladder types. Stiffness at higher stretches was greater in aged bladder when compared with young bladder; demonstrated by a steeper gradient. Additionally, two subsets of young and aged bladder mechanical response were demonstrated, labelled 'compliant' and 'non-compliant' (See Figure 3.5). No identification of the possible cause was found.

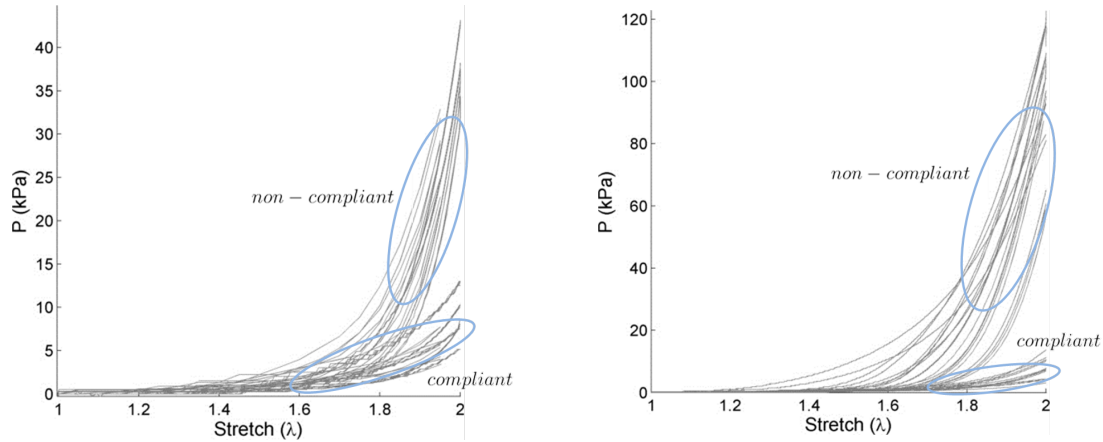


Figure 3.5: Separate 1st Piola-Kirchoff vs. stretch uniaxial tests of young (left) and aged (right) hi-lighting two identified sub-groups seen in uniaxial testing.

MPM Imaging of Mouse Bladder under Uniaxial Stretch

Bladder tissue strips were imaged using MPM under incremental stretch in a uniaxial testing device is seen in Figure 3.6. Figure 3.6a shows the inner surface of the bladder wall. Initially, large undulations of the rugae are seen with a fine, dense collagen network forming the surface. At a stretch of 1.4 rugae begin to flatten and at 1.6 have almost completely flattened. Collagen at this stretch remains loose and with no observable preferred orientation. At a stretch of 2 the rugae flatten entirely and collagen reorientates in the direction of loading. Fibrils straighten across the wall.

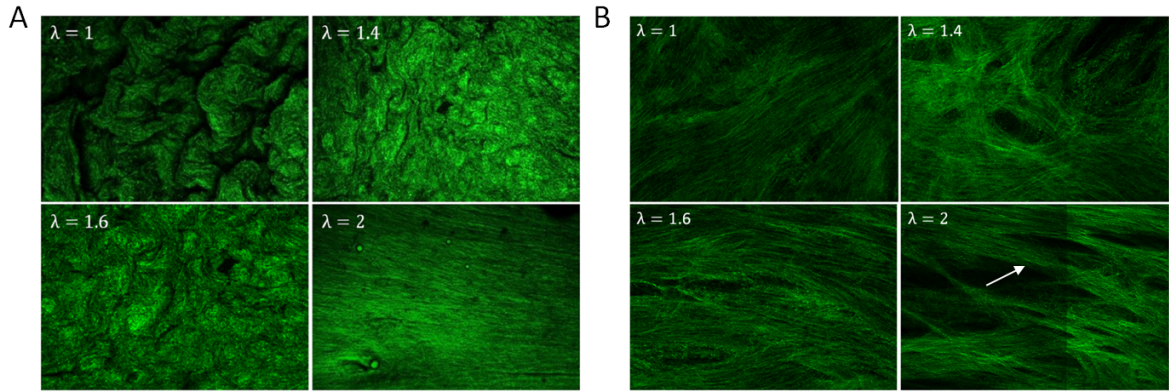


Figure 3.6: MPM images of collagen in the lamina propria surface (A) and detrusor (B) layers under incremental uniaxial stretch. Arrows indicate visible lateral thinning of fibre bundles in the detrusor.

In the detrusor layer initially fibres appear straight with a strong local alignment specific to each bundle (Figure 3.6b). A stretch of 1.4 shows fibrils to retain their straightness. Multiple grouped bundles of fibrils become visible in the same plane. Fibres begin to reorientate in the direction of loading at a stretch of 1.6. A stretch of 2 shows fibres pulled highly taut in the direction of loading and bundles undergo lateral thinning.

Stress-stretch response of young murine bladder exhibits the highly non-linear behaviour seen in many soft collagenous tissues (Figure 3.7). The compliant toe region occupies a large stretch region to approximately $\lambda = 1.6 - 1.75$. Throughout the toe region rugae are highly tortuous and loose. At the initiation of the heel region a significant increase in stiffness occurs, coinciding with flattening of the rugae. Further stretch shows further stiffening of the tissue and images reveal collagen to realign in the direction of loading and straighten completely.

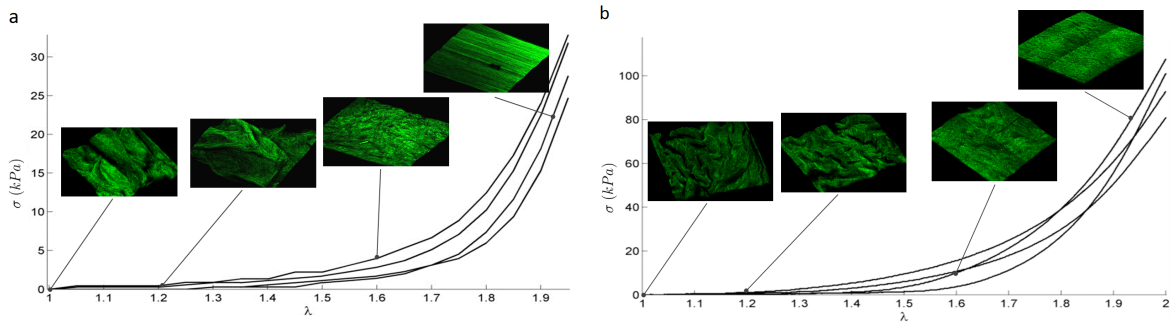


Figure 3.7: Average uniaxial stress-stretch curves for young (a) and aged (b) murine bladders ($n = 4$ per group) with corresponding 3D reconstructions of representative MPM images of the inner surface of the lamina propria at stretches of (a) 1, (b) 1.2, (c) 1.6, (d) 2.

After imaging the lamina propria bladders were flipped and imaged from the detrusor side during uniaxial testing. Figure 3.8a and 3.8b show representative aged and young MPM images under tensile testing at incremental stretch. The aged sample shown shows fibres to rapidly become taut in the direction of loading. Perpendicular fibres are loose and wavy at all stretches. Fibres in young detrusor appear more dispersed when tissue was at rest. Fibres realign under small stretches (approximately 1.2) and are subsequently pulled taut. No perpendicular fibres are seen in this image series.

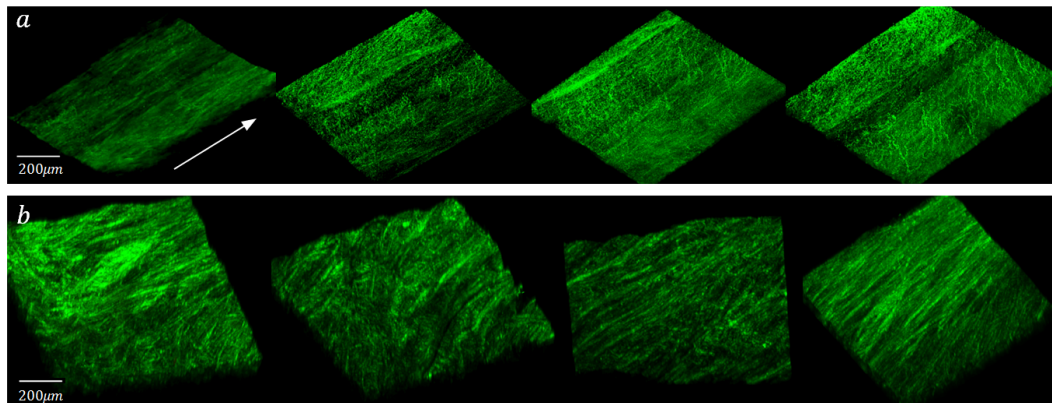


Figure 3.8: Representative MPM images of aged (a) and young (b) bladder outer wall surface imaged from detrusor side under incremental uniaxial stretch. Stretch intervals of $\lambda = 1, 1.4, 1.6$ and 2 are shown. Arrow shows approximate direction of loading.

Circumferential strips of the bladder body were dissected to obtain rings of tissue. Rep-

Table 3.1: Measured thickness of lamina propria and detrusor in cross sections of ($n = 4$ young, $n = 4$ aged) uni-axially stretched bladder wall. Measurements shown with $\pm$ SD. Percentages show proportion of wall thickness occupied by detrusor.

Stretch	Aged			Young		
	Det (μm)	LP (μm)	(%)	Det (μm)	LP (μm)	(%)
1	342 ± 112	224 ± 63	59	431 ± 54	272 ± 69	62
1.13	333 ± 126	237 ± 53	57	407 ± 62	256 ± 61	62
1.25	309 ± 93	195 ± 64	61	360 ± 47	219 ± 31	62
1.38	265 ± 97	225 ± 75	54	354 ± 56	201 ± 42	64
1.5	277 ± 83	191 ± 71	59	300 ± 38	176 ± 23	63
1.63	255 ± 87	204 ± 58	56	277 ± 20	150 ± 25	65
1.75	245 ± 55	146 ± 25	63	243 ± 40	133 ± 24	65
1.86	247 ± 77	176 ± 35	57	239 ± 55	119 ± 29	67
2	243 ± 54	144 ± 31	62	223 ± 63	117 ± 12	65

representative through-wall MPM images of aged (a) and young (b) murine bladder cross sections under incremental uniaxial stretches are shown in Figure 3.9. Measurements from all ($n = 5$ aged, $n = 5$ young) samples of lamina propria and detrusor thickness with standard deviation are shown in Table 3.1.

Bladder wall dimensions and percentage wall thickness of the detrusor and lamina propria given Table 3.1 illustrate relative layer thinning in response to stretch. The ratio between lamina propria and detrusor in aged tissue fluctuates during stretch and neither layer preferentially thins under stretch. Several incidences are observed where the detrusor appears to preferentially thin under stretch, however these may

be within lower margins of measuring errors. Consistent preferential thinning of the lamina propria was observed in young bladder wall, however only by several percent (and may be subject to measuring error). Consistent overall wall thinning was seen in both tissue types. Young bladder wall shows the largest reduction in thickness of detrusor and lamina propria (49% and 57%) compared with 29% and 50%, respectively, in aged bladder.

Cross sections of bladder wall in young and aged tissue in Figure 3.9 show collagen through the entire wall depth under incremental stretches. A distinct boundary can be observed between the detrusor and lamina propria, typically the lamina propria appears to have high collagen content and high contrast. The detrusor typically has fewer collagen fibres, and fibres are more aligned. At low stretches small bundles of folds can be seen along the wall and are visible until higher stretches.

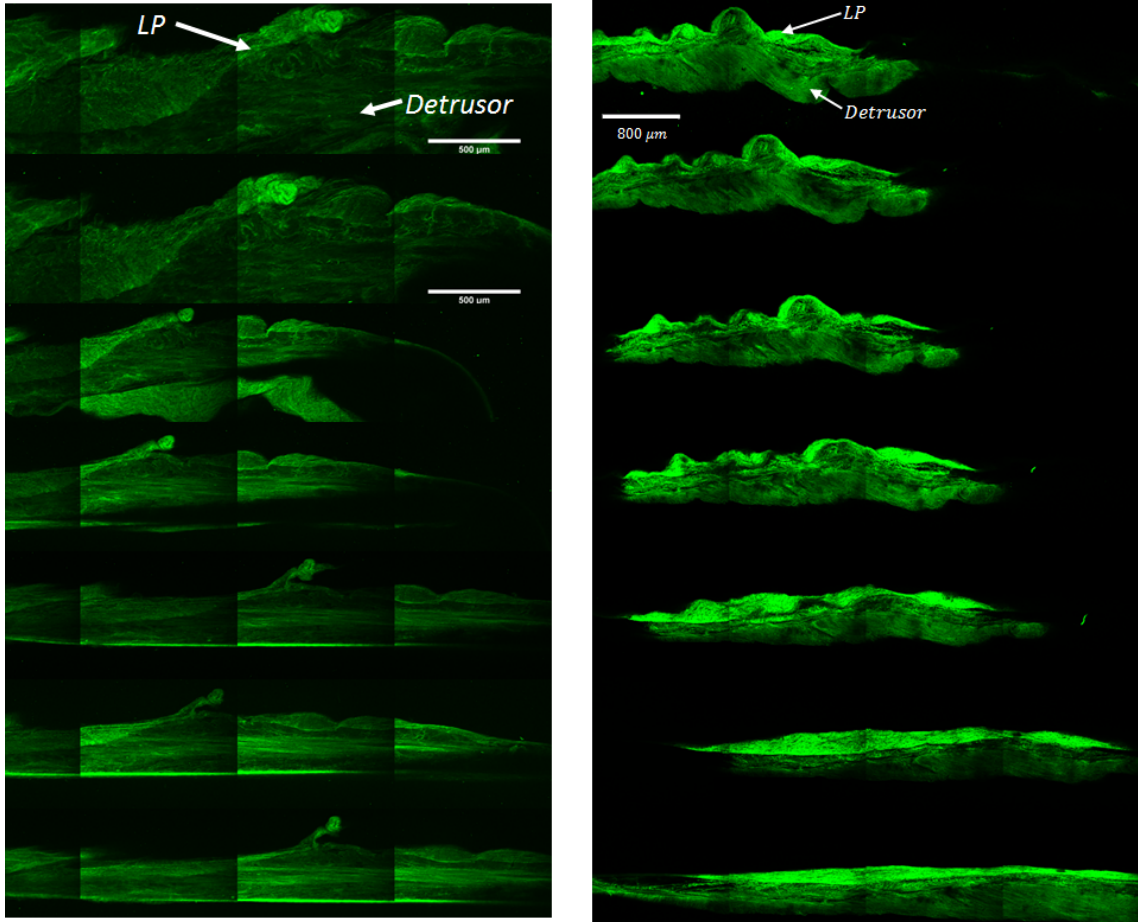


Figure 3.9: 2D MPM images of collagen in young (a) and aged (b) murine bladder body wall cross section under uniaxial stretch; ranging from $\lambda = 1$ to $\lambda = 2.2$ in increments of 0.2. Detrusor and LP tags denote orientation of bladder strips. Note, vertical lines seen in MPM tiles are artefacts caused by poor merging of imaging in MPM software.

3.4 Biaxial Mechanical Testing

Biaxial testing was performed to gain a more physiologically relevant method of loading the bladder wall, i.e. circumferential and longitudinal stretching, and to measure anisotropy. A custom in-situ biaxial device fitted under MPM was constructed at the University of Pittsburgh, Mechanical Engineering & Materials Science, Bioengineering Department (Prof. Anne Robertson, Fangzhou Cheng). This system was pre-designed by the group in Pittsburgh, however I was responsible for tailoring software and protocols for the bladder specimens. This system was used to image young and aged murine bladder wall strips obtained from University of Pittsburgh, Department of Pharmacology and Chemical Biology (Prof. Lori Birder, Dr. Aura Kullmann) under equibiaxial loading. Collagen microstructure, including recruitment, orientation and dispersion was quantified from MPM image stacks taken at discrete stretch increments.

Tissue strips extracted from the bladder wall are considered to be planar as the thickness is typically orders of magnitude smaller than length and width dimensions. Second order shear and bending effects are considered negligible under typical physiological loading. For the following, a planar stress state was assumed for analyses, whereby stress in perpendicular axes was zero.

In-plane stretches (λ_x and λ_y corresponding to stretches in x and y axes, respectively) are known for analyses and prescribed by actuators; local stretches can be measured optically at discrete time points. Surfaces in the z-plane are assumed to be traction free. X and x are points in initial reference states and deformed states, respectively. Other components of the deformation gradient, κ_i , describe the in-plane shear. Incompressibility was assumed as soft tissues largely consist of water, from which out of plane stretch λ_z may be calculated from stretch components of the deformation gradient, $\mathbf{F}$. Deformation gradient components may be approximated locally by linear interpolation,

which in this case was based on displacement of a four-node quadratic element,

$$u(\xi, \eta) = \phi_1(\xi, \eta)u_1 + \phi_2(\xi, \eta)u_2 + \phi_3(\xi, \eta)u_3 + \phi_4(\xi, \eta)u_4, \quad (3.2)$$

and,

$$v(\xi, \eta) = \phi_1(\xi, \eta)v_1 + \phi_2(\xi, \eta)v_2 + \phi_3(\xi, \eta)v_3 + \phi_4(\xi, \eta)v_4. \quad (3.3)$$

Here, $u(\xi, \eta)$ and $v(\xi, \eta)$ describe displacement in x and y directions as a function of the local coordinates, ξ and η . $\phi_i(\xi, \eta)$ is the shape function corresponding to each of the local node coordinates; 1,2,3 and 4. (u_i, v_i) is displacement of node i .

$$\begin{aligned} \phi_1(\xi, \eta) &= \frac{1}{4}(1 - \xi)(1 - \eta) \\ \phi_2(\xi, \eta) &= \frac{1}{4}(1 + \xi)(1 - \eta) \\ \phi_3(\xi, \eta) &= \frac{1}{4}(1 + \xi)(1 + \eta) \\ \phi_4(\xi, \eta) &= \frac{1}{4}(1 - \xi)(1 + \eta) \end{aligned} \quad (3.4)$$

Displacements obtained are used to approximate a position vector field, from which elements of the deformation gradient matrix can be populated to obtain local strain. As shape functions are not direct functions of x and y a Jacobian matrix is required to relate global coordinates to local coordinates ξ and η ,

$$J = \frac{\partial(x, y)}{\partial(\xi, \eta)}, \quad (3.5)$$

giving derivatives of shape functions in terms of the global coordinates,

$$\begin{pmatrix} \frac{\partial \phi(\xi, \eta)_i}{\partial x} \\ \frac{\partial \phi(\xi, \eta)_i}{\partial y} \end{pmatrix} = [J]^{-1} \begin{pmatrix} \frac{\partial \phi(\xi, \eta)_i}{\partial \xi} \\ \frac{\partial \phi(\xi, \eta)_i}{\partial \eta} \end{pmatrix} \quad (3.6)$$

Subsequently, local strain measurements can be computed using the following strain-displacement relation,

$$\mathbf{B}_i = \begin{bmatrix} \frac{\partial \phi(\xi, \eta)_i}{\partial x} & 0 \\ 0 & \frac{\partial \phi(\xi, \eta)_i}{\partial y} \\ \frac{\partial \phi(\xi, \eta)_i}{\partial y} & \frac{\partial \phi(\xi, \eta)_i}{\partial x} \end{bmatrix} \quad (3.7)$$

Then Lagrange strains in x and y approximated as follows,

$$\varepsilon_{L,xx} = \frac{\partial \phi(\xi, \eta)_x}{\partial x} + \frac{1}{2} \left[\left(\frac{\partial \phi(\xi, \eta)_x}{\partial x} \right)^2 + \left(\frac{\partial \phi(\xi, \eta)_y}{\partial x} \right)^2 \right] \quad (3.8)$$

$$\varepsilon_{L,yy} = \frac{\partial \phi(\xi, \eta)_y}{\partial y} + \frac{1}{2} \left[\left(\frac{\partial \phi(\xi, \eta)_y}{\partial y} \right)^2 + \left(\frac{\partial \phi(\xi, \eta)_x}{\partial y} \right)^2 \right] \quad (3.9)$$

Stretch is then calculated from Lagrange strain ($\lambda = \varepsilon + 1$). These steps allow calculation of local strain measures obtained directly from optical measurement of strain markers placed on tissue. The following sections will describe the experimental methodology used in this study.

3.4.1 Methods

Specimen Preparation

Intact bladders ($n = 10$; 5 young and 5 aged) were excised from Sprague-Dawley female rats approximately 10 months old, and immediately placed in HBSS with no calcium or magnesium for 15-30 minutes (composition in mM: 140 NaCl, 5 KCl, 0.3 KH₂PO₄, 4 NaHCO₃, 0 CaCl₂, 0 MgCl₂, 10 HEPES, 5.6 Glucose, 0.5 EGTA, pH 7.4, Osm 290-300mOsm/l). 10 μ M thapsigargin was added. Samples are then washed in PBS and kept chilled on ice until testing. These steps ensured internal calcium stores were depleted, eliminating spontaneous contractions of the smooth muscle. Prior to testing the dome and trigone regions of the bladder were removed and the remaining body cut open along the longitudinal axis. Samples were then trimmed to approximately 6 mm² before mounting (See Fig 3.10).

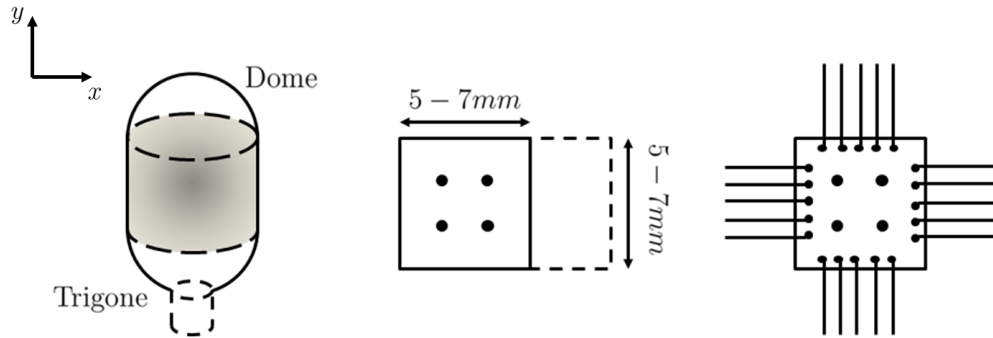


Figure 3.10: Schematic of steps in specimen preparation to mounting in the biaxial testing device. Excised bladders are cut longitudinally after the dome and trigone was removed. Samples are then trimmed to size and have microsphere markers attached before mounting.

Biaxial Mechanical Testing Protocol

A custom in-situ biaxial testing device was designed to be coupled with MPM imaging (University of Pittsburgh) for synchronised mechanical testing and scanning of bladder

tissue collagen interactions under loading (See Fig 3.11). The design of the biaxial device consists of a CCD camera and a 45° offset mirror system to be used to track strain markers on the bottom of the tissue whilst the MPM lens could be lowered overhead to scan tissue simultaneously.

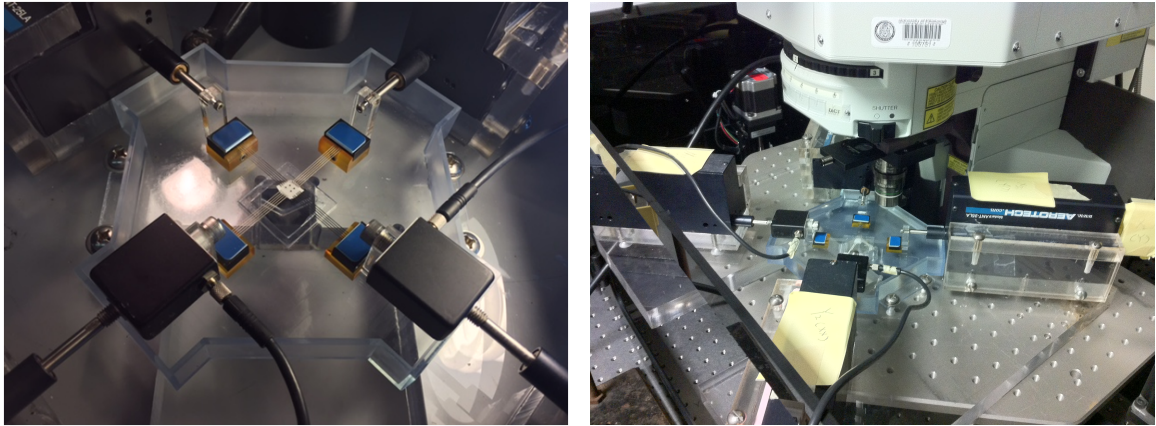


Figure 3.11: (Left) Biaxial device used to test square strips of bladder tissue. Each actuator provides displacement independently. 2 load cells measure forces across circumferential and longitudinal axes. 4 equally spaced microsphere markers are used to track local strain of the tissue under a CCD camera. (Right) Biaxial system in use with multiphoton imaging.

After specimen preparation they were mounted on a custom biaxial testing device and submerged in PBS at room temperature. Nefediprine ($5 \mu\text{M}$) was added to the solution to prevent further calcium entry. The loading directions x and y correspond to the circumferential and longitudinally axes, respectively. Each edge of the square sample was secured by 5 prong rakes (Worldwide Precision Instruments, FL, USA) pressed into the tissue. Rubber padding was placed underneath tissue during mounting to keep samples in a resting state and allow prongs to be pressed securely into the tissue. Force was measured in circumferential and longitudinal axes by two load cells and displacement controlled by 4 independent linear actuators. Local strain was obtained by imaging of 4 approximately evenly spaced black microspheres glued onto the surface of the tissue. Custom LabVIEW (National Instruments, Texas, US) software was designed to track microsphere markers (credit to F. Cheng, University of Pittsburgh)

imaged using a CCD camera system and derive local strain measures in longitudinal and circumferential axes. Force measurements sampled at the same frequency were paired with strain data.

The tissue was subjected to 5 consecutive preconditioning cycles of non equibiaxial stretch ratios i.e. $\lambda = 1.4:1.8, 1.6:1.8, 1.8:1.8, 1.8:1.6$ and $1.8:1.4$ at a strain rate of $1\%s^{-1}$. This was designed to condition the tissue in all loaded states and is a technique previously used in testing by [13]. Following preconditioning the sample was extended until reaching a load tare of 0.02 N. Dimensions and initial marker coordinates were measured in the preconditioned and load tared configuration (i.e. $\Omega_0, \lambda = 1$) and used to formulate all stress and strain calculations (See Fig 3.12).

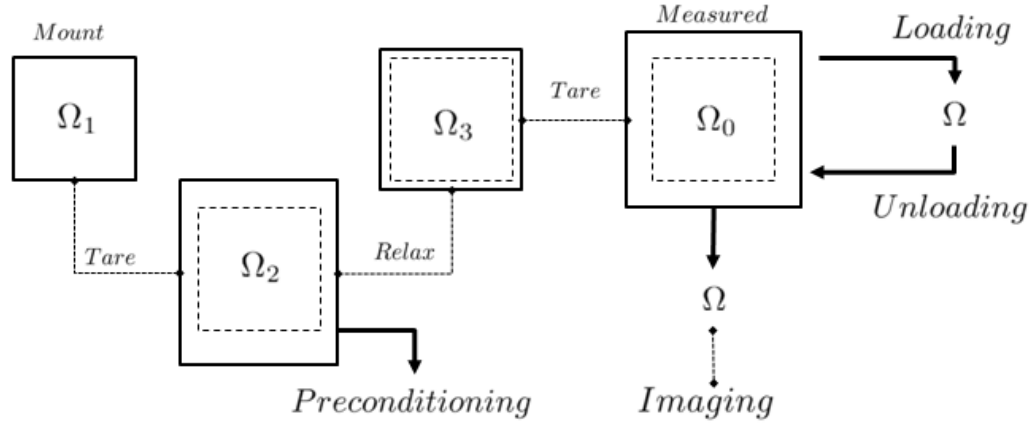


Figure 3.12: Schematic of individual loading states considered in testing protocols. Here, tissue mounting (Ω_1), initial tare (Ω_2), post-preconditioning (Ω_3) and reference states (Ω_0) are illustrated. (Ω) corresponds to the current loaded configuration during testing and is used as notation for both mean curve acquisition and deformation during imaging.

Biaxial testing was then performed over 5 cycles at a strain rate of $1\%s^{-1}$ to a maximum equibiaxial stretch of $\lambda = 2$. Average data curves were obtained over the five tests. Following biaxial testing, samples were distended at 5 intervals of stretch from the initial reference state ($\lambda = 1$) to $\lambda = 2$. At each stretch increment bladders were held

for 2 minutes before imaging at the extended state to allow the tissue to settle (and to reduce viscous movement during imaging).

Stretch Configurations

Stretch measures are obtained through applied stretch (clamp-clamp distance) and measure, local stretch (tissue markers). Stress-stretch curves were calculated from measured local stretches. MPM imaging was taken at intervals related to applied stretch. For consistency, this stretch was converted to local stretch. Stretch was applied linearly with a known strain rate, meaning linear interpolation could be used to match local stretches to a given applied displacement. MPM imaging intervals can then be expressed in terms of local stretches for modelling purposes. This section describes images taken in terms of applied stretch. Section 3.4.2 implements matching of stretch configurations, given in terms of local stretch.

Imaging Protocol

Imaging was performed using an Olympus FV1000 MPE (Tokyo, Japan) with a Spectra-Physics DeepSee Mai Tai Ti-sapphire laser (Newport, Mountain View, CA) and 1.12 NA MPE water immersed objective (25x magnification). Non-descanned epi-detectors captured second harmonic signal. Emission excitation wavelength of 870 nm with a 400 nm emission filter (Chroma, Brattleboro, VT) and ± 50 spectral bins was used to collect second harmonic signals over a 1024 x 1024 pixel region.

In-plane images were obtained from a mucosal perspective with following images scanned towards the detrusor in the z-direction in 2 - 3 μm increments (depending upon the total imaging depth). Images were acquired over an approximate time period of 20 minutes, where the tissue was held stretch for this period. Due to the thickness of the tissue, entire depth of the tissue could not be captured.

3.4.2 Results

Mechanical Response

Rat bladder strips from $n = 3$ young and $n = 3$ aged rats were subjected to equibiaxial stretch and force recorded in longitudinal and circumferential axes (Actual $n = 6$ for each specimen type, however results included artefacts or marker tracing was not possible). Figure 3.13 illustrates representative biaxial tests and compares direction dependant differences in young and aged bladder strips. Biaxial tests show anisotropy in young bladder strips and differences in onset of heel region. Aged bladder strips, in comparison, are more isotropic with similar directional response. Initial toe region compliance was lower in circumferential axes for young and aged tests when compared to longitudinal.

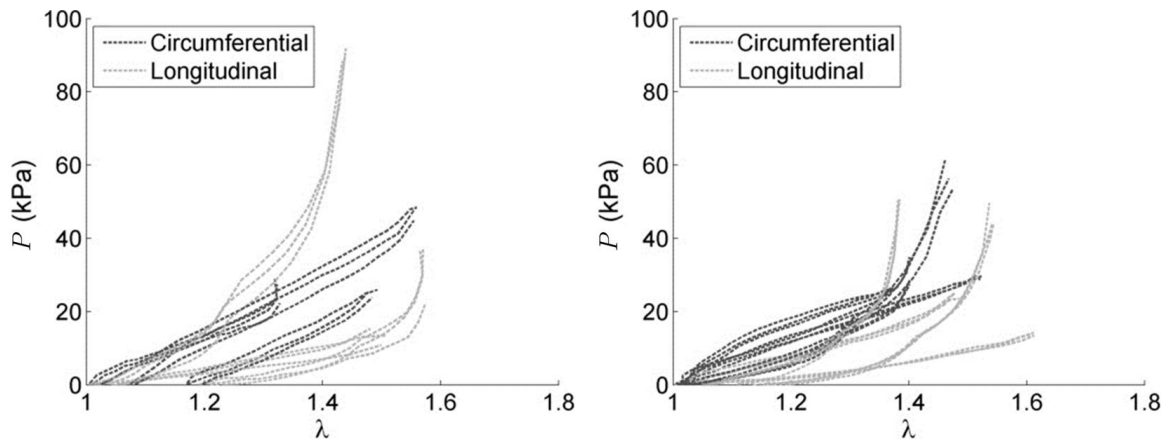


Figure 3.13: Representative 1st Piola-Kirchoff stress vs. stretch plots of $n = 6$ (3 aged, 3 young) individual biaxial tests showing longitudinal vs. circumferential stress vs. measured stretch in young (left) and aged (right).

Mechanical differences in young and aged tissues were compared according to direction in Figure 3.14. Circumferential stress vs. stretch in Figure 3.14(left) shows young and aged bladder to have similar compliance, particularly in low stretches up to 1.4. Differences in heel region are seen at higher stretches. Aged tissue increases in stiffness after

the heel region. Young samples did not show a steep increase in stress in comparison. Longitudinal stress shows a greater variation in sample compliance. Large differences are seen in onset stretch of heel region. Stress varies greatly between samples, for example at $\lambda = 1.4$ stress ranges from 4.5 to 58.6 kPa and 7.1 to 48.3 kPa in young and aged tests, respectively.

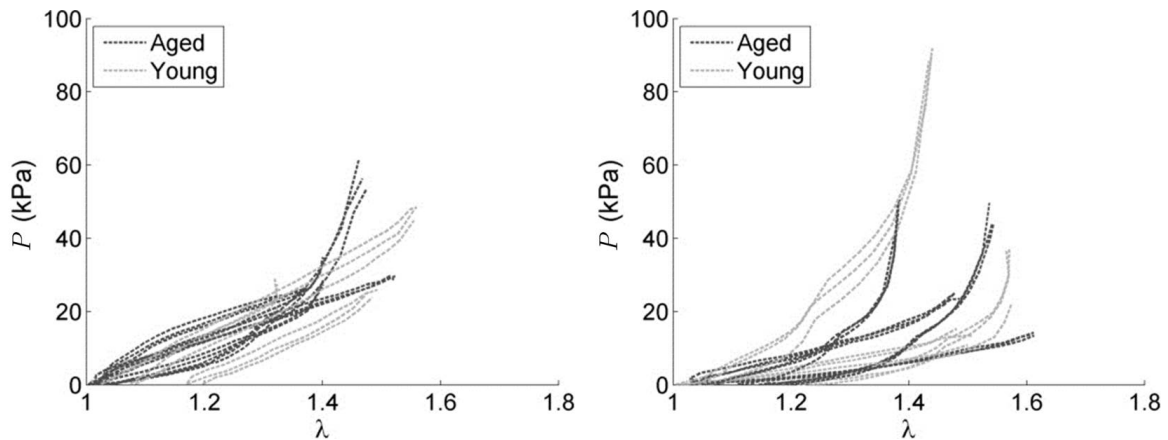


Figure 3.14: Representative 1st Piola-Kirchoff stress vs. stretch plots of $n = 6$ (3 aged, 3 young) individual biaxial tests showing differences between young and aged stress in circumferential (left) and longitudinal (right) directions.

Quantification of Collagen Recruitment of Young and Aged Rat Bladder under Biaxial Load

Collagen fibre recruitment was quantified at incremental biaxial stretches. Due to imaging constraints only the first layer of the detrusor (from here called 'shallow detrusor') was used for tracing.

Young specimens

MPM images of Fischer rat bladder, aged 12 months, stretched incrementally from $\lambda = 1.8$ to $\lambda = 2.15$ and imaged from a planar perspective are shown in Figure 3.15. Collagen fibres are seen to lie in a wavy state at $\lambda = 1.8$ in the lamina propria. A majority of fibres remain undulated until a tissue stretch of 2, where a progressive

straightening was seen. Further stretches up to 2.15 show incremental straightening of the remaining undulated fibres, and stretching of those fibres already recruited.

In the layers possible to capture using MPM a majority of fibres appear to predominantly align with the circumferential axis. Whilst appearing relatively isotropic even at stretches of 1.8, collagen fibres become gradually more aligned with the circumferential axis with increasing stretch. This indicates the fibres do not undergo any alignment changes until higher stretches; therefore they are unlikely to be playing a load bearing role until re-orientating and subsequent recruiting occurs.

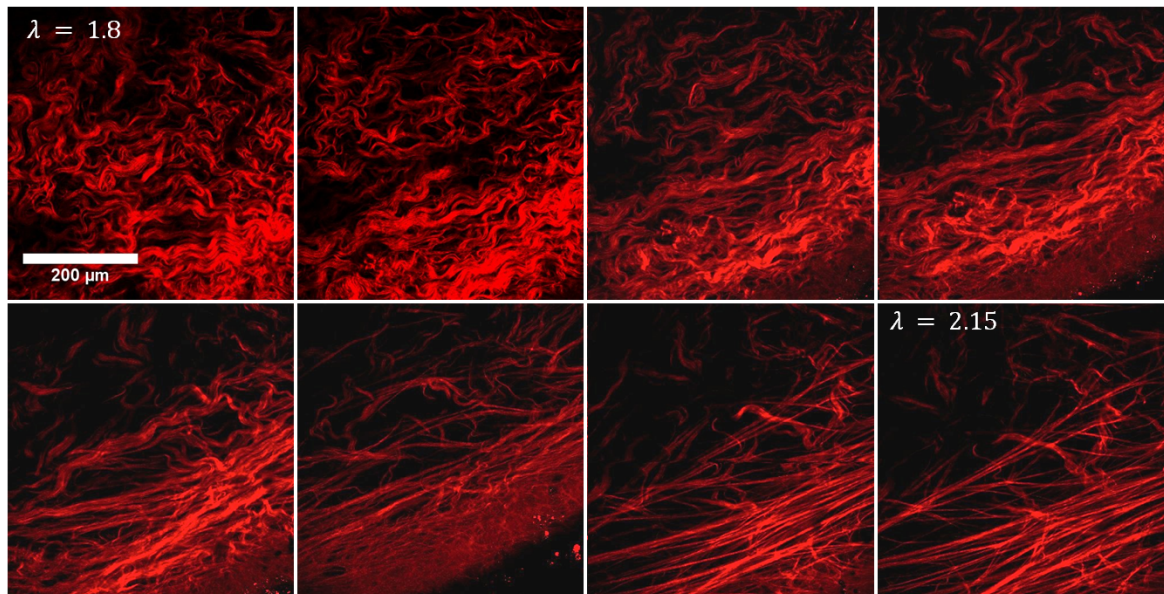


Figure 3.15: Series of planar images of collagen fibril bundles in the first detrusor layer taken by MPM over intervals of 0.05 with range of stretches from 1.8 (top left) to 2.15 (bottom right) in sample; Fischer (Young) 1. Increasing stretches are shown from left to right. It can be seen in this particular region that individual collagen fibres become increasingly straight towards of stretches of approximately 2 where they eventually undergo stretch. (circumferential axis corresponds to horizontal)

Detrusor fibril bundle waviness corresponding to the images in Figure 3.15 is shown in histograms in Figure 3.16. The distribution shifts to higher ratio values with incremental stretches; indicating straightening of fibres. Additionally, towards maximum applied stretch of 2.15 the distribution of ratios narrows; suggesting fibres become uniformly

straightened.

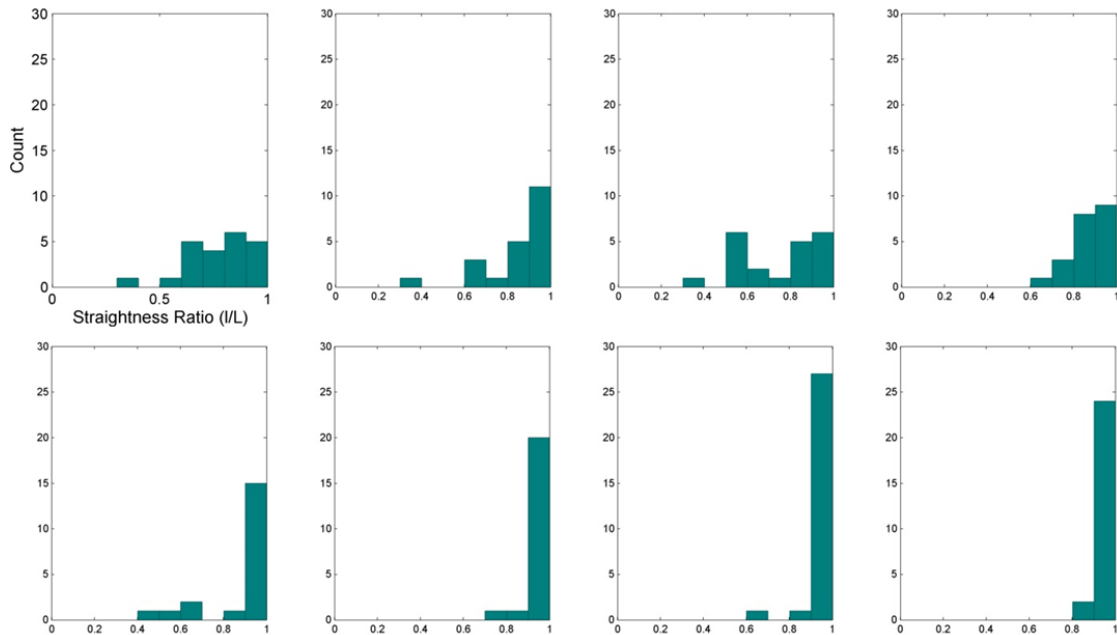


Figure 3.16: Histograms showing straightness ratio counts of individual collagen fibres traced from images in figure 3.15 (stretches from 1.8 to 2.1). Measured straightness increases with stretch.

Aged Specimens

MPM images taken from the lamina propria side in aged rat bladder at stretches of 1.8 to 2.1 are shown in Figure 3.17. At a stretch of 1.8 collagen appears relatively wavy with a noticeable preferential alignment to the circumferential axis. An abrupt change in tortuosity of the fibres was observed with a 0.05 stretch increment to 1.85 as the fibre network appears much sparser with many fibres beginning to straighten. Straightening continues until several fibres can be seen completely straightened at 1.95. Further stretches indicate a majority of fibres are recruited towards stretches of 2, and almost all fibres straightened at the maximum applied stretch of 2.1.

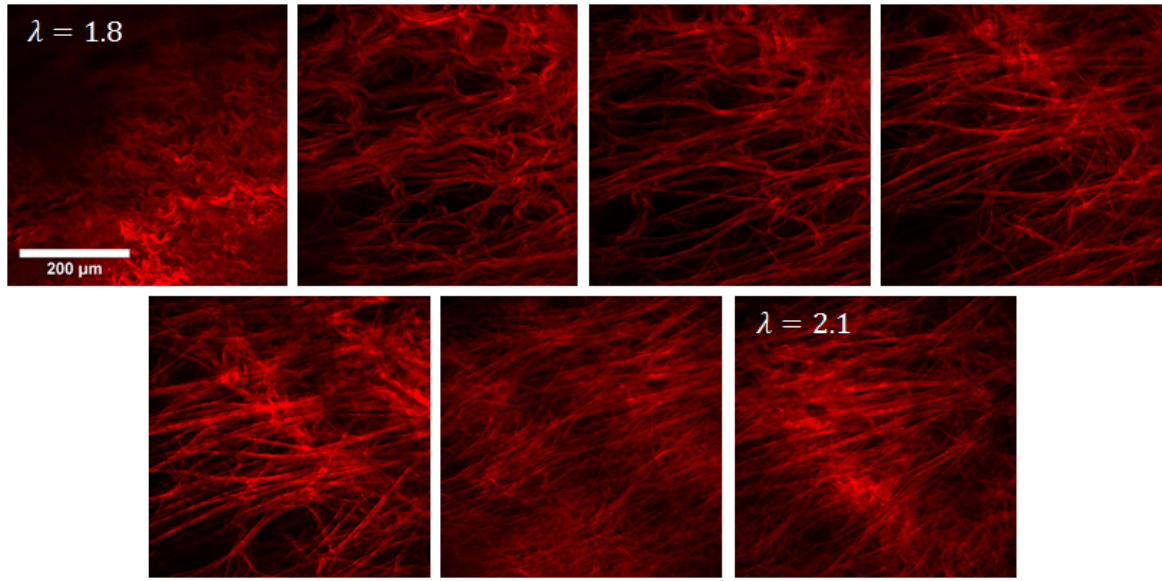


Figure 3.17: Collagen fibres in the inner wall of 24 month old Fischer rat (Fischer (Aged) 3) under increasing stretch intervals of 0.05 (80 % strain seen in top left, 110 % in bottom right). Fibril bundles in the detrusor can be seen to increasingly straighten and begin recruitment from approximately 90 % stretch onwards. (circumferential axis corresponds to horizontal)

Corresponding plots of straightness ratio in aged rat bladder detrusor are shown in Figure 3.18. Distribution of straightness ratios shifts to the right and narrows; indicating increasing straightness of fibres and increased uniformity of straightness with increasing stretch.

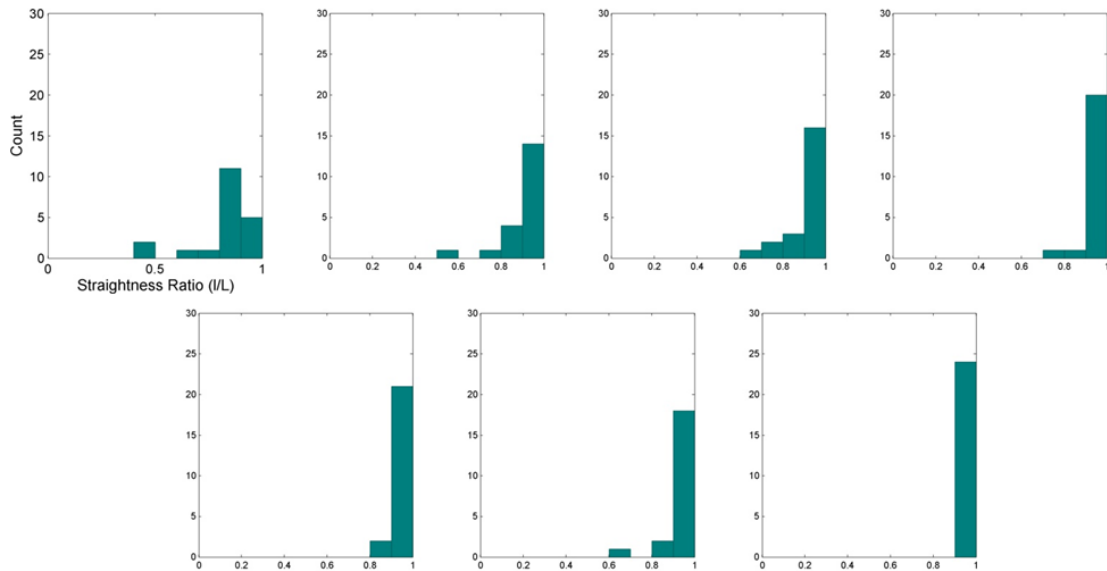


Figure 3.18: Histograms of straightness ratios of individual collagen fibres over increasing stretch from corresponding MPM images in Figure 3.17, i.e. $\lambda = 1.8$ to 2.1. Fibres seen to consistently increase in straightness with stretch.

Results from quantified collagen recruitment in young and aged samples were compiled. Average straightness ratio plots of all young and aged bladders stretched and imaged are shown in Figure 3.19. The trend of increasing fibre straightness and a decreasing variation of fibre waviness was observed with increasing stretch in both young and aged bladder. Onset of recruitment occurs at a stretch of 1.15 and 1.25 in young and aged, respectively. Percentage of recruited fibres remains relatively low in aged tests until $\lambda = 1.4$, where recruitment steeply increases. In comparison recruitment of fibres in young tissue occurs over a greater range of stretches with a milder gradient. Some samples reach 100 % recruitment by $\lambda = 1.45$ in aged tissue, compared with $\lambda = 1.5$ in young, despite a later onset of recruitment in aged tissue.

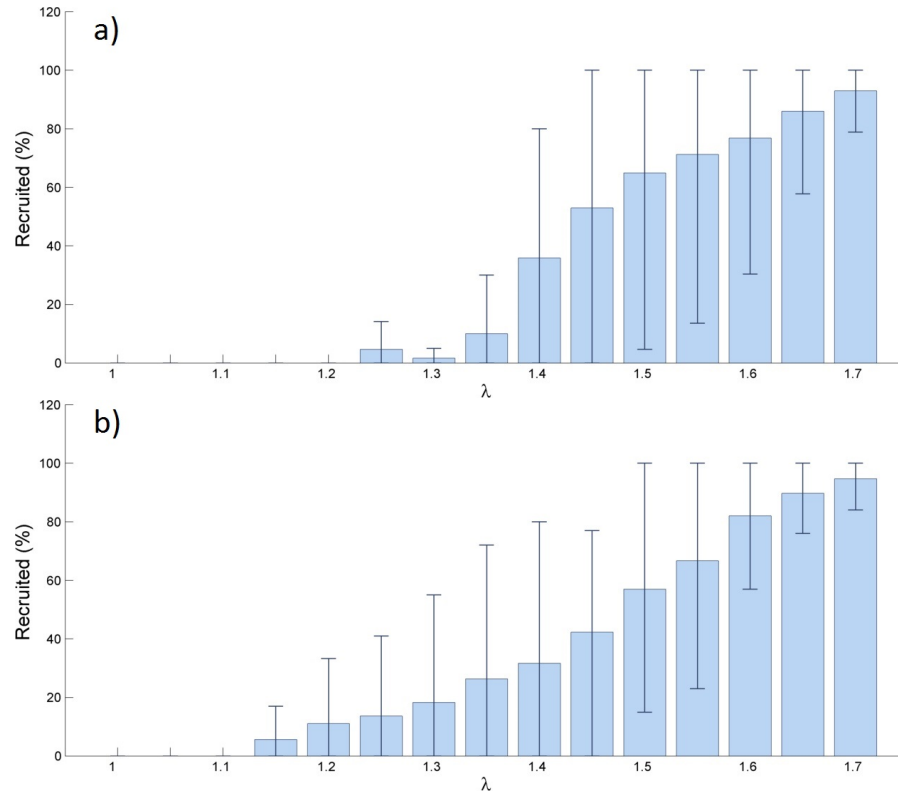


Figure 3.19: Plots of average fibre recruitment vs. stretch of samples quantified in aged (a) and young (b) rat bladder. Range bars indicate minimum and maximum range of percentiles.

Collagen Fibre Recruitment

Recruitment of collagen fibres was calculated by thresholding individual collagen fibre straightness ratios (0.95) and mapping fibre's orientation back to the initial configuration. Individual mapped fibre orientation and tissue stretch was then used to calculate fibre specific stretches, which were divided into stretch bins of 0.05. Results shown in this section were used for all model fitting in subsequent modelling sections.

Figure 3.20 shows two examples of collagen fibre recruitment percentages versus fibre stretch in young rat bladder detrusor (Fischer (Young) 1 & 3). Collagen fibre recruitment was seen to initiate at a fibre stretch of 1.3 and 1.1, respectively. Straightening of collagen fibres after the initial recruitment stretch occurs at a similar rate in relation to fibre stretch. Recruitment in Fischer (Young) 1 shows all collagen fibres to have

been recruited at a fibre stretch of 1.65, compared with approximately 80% of fibres recruited at $\lambda_f = 1.4$ in Fischer (Young) 3.

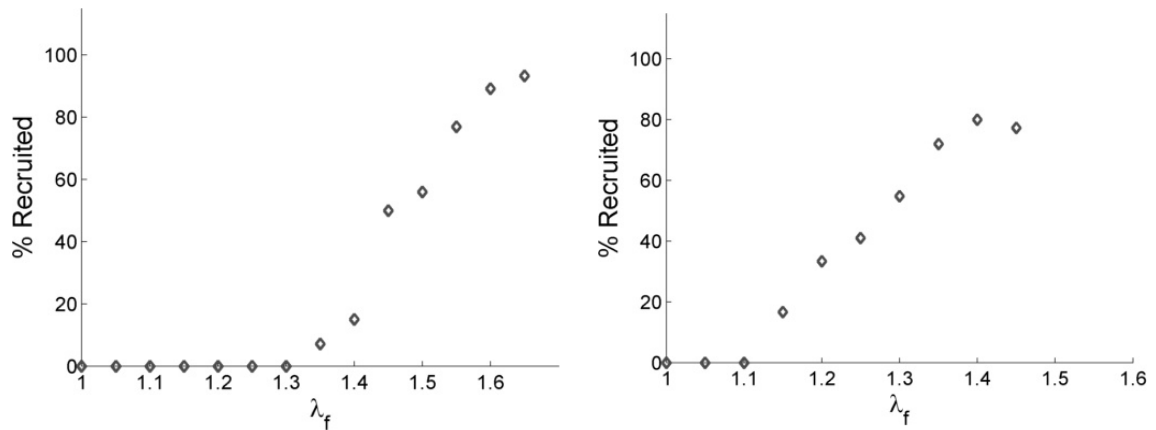


Figure 3.20: Plots of collagen fibre recruitment in the shallow detrusor of young rat bladder against fibre stretch. Samples shown correspond to Fischer (Young) 1 (left) and Fischer (Young) 3 (right) with initial fibre recruitment occurring at stretches of 1.3 and 1.1, respectively. Total fibre recruitment was observed in Fischer (Young) 1 at approximately 1.65, whereas Fischer (Young) 3 shows not all fibres are recruited even at the maximum applied stretch.

Fibre recruitment over increasing fibre stretch in aged Fischer rat bladder shallow detrusor is shown in Figure 3.21. Initiation of recruitment occurs at a fibre stretch of 1.35 in Fischer (Aged) 3 and 1.2 in Fischer (Aged) 4. 100% of fibres are recruited at $\lambda_f = 1.55$ and $\lambda_f = 1.45$, respectively.

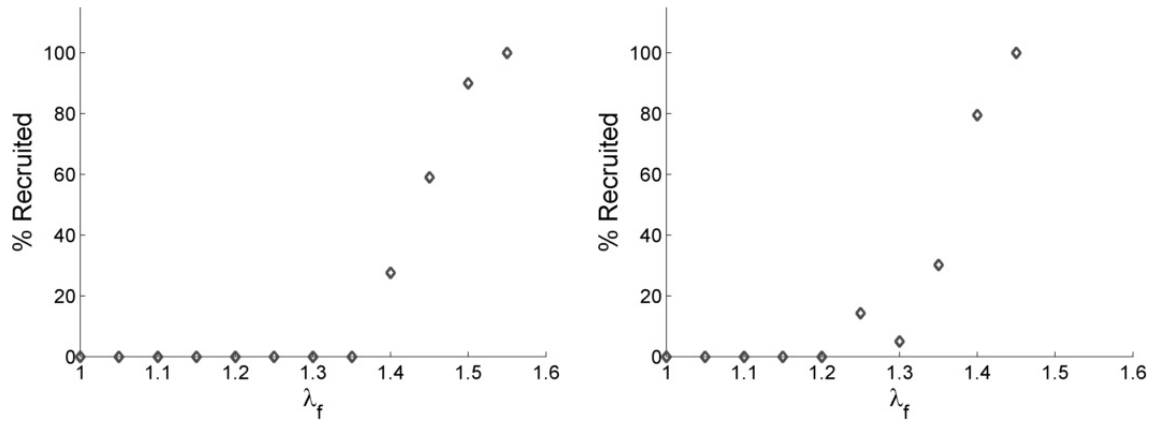


Figure 3.21: Plots of collagen recruitment percentages over increasing stretch in shallow detrusor of aged urinary bladder. Samples shown are Fischer (Aged) 3 (left) and Fischer (Aged) 4 (right). Initial recruitment occurs at fibre stretches of approximately 1.35 and 1.2, respectively. Recruitment of all load bearing fibres occurs at approximately 1.55 and 1.45, respectively.

The collagen recruitment mechanism occurs over a stretch range of 0.2 in both aged bladder samples, compared to a longer interval of 0.35 in young bladder (Fischer (Young) 1).

Collagen Fibre Orientation

Collagen fibre orientation was quantified through the observable wall thickness (from the lamina propria side) at each stretch increment to visualise the extent of collagen re-orientation under biaxial load. Several individual examples are given of the young and aged rat sample tests. Imaging depth was limited to approximately 200 μm , although was subject to stretch and collagen density. Plots are presented as orientation magnitude through wall depth, illustrated as contour plots where red indicates regions of high anisotropy (strong alignment) and blue regions are isotropic (random alignment).

- Note: All plots given in this section show longitudinal axis corresponding to 0 and 180° and circumferential to 90°.

Representative contour plots of collagen orientation through wall depth (imaged from lamina propria side) in young samples at increasing stretch are shown in Figure 3.22 and Figure 3.23. Image penetration depth decreases towards higher stretches. Compression of the wall during stretch meant collagen density was higher, subsequently increasing signal attenuation and limiting visible depth to approximately $80\text{ }\mu\text{m}$.

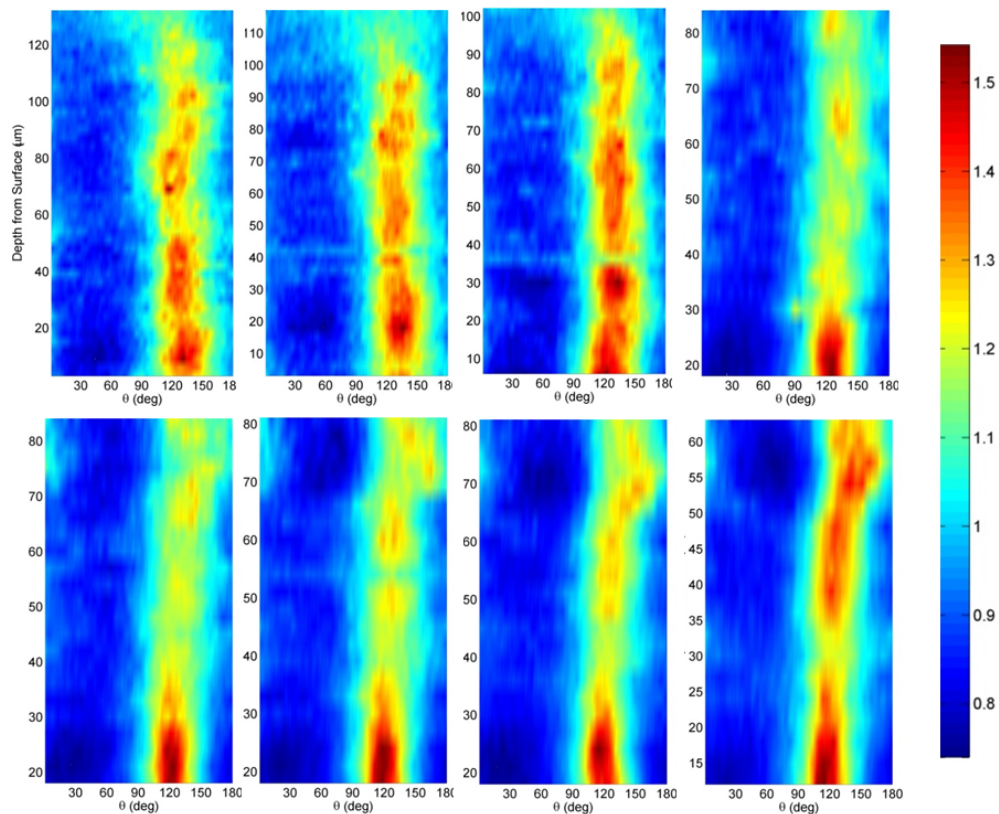


Figure 3.22: Illustrative example of collagen orientation throughout up to $120\text{ }\mu\text{m}$ of young bladder wall (FY02) under incremental stretches (left-to-right) from 1.8 (top left) to 2.15 (bottom right) imaged from lamina propria side. Colour bar indicates strength of alignment, where red is strong and blue is weak.

Mean fibre angle of sample FY02 (Figure 3.22) in the lamina propria at $\lambda = 1.8$ was approximately 130° , compared with approx. 50° seen in sample FY03 at $\lambda = 1.65$. Mean fibre alignment was stronger near the lumen in FY02, whilst increased anisotropy was observed deeper in FY03 bladder. Mean fibre angle in both samples remains

consistent at increased biaxial stretch, anisotropy increases. At stretches of 2.05 and 1.9 in FY02 and FY03, respectively, minor reorientation of fibres was observed. Collagen in LP of FY02 begins to reorientate approximately 70 μm from the surface as some fibres shift 10 - 20° towards the longitudinal axis. FY03 bladder wall shows significant fibre reorientation with mean fibre angle increasing to 80° at $\lambda = 1.85$. At peak stretches deeper fibres over 60 μm into the wall show increased isotropy with mean orientation strongly aligned with the circumferential axis; perpendicular with final mean alignment seen in FY02.

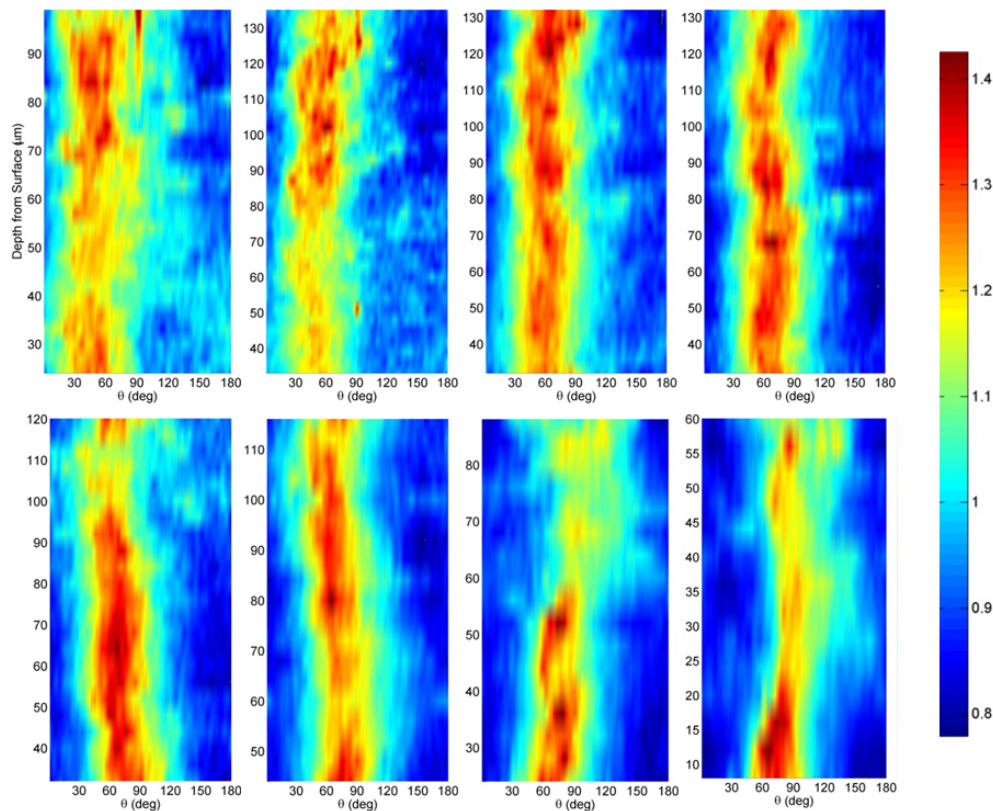


Figure 3.23: Illustrative example of collagen orientation throughout up to 140 μm of young bladder wall (FY03) under incremental stretches (left-to-right) from 1.65 (top left) to 2 (bottom right) imaged from lamina propria side.

Collagen orientation throughout visible wall depth from the lamina propria side was quantified in aged rat bladder under incremental stretch. Representative samples FA03

and FA04 are shown in Figure 3.24 and Figure 3.25, respectively.

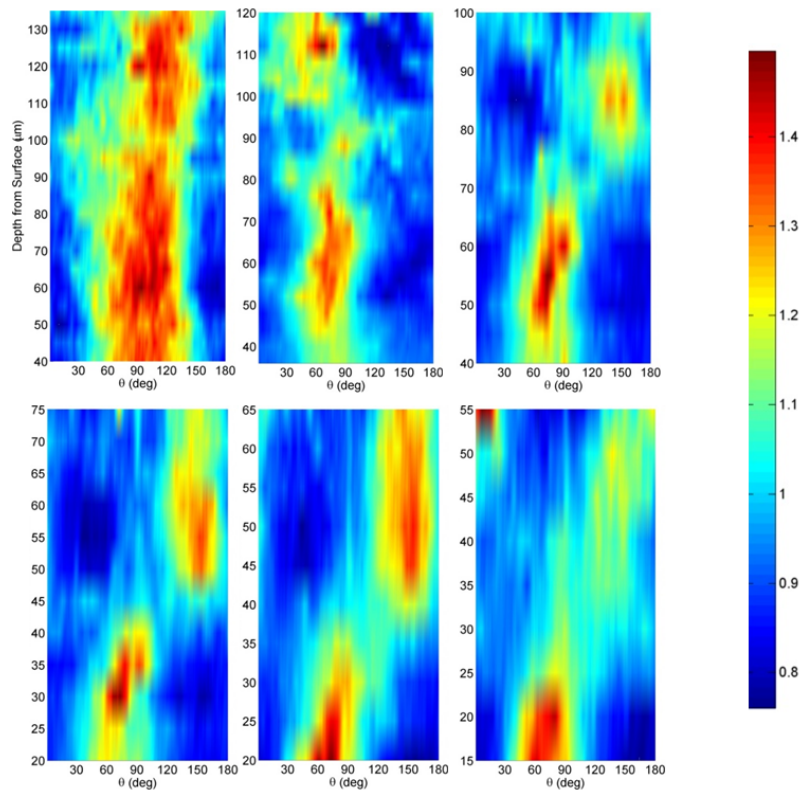


Figure 3.24: Illustrative example of collagen orientation throughout up to 140 μm of aged bladder wall (FA03) under incremental stretches (left-to-right) from 1.8 (top left) to 2.05 (bottom right) imaged from lamina propria side.

Contour plots of collagen orientation through-wall in both samples shows collagen in the visible depth aligned longitudinally at an initial stretch of 1.8. An initial anisotropic arrangement of collagen was followed by a relatively isotropic dispersion at 80 μm in FA04. Biaxial stretching of FA03 reveals two like-orientated independent 'layers' forming orientated at approximately 60 and 150°. These two layers initially have a small amount of dispersion which reduces with further stretch. Additionally the first layer appears to thin, occupying approximately 80 μm at $\lambda = 1.85$ and reduces to approximately 30 μm at peak stretch of 2.05.

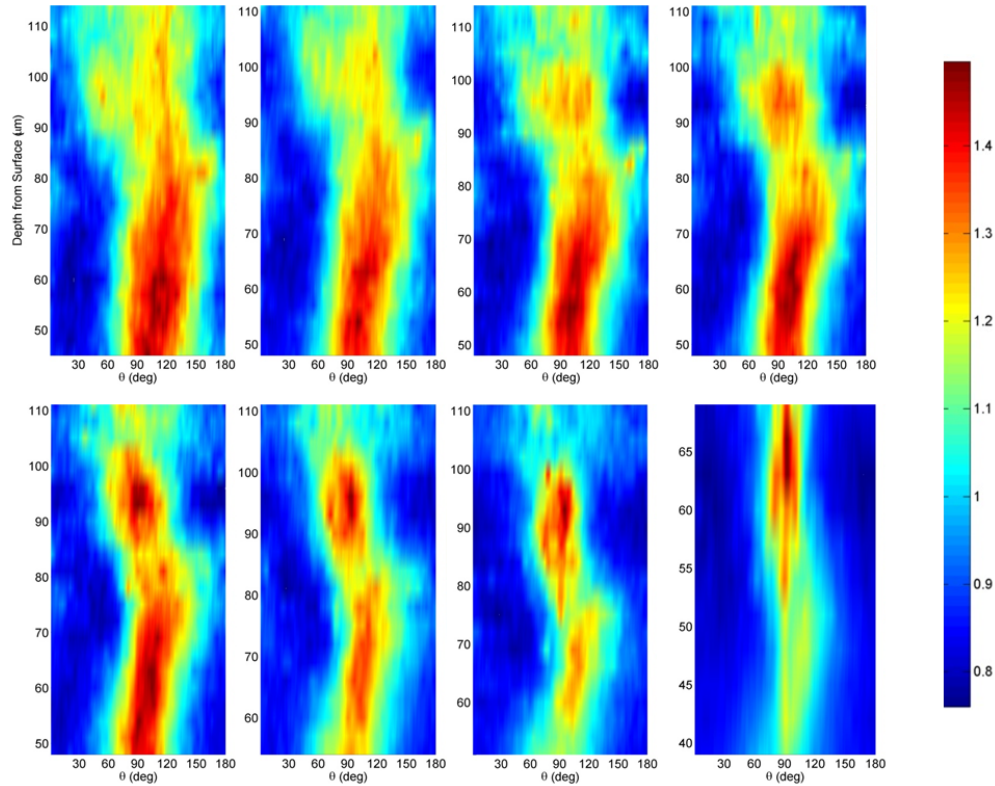


Figure 3.25: Illustrative example of collagen orientation throughout up to 120 μm of aged bladder wall (FA04) under incremental stretches (left-to-right) from 1.8 (top left) to 2.15 (bottom right) imaged from lamina propria side.

In contrast to FA03, a single orientation was seen through observable wall thickness. Initial stretches from 1.8 to 1.95 show fibres to lie near the circumferential axis, at approximately 100° . Small variation from the mean are seen throughout depth. Higher biaxial stretch incrementally reduces dispersion as fibres are pulled taut. The final fibre direction was approximately 90° . Strongest alignment was initially observed near the surface, however as stretch increases, anisotropy was greatest approximately $70\mu\text{m}$ from the lumen surface.

3.5 Bladder Inflation

The mechanical behaviour of the bladder during inflation has been of interest for modelling [20], tissue engineering [17] and clinicians [47] as it provides the most clinically relevant method of distending the bladder both in-vivo and in-vitro. Whilst uniaxial and biaxial stretching have certain advantages, inflation is the primary method of clinically diagnosing pathologies; meaning mechanical data obtained from in vitro studies can be more readily applied clinically. Additionally, loading that the bladder undergoes during inflation closely simulates that of normal physiological functioning. In this section intact murine bladders are inflated and pressure recorded. After testing, bladders are held at a prescribed volume, fixed and sectioned. MPM imaging was used to image the stretched tissue in base, body and dome regions.

3.5.1 Methods

Tissue Preparation

Whole intact LUT (bladder, urethra and ureters) were dissected from 10 4 week old male Wistar rats. 8 bladders were used for inflation testing and imaging and 2 imaged in a resting state. Urethras were catheterised in a PBS bath using a blunt 20 gauge needle tip and sutured to secure and provide an air tight seal. Bladders were completely drained and ureters sutured to prevent back-flow of fluid.

Inflation Protocol

Air was cleared from bladders and tubing prior to testing by opening stop taps and bleeding the system. Bladders were drained completely using a syringe and stop taps shut to close the system. Pressure was allowed to equilibrate to approximately zero

before testing. A NE-300 Just Infusion syringe pump (New Era Pump Systems Inc, NY, USA) with a 5 mL syringe was used to inflate bladders with PBS (See Figure 3.26). An Omega PX409-USBH pressure transducer (15 psi max, error 0.5%, 5 significant figure resolution) (Omega Engineering, Manchester, UK) was used to measure system pressure. Prior to testing all bladders were preconditioned with 3 inflation cycles to a volume of 0.8 *ml* at a fill rate of 0.4 *ml/min* with 3 minutes between each cycle.

Bladders were allowed to rest for 5 minutes post-preconditioning. After, mean pressure-volume curves were obtained at a fill rate of 0.4 *ml/min* until fill volume reached 0.8 *ml*. After each test the system was opened using a stop tap and bladders drained completely. Pressure was zeroed and allowed to equilibrate for 5 minutes before performing the next test. The initial stretch configuration (i.e. $\lambda = 1$) was defined where pressure exceeded 20 Pa from the equilibrated pressure value.

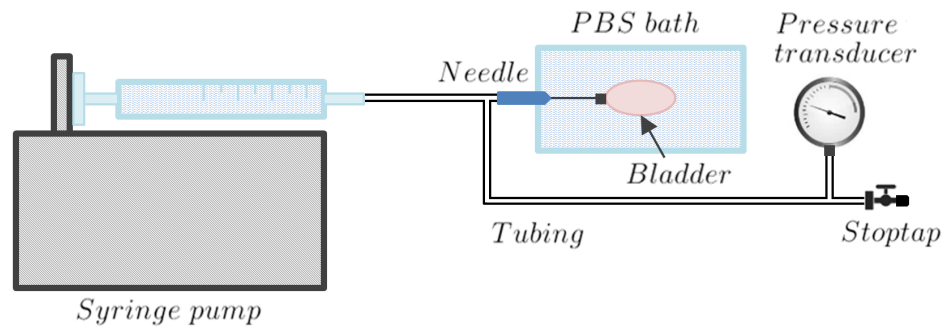


Figure 3.26: Schematic of inflation system used to inflate urinary bladders. A syringe pump controls input flow of PBS into the bladder and a pressure transducer records elevation in pressure (placed at same height as bladder). Stop taps allow the system to be bled and open/close when required.

The diameter of the initial sphere configuration, i.e. at $\lambda = 1$ was calculated by assuming an idealised sphere using volume measurements from integrated flow rate. Wall thickness was taken as an average of resting wall thickness measured in MPM studies as measured from fixed samples post-mechanical testing. Stress was approximated by assuming bladders to be thin-walled spheres, as it is agreed to provide a reasonable

approximation of the bladder wall, although becomes poorer as Volume $\rightarrow 0$ [103] [31], (Further details in Section 5.3.2). 1st Piola-Kirchoff was calculated as,

$$P = \frac{\bar{p}R}{2H} \quad (3.10)$$

Where $\bar{p}$ is the internal pressure acting on the wall and R and H are initial unloaded sphere radius and initial wall thickness, respectively. An approximation of stretch was taken using the radius of the initial volume, V_0 , of the bladder when $\lambda = 1$. As flow rate was constant and volume in, V_{in} , was known an idealised sphere was calculated at discrete time points based on current volume, V_1 of the sphere, i.e. $V_1 = V_0 + V_{in}$. Principal stretches were obtained by calculating current radius r ,

$$\lambda = \lambda_1 = \lambda_2 = \frac{r}{R} \quad (3.11)$$

Then assuming incompressibility, the following relationship exists,

$$\lambda_1 = \frac{1}{\lambda_2\lambda_3} \quad (3.12)$$

Imaging Protocol and Acquisition

Following mechanical testing individual bladders were held at volumes of 0.2, 0.4, 0.6 and 0.8 *ml*, i.e. single bladder per volume. The PBS bath was drained and filled with 10% formalin and sealed for approximately a week. Bladders were washed and sectioned using protocols outlined in Section 2.2. Tissue sections of the base, body and dome of the bladder were imaged using MPM imaging protocols described previously in Section 2.3. All image measurements were made using *ImageJ*; sampled 5 times per image at random intervals along the bladder wall and averaged.

3.5.2 Results

Mechanical Testing

Whole intact rat bladders ($n = 8$) were inflated 3 times with PBS to obtain pressure-volume curves. Using bladder dimensions stretch-stress response was calculated. Bladders were fixed in an inflated state (0.2, 0.4, 0.6 and 0.8 *ml*) and sectioned for MPM imaging. observations of layer structure and mechanical response were made. Figure 3.27 shows $n = 5$ representative stress-stretch curves obtained from inflation testing.

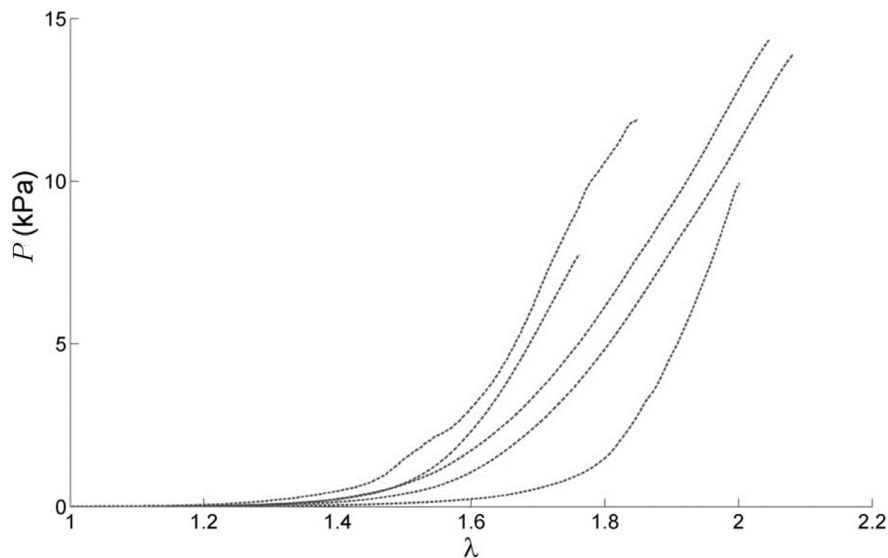


Figure 3.27: Representative $n = 5$ stress-stretch curves from inflation testing of whole intact murine bladder. Maximum fill volume was 0.8 mL, filled over 2 minutes with a flow rate of 0.4 mL/min.

Stress-stretch curves in Figure 3.27 show the familiar soft toe region of the bladder wall up to stretches of approximately 1.4 to 1.5, followed by the transition into the heel region. Stress-stiffening continues through the collagen phase of the stress-stretch curve with peak stresses between approximately 7.7 and 14.3 kPa. Small variations in the maximum stretch achieved were accounted for by differences in the configura-

tion at which an increase in pressure was observed, i.e. small differences in initial configuration.

Initial configuration was approximated by tared pressure of 50 Pa above baseline (volume = 0). This value exceeded the noise in pressure data and gave an accurate representative of where the bladder wall begins bearing load. Volumes at which a stretch of 1 was calculated were 0.42 ± 0.07 mL across all samples.

MPM Imaging of Fixed Sections

Tissue samples fixed at incremental filled volumes were sectioned and imaged using MPM. Cross sections of 10 (8 fixed during inflation, 2 at resting state) bladders were taken from base, body and dome regions of the bladder, e.g. see Figure 3.28.

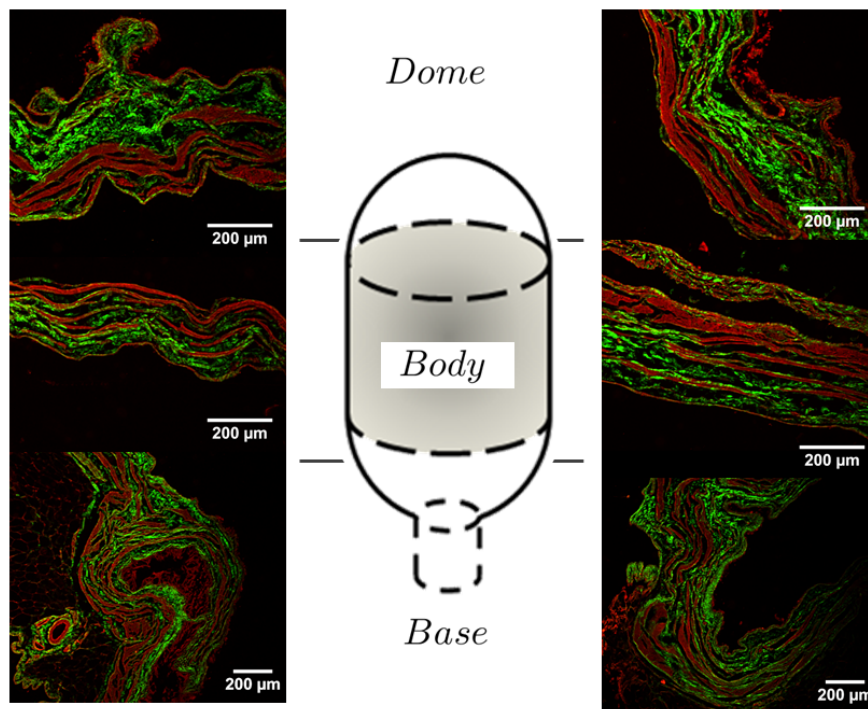


Figure 3.28: Example MPM images of inflated bladder wall ($800 \mu\text{l}$) cross sections taken from the base, body and dome of the bladder to illustrate choice of imaging locations and regional differences. Collagen signal (430 nm) seen in green and elastin seen in red (520 nm) with enhanced contrast.

Bladders were fixed individually at a range of volumes from 0.2 to 0.8 *ml*. Representative MPM images of collagen and elastin taken from the base, body and bladder at each volume are shown in Figure 3.29.

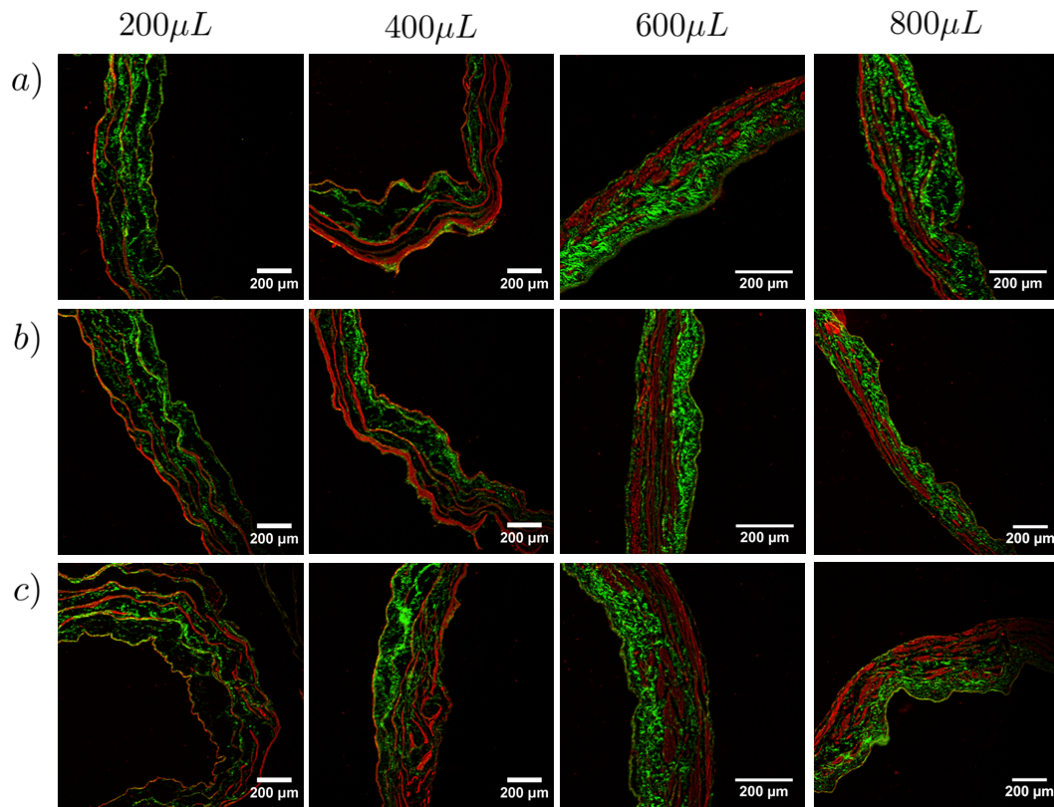


Figure 3.29: Representative MPM images of rat bladder dome (a), body (b) and base (c) cross sections inflated to 200, 400, 600 and 800 μ L. Collagen (430 nm) shown in green channel and enhanced contrast elastin (520 nm) shown in red. The inner lumen can be identified by increased collagen content of the lamina propria compared with muscular and elastin content of the outer wall.

Measurements of bladder wall thickness at random intervals along the bladder wall were taken from section tissue samples under MPM. Average measured wall thickness of the dome, body and base at filled volumes with SD are illustrated in Figure 3.30. Wall thickness in the body and base regions thin progressively at higher volumes, decreasing by approximately 34 % and 18 %, respectively. The dome region remains a similar thickness when deformed. Wall measurements show high SD due to folds formed by

the rugae of the mucosal layer.

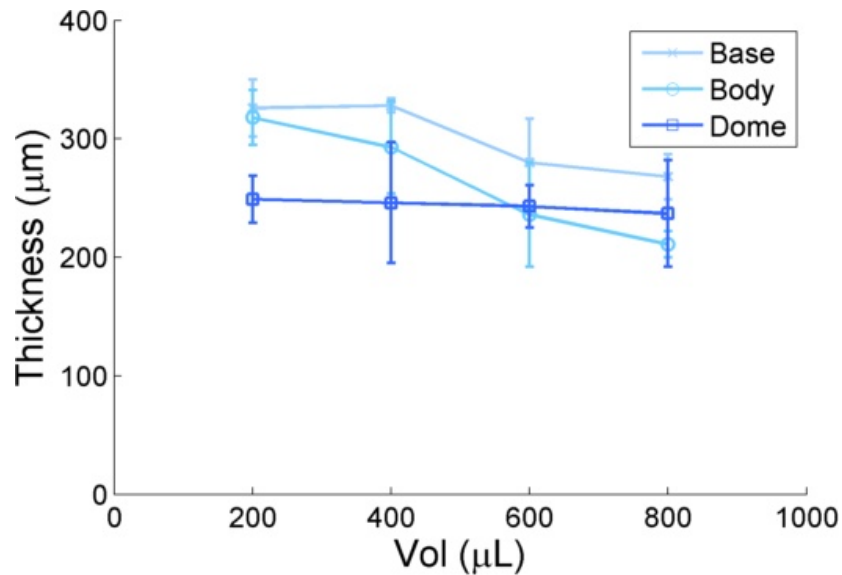


Figure 3.30: Illustrative plot of average measurements and SD from the base, body and dome of the bladder under increasing inflation.

3.6 Discussion

MPM imaging and mechanical testing in this study demonstrates for the first time anatomical layer specific collagen morphology and orientation across the entire bladder wall and its response to loading by uniaxial, biaxial and inflation mechanical testing. This approach allowed MPM image snapshots to be correlated with stress-stretch response to identify the mechanical roles of collagen and smooth muscle in the detrusor and lamina propria. It is known functional and mechanical changes occur to the LUT during aging and disease. For the first time, an attempt to quantify the microstructural changes that may occur in murine bladder due to age and relate these to the macroscopic mechanical behaviour.

Mechanical and microstructural differences between young and aged murine bladder were examined by uniaxial and biaxial testing under MPM. Aged circumferential strips

are significantly stiffer than the 3-4 month old bladders under uniaxial tension. In rats, biaxial testing shows young tissue to be more compliant in the circumferential axis, however differences are smaller between young and old tissue types. Increased deposition of collagen that is often seen in aged bladder wall may be responsible for increased stiffness [10]. Increased collagen in the detrusor was also observed in the previous chapter (30 % vs. 37 % in young and aged). The lower collagen/muscle ratio may also reduce the relaxation and contractility capacity of the detrusor, although inactive bladder tested here may not be affected [34]. Interestingly, two sub-types (compliant and non-compliant) of stress-stretch curves were identified in both young and aged bladder. No identification of the cause of this was made, however may be intra-specimen variation and would require increased samples to improve significance.

Inflation is arguably the most accurate method to replicate in-vivo loading of the bladder, and certainly the most clinically relevant (to cystometry). However, whilst accurate mechanical behaviour is obtainable by inflation, the ability to identify direction specific behaviour is limited. Biaxial testing allowed anisotropy of tissue to be studied and gave improved physiological relevance over uniaxial testing. However, of the 10 (5 young, 5 aged) rats originally tested results were given for 2 of each due to marker tracing difficulties or poor MPM image quality (due to innate constraints of the biaxial testing apparatus).

Combined MPM and biaxial testing allowed orientation and dispersion of visible fibres to be related to the direction specific mechanical response. The orientation was quantified across the entire observable wall depth to identify changes in fibre orientation. A majority of fibres from the lamina propria surface inwards were seen to align predominantly in the circumferential axis, with this orientation consistent deeper into the wall. Correspondingly, increased stiffness was generally seen in the circumferential plane. [104] report average fibre direction is longitudinal across the bladder, although

this was measured from the outer layer. This is as expected as the second muscle bundle aligns perpendicular to the other [79]. There were several incidences where a distinct boundary in fibre orientation was observed; for example, a 90 degree shift in mean fibre direction. This occurred at the interface of the lamina propria and detrusor. It is also reported fibre orientation is region specific [105]. Slightly different imaging locations may therefore account for differences in orientation observed. Additionally, fibres reorganised under distension. As stretch is applied fibres initially realign, then become straighter and eventually pull taut. Fibre reorganisation is observed as distributions of dispersion narrow, becoming more anisotropic.

MPM imaging of wall layers under deformation illustrate the difference in structure and give insight into the specific mechanical roles they play. MPM images were correlated with stress-stretch curves obtained from uniaxial, biaxial and inflation testing. Uniaxial stress-stretch response of murine bladder wall has a predominantly long toe region with high compliance. At these low stretches rugae of the inner lumen retain their loose, wavy appearance. Images support the role of the lamina propria as a capacitance layer. [4] propose that the disproportionate initial thinning of the lamina propria versus the detrusor is to accommodate increased intravesical volume and supported in earlier work by [9]. Additionally, the long toe region acts to minimise intravesical pressure during filling as not to damage components of the upper urinary tract; an outcome observed in severe obstruction [106].

Observations here suggest disproportionate thinning of layers may be a result of rugae unfolding and flattening rather than stress-thinning as a result of bearing tension in the wall. It is likely this is a result of the loose rugae being 'pulled along' with the detrusor as it stretches. It is not until the linear portion of the stress-stretch curve that the rugae completely flatten and collagen straightens in the lamina propria; suggesting it is likely playing a load bearing role. Additionally, results here suggest the bladder wall

thins heterogeneously as the body and base thin proportionately more than the dome during inflation. The highly undulated geometry of the rugae require large strains to straighten before the tissue begins to bear load; meaning these layers have to be pulled taut before bearing tension. It is hypothesised during the toe region smooth muscle bundles elongate, unfold and reconfigure while collagen plays a capacitance role in a coiled state. It is not until the heel region that collagen uncoils and becomes a load bearing constituent. In contrast to the lamina propria, the detrusor muscle rests in an unfolded state that follows the perimeter of the bladder wall, so collagen and muscle bear load. The result of two very different configurations (between lamina propria and detrusor) means the two layers are subjected to different stretches during whole wall expansion (See Figure 3.31). The highly coiled collagen of the lamina propria may accommodate the different stretch configurations to minimise shear stress at the layer interface and allows the rugae to unfold with minimal resistance.

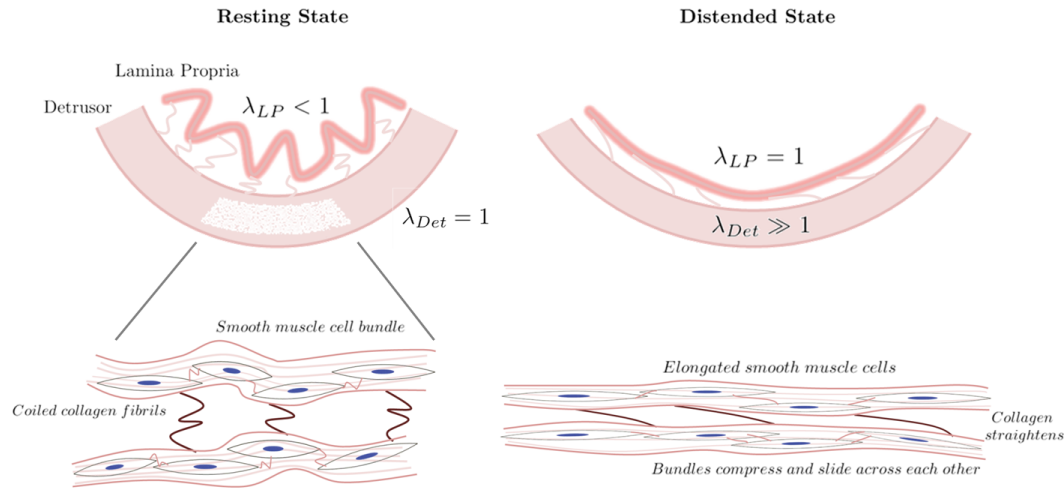


Figure 3.31: Schematic of the initial configurations and relative stretch of the rugae and detrusor showing illustration of smooth muscle and collagen changing states from empty to distended. Collagen acts as a capacitance mechanism to allow smooth muscle reconfiguration and elongation and a limiting 'girding' to prevent over-distension. λ_{LP} and λ_{Det} are stretches in the lamina propria and detrusor, respectively (assuming $\lambda = 1$ is the point at which the layer unfolds). It is hypothesised highly coiled collagen in the lamina propria accommodates the different stretch configurations and minimises shear between layers.

Thinning of the bladder wall occurred heterogeneously across the height. Body and base thinned progressively at higher distension. The thickness of the dome region remained relatively consistent across each volume. Contrary to results presented here, [104] use optical coherence tomography to image distended bladder regions and find the wall thickness of the dome to decrease more than ventral and dorsal regions up to pressures of 3.3 kPa [105]. It is important to note that intra-specimen variation could account for the lack of visible thinning observed in the dome, additionally there may be variation in location chosen to image. It is therefore necessary for future studies to evaluate wall thickness across a greater number of samples to reach statistical significance.

Collagen recruitment occurs during the expansion of the bladder wall and is a structural mechanism that has not been previously studied in the bladder, nor is there knowledge of how aging influences this function. Measurements of collagen recruitment suggest collagen recruitment begins at higher stretches in aged bladder compared with young. Additionally, recruitment of fibres in young bladder occurs gradually, compared with a steep recruitment curve of aged tissue. The expected effect of this would be a longer toe region, followed by a steep heel region in aged bladder. However, stress-stretch curves show high variance and only in the circumferential plane aged tissue shows noticeably decreased compliance. A steep recruitment curve may hinder the bladder's ability to reach normal capacity. Whilst fibres begin to recruit later in aged tissue, they also reach total recruitment earlier suggesting tissue becomes stiffer at lower stretches. This gives a potential mechanism for loss of compliance and capacity that is known to occur with age [67]. However, due to the low numbers future studies would look to increase sample size to improve comparability and significance between young and aged collagen recruitment.

Collagen recruitment and orientation was quantified in the detrusor as this was identified as the main load bearing layer. However, through-wall depth imaging reveal very

different collagen structure and response to biaxial loading (See Figure 3.32). Figure 3.32 (top) shows the fine, dense stroma on the surface of the lamina propria with smaller fibres. At peak strains of 100 % these fibres remain relatively wavy. Larger fibres deeper in the lamina propria retain their loose appearance at 80 % and 90 % strains. At 100 % most fibres realign, however most are not yet fully uncoiled. Fibres in the first detrusor layer are recruited at lower strains, with some fibres starting to recruit at 90 % strain. Fibres appear highly strained at 100 % biaxial strain. These layer specific images illustrate the complexity of the bladder wall and heterogeneity across wall thickness. This, in addition to regional heterogeneity observed by [104], mean quantification of microstructure and the wall structure, and subsequent modelling, must be simplified to an extent.

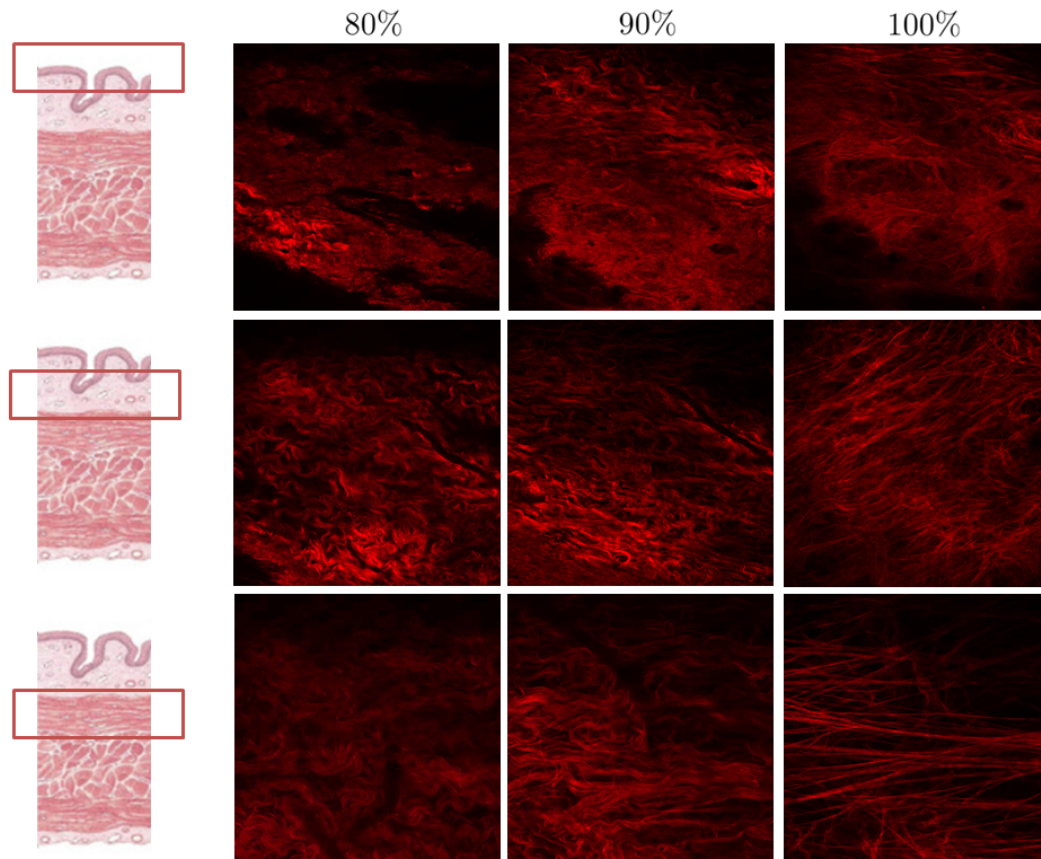


Figure 3.32: MPM images of through-wall depth collagen taken from the inner surface of rat bladder wall at increasing biaxial strains. (Top) Inner surface of the lamina propria. (Middle) Mid-region of the lamina propria. (Bottom) First layer of the detrusor muscle.

A key advantage of the biaxial testing performed in this study was the use of novel 5 pronged rake grips to hold tissue. These clamps alleviated the issues associated with solid metal clamps as they allowed near-free lateral movement and therefore reduced unwanted shear. Additionally, they were extremely time efficient to insert, as after correctly positioning the tissue the prongs could be inserted within seconds, giving benefits over typical suture / hook methods. To date, this is the first study to the author's knowledge that employs the raked prong design for clamping.

Limitations

In terms of quantifying collagen recruitment a threshold was assigned to determine

whether a fibre is considered straight enough to have been recruited. Ideally, this value would be 1, however this would never be the case as a fibre never becomes perfectly straight. Therefore a threshold of 0.95 was assigned based on parameter studies and previous values in the literature, i.e. 0.95 [92], 0.998 [99]. This parameter is highly dependant upon the imaging resolution, tracing skill and method used. Ideally, future studies would look to keep this threshold and tracing and imaging methods similar.

An important aspect of mechanical testing that requires further attention is establishing a consistent reference configuration. Preliminary studies performed (not shown here) and [15] demonstrate, with biaxial testing, the significant difference in mechanical behaviour that can be obtained with respect to two different reference configurations (for example, one configuration shows circumferential direction is more compliant than longitudinal, and another where longitudinal is more compliant with the same mechanical data) [15]. In this present study a consistent reference state was established for each mechanical testing method allowing comparison between samples tested here. However, when comparing to existing literature care must be taken that data are compared on the basis of a consistent reference configuration as significant differences in mechanical response can be achieved. Future work should look to bring in consistent and standardised mechanical reference states for each type of mechanical testing to ensure all studies are comparable and that data are not being misinterpreted.

To overcome the limitations of each mechanical testing method; uniaxial, biaxial and spherical inflation testing were performed. However, the combination of mechanical testing and MPM imaging has limitations. As discussed previously the signal penetration depth is a limiting factor. Attempting to improve penetration depth by methods such as optical clearing dramatically changes mechanical behaviour, meaning a more viable option needs to be sought. Preliminary studies were carried out using microCT

scanning and it was found the entire wall thickness was observable with sufficient resolution and contrast between wall features. Further studies may wish to investigate microCT scanning as viable alternative to quantify bladder wall structure.

Inflation testing was not possible to image during live mechanical testing meaning tissue required fixing. Multiple samples were therefore required. A single sample tested across all stretch ranges would be preferable so that mechanical response is consistent with images. It was possible to achieve this with uniaxial and biaxial studies, mitigating some of the limitations with inflation / MPM studies. Tissue availability meant rat and mouse bladder were used for different tests. Results are therefore not directly comparable, however trends and features observed provide valuable insight into animal specific variations, i.e. differences in layer anatomy and microstructure and how this correlates with mechanical response. Future studies would preferably look to have a consistent animal model across all mechanical testing.

3.6.1 Conclusions

The methods proposed in this study provide valuable insight into the deformation mechanisms of the urinary bladder and the structural roles of the lamina propria and detrusor. Mechanical testing reveals striking differences between differences in compliance of young and age murine bladder; which may be accounted for by increased area fraction and increased wall thickness quantified in aged bladder. Collagen orientation may be responsible for direction dependant differences observed between young and aged murine bladder strips.

Large changes in volume are accommodated in the lamina propria by a highly coiled hierarchy of collagen that provides minimal resistance and a long toe region in the stress-stretch response. The lamina propria also acts to accommodate vastly different stretch

configurations and reduces shear between the dense lumen surface and detrusor. The detrusor likely accommodates for large changes in volume by coiled collagen connecting SMCs; allowing bundles to slide across each other. Peak stretches indicate the detrusor changes role to a girding structure to limit further distension and subsequent damage to the bladder.

Development of a Microstructural Constitutive Model for Passive Bladder Wall Tissue

A constitutive model provides quantification of the relationship between stresses that a material is subjected to and the stretch behaviour. This response is dependent upon the individual mechanical behaviour of the material's constituents and their interactions. To date, constitutive modelling of the bladder has been largely phenomenological [107] [108]. A single study, to the authors knowledge, exists in which microstructural modelling of collagen recruitment in the bladder was performed [15]; however this was a theoretical exercise with little experimental validation or quantification. Microstructural mechanical models allow individual constituents and layers of a tissue to be attributed to specific characteristics and behaviours of the gross material mechanical response. Hence, further insight into the impact of tissue composition on mechanical function can be attained.

In this chapter a novel microstructurally motivated constitutive model was developed with application to the bladder wall. The purpose of this was to improve understanding of how individual constituents and wall layers bear tension in the bladder wall and whether this behaviour could be captured in a constitutive model. For the first time MPM imaging and biaxial mechanical testing studies are used to quantify microstruc-

ture and mechanical response to which the model could be fit and experimentally validated.

4.1 Constitutive Modelling

A material that can be described using hyperelastic constitutive behaviour will have elastic response even at large strains (> 0.25). An elastic response requires that forces deforming the material do not exceed its yield strength and therefore no plastic deformation occurs. A purely elastic deformation will mean elastic (potential) energy is stored in the material during deformation, and once released can recover its original configuration.

These material properties can be described by a strain energy function (SEF), with which nonlinear material behaviour and large changes in dimensions can be accounted for. In the case of an isothermal deformation the strain energy of the material is zero in the unloaded state, i.e. free energy per unit volume (in reference state). For an overview of nonlinear elasticity see Appendix A.

An extension of Hooke's law for the case of large deformations is the Neo-Hookean material model, as proposed by Rivlin in 1948 [109]. This hyperelastic model is used to describe non-linear rubber-like responses. In biomechanic applications it is commonly implemented to model the response of elastin in soft biological tissues [110], [111]. The key limitation of using this type of model to solely describe soft tissues is that it is unable to model the collagen phase, and therefore is typically used in conjunction with exponential additive terms, such as Fung type models [112] [75]. A Neo-Hookean material will have an initial linear phase, but towards higher strains will plateau. The Neo-Hookean model does not account for dissipation of energy into heat; assuming a perfectly elastic material during all deformation. An isotropic, incompressible Neo-

Hookean model is as follows,

$$\Psi(I_1) = \frac{\mu}{2}(I_1 - 3)^2 \quad (4.1)$$

The Fung model is perhaps the first widely used soft tissue specific constitutive model and has been extensively implemented in literature and finite element analysis, particularly with application to arterial tissue [113], [114], [112]. Unlike the Neo-Hookean model, a Fung type model includes an exponential component, allowing it to describe high strain collagen phase soft tissue responses. Although this model typically provides good fits to soft tissue experimental data, the key limitation with this type of model is the lack of a structural description, i.e. it is phenomenological. Recent application of advanced imaging technology has allowed models to take into account the microstructure, thus allowing correlation between tissue constituents and macroscopic behaviour. The SEF for a Fung model may be written in a generalised, isotropic form as,

$$\Psi = \frac{1}{2}[c_1(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) + c_2(e^{c_3(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3)}) - 1] \quad (4.2)$$

Where $\lambda_i, i = 1, 2, 3$ corresponds to primary stretches in the x, y and z planes. Then, further simplification by substituting in the invariant I_1 ,

$$\Psi = \frac{1}{2}[c_1(I_1 - 3) + c_2(e^{c_3(I_1 - 3)}) - 1] \quad (4.3)$$

The GOH model [110] [75], describes collagenous soft tissues in a microstructural fashion, and is used in arterial wall mechanics [115]. This constitutive model assumes an isotropic compliant material representing elastin with the addition of two orientations of families of stiff fibres describing an anisotropic response due to collagen fibres. The

stiff fibre direction is defined with respect to the reference configuration of the material by a set of unit vectors. Elastin is assumed to bear load at all stretches, using a Neo-Hookean SEF with a single modulus-like material constant (μ) to describe response. The SEF is related to the deformation gradient by the first invariant (I_1) of the Cauchy deformation tensor and the fourth and sixth invariant (I_4, I_6) which denote stretch squared in the fibre direction. The increased stiffness associated with collagen recruitment to load bearing is described by an exponential response to the fourth invariant of the Cauchy deformation tensor. Two material constants (k_1, k_2) then control the shape and magnitude of the exponential collagen stiffness response.

$$\Psi(I_1, I_4, I_6) = \frac{\mu}{2}(I_1 - 3) + \sum_{i=4,6} \frac{k_1}{2k_2} \exp(k_2(I_i - 1)^2) - 1 \quad (4.4)$$

4.1.1 Microstructural Modelling

The tissue's response to loading is fundamentally dependent upon the multiple structural constituents that the tissue is composed of and their combined interactions. Many of the recent constitutive models of soft tissue consider both isotropic elastin components and anisotropic contribution due to fibrous constituents. Most recently, models such as [92], [116] and [117] include microstructurally relevant parameters, such as collagen fibre recruitment and orientation distributions based upon data obtained from microscopic imaging modalities. Microstructural models aim to capture the response of the various constituents and hierarchies present in soft tissue and give input parameters a physically meaningful capacity to better understand the mechanical interactions.

4.1.2 Collagen Recruitment Modelling

Modelling of individual collagen fibre response is a key aspect of multi-scale microstructural models when capturing the macroscopic tissue properties. Previous models by [92], [118] and [117] all consider individual collagen fibres to have a linear mechanical behaviour. The non-linear heel region at collagen recruitment initiation then arises due to integration of the recruitment probability density function and the collagen response. Upon 100% fibre recruitment a linear region arises where the modulus is determined by the individual collagen fibre stiffness and collagen density (See Figure 4.2).

Zulliger and colleagues propose a structural constitutive model with application to arteries [116]. The SEF is defined starting from the Holzapfel model as a basis with a modified elastin response using a single material constant and a novel fraction constant to determine the proportion of elastin. The major development step they report is the introduction of a statistical fibre recruitment model implemented using a log-logistic probability distribution function (ρ_{fibre}). This attribute then allows direct inclusion of experimentally relevant fibre recruitment parameters.

$$\Psi_{col} = \int_{-\infty}^{\infty} \Psi_{fibre}(x) \rho_{fibre}(\epsilon - x) dx \quad (4.5)$$

The strain, x denotes the finite point at which collagen fibre recruitment initiates. Ψ_{fibre} is the mechanical strain energy function of an individual collagen fibre as a function of strain (ϵ), with μ denoting a stress-like modulus. Given as

$$\Psi_{fibre}(\epsilon) = \mu[\epsilon - \log(\epsilon + 1)] \quad (4.6)$$

A similar approach is taken by [119], however a triangular distribution function is im-

plemented. Both these models provide a good description of the nonlinear stiffening of soft collagenous tissue. However, these models do not consider a method of incorporating measured collagen fibre orientation dispersion; instead taking a similar approach to the Holzapfel model, whereby a dominant fibre family direction is specified.

A microstructural model proposed in [92] uses a similar collagen strain energy function as [116], however provides additional information from experimentally derived values for both recruitment and fibre orientation. Instead of a log based mechanical response for collagen fibres, a linear material is assumed i.e, $\Psi_f = \frac{\mu}{2}(\lambda_f - 1)^2$. As a function of true fibre stretch λ_f , the collagen model does not bear load until the recruitment threshold is exceeded. Collagen fibre recruitment distribution is represented by a Gamma function and provides a good fit to a range of arterial mechanical properties, although this presented a great deal of variability within fitted parameter values; motivating further investigation into this type of model. An alternative distribution function as suggested by [120] uses a triangular PDF to represent the range of recruitment stretches by a minimum, modal and maximum value. This model has the distinct advantage over traditional Gamma [92] and log-logistic [116] distributions by using physically meaningful distribution parameters than can be directly quantified from imaging studies. This model forms the basis of the passive SEF proposed in this section.

4.1.3 Collagen Fibre Dispersion

It is often seen as a modelling assumption that embedded fibres in a medium will align perfectly to one or more directions, as expressed by the invariants I_4 and I_6 . However, studies such as [26] and [100] demonstrate and quantify in arteries and skin, respectively, that collagen in soft tissues will disperse to varying extents about the dominant fibre direction depending upon anatomical location and layer. To date there

are no studies that quantify this dispersion nor dominant fibre direction in urinary bladder and presents a critical limitation to implementing more sophisticated structural models.

To account for this observed fibre dispersion, a recent extension of the GOH model was formulated [75]. This model aims to include a varying extent of fibre dispersion around one or more mean orientations of fibre families. To achieve this a new invariant is defined,

$$I_4^* = \kappa I_1 + (1 - 2\kappa)I_4, \quad (4.7)$$

This invariant scales the degree of isotropic stretch and directional dependant stretch that a fibre family is undergoing. The variable κ is a dispersion parameter that describes the extent of fibre spread around the mean direction, and may be obtain from the integrated area of a distribution function. For a 3D case, κ ranges from 0 to $\frac{1}{3}$ and in 2D, from 0 to $\frac{1}{2}$. For example, in this case a Gaussian distribution is described,

$$\rho(\theta) = 4 \left(\sqrt{\frac{b}{2\pi}} \right) \frac{\exp[b(\cos(2\theta) + 1)]}{\operatorname{erfi}(\sqrt{2b})}. \quad (4.8)$$

Where erfi is the imaginary error function. b is a fitting or scaling parameter to adjust extent of dispersion. κ is then obtained by integrating over the range of possible fibre angles,

$$\kappa = \frac{1}{\pi} \int_{-\pi/2}^{\pi/2} \rho(\theta) \sin^2(\theta) \, d\theta \quad (4.9)$$

4.1.4 Bladder Specific Constitutive Models

An overview of constitutive models for soft tissues in general has been given in previous sections. The main progression in this area has been directed at vascular tissue, however limited studies have applied more recent constitutive models to the bladder. Whilst LUT models often provide a description of the bladder wall's mechanical behaviour, they are often use simple non-linear elastic phenomenological constitutive models [108] [121]. This section will provide an overview of some of the more recent models that aim to introduce microstructural information.

Following a similar modelling direction to [110]; [122] use a decomposition of bladder wall constituents into ground matrix and collagen components. The form used to describe the ground matrix consists of an isotropic exponential, phenomenological model to describe the non-linear stress-stiffening at higher strains,

$$\Psi(I_1) = \frac{c_1}{c_2}(\exp[c_2(I_1 - 3)] - 1) \quad (4.10)$$

Near incompressibility is assumed as a kinematic constraint using the Lagrange multiplier, p , and third invariant, I_3 . As with the GOH model, the matrix response is governed by stress-like term c_1 and constant c_2 . Fibre reinforcements to the model are composed of additive exponential terms of two families of fibres,

$$\Psi(I_i) = \frac{k_{1,i}}{k_{2,i}^2}(\exp[k_{2,i}(I_i - 1)] - c_{2,i}(I_i - 1) - 1) \quad (4.11)$$

Here, i denotes the invariants 4 and 6 and the corresponding mechanical constants to each fibre family. The model appears to capture the non-linear response of bladder wall tissue well and is applied successfully to uniaxial testing over a wide range of

strain rates (10, 50, 100 and 200 %/s). However, this model is defined by a large number of parameters, including 2 terms per family of fibre and 2 additional constants to describe the ground matrix. In cases this may lead to over fitting of the data and ultimately, parameters of limited meaningfulness. Additionally, this model has very little microstructural information incorporated.

The microstructural theme is proposed by [15] for bladder specific constitutive modelling. A collagen recruitment model, similar to the approach taken by [92], is proposed to model normal and spinal cord injury rat bladder under biaxial tension. Fibre recruitment with increasing stretch is modelled by a uni-modal beta distribution, modified to span across the strain range. The individual collagen fibre stress is linear and given as $S_f = \eta E_f$, where η is the modulus and E_f the true fibre strain. The constitutive response is obtained by integrating the product of the distribution and collagen fibre response.

The use of a beta distribution function may be adequate for fitting purposes, however does not give the most effective insight into the microstructure. For example, the shape parameters used for fitting have no physical meaning in terms of the microstructure. The distribution model with a lack of elastin representation may also lead to fibres being 'recruited' earlier than in the actual tissue when fitting as stress is now only governed by collagen fibres. Therefore, this type of approach looks to improve the microstructural representation of the tissue, however lacks quantification of the measurable parameters.

4.1.5 Summary and Aims

Modelling of soft tissues is a well researched field and has been studied over decades, and is supported by experimental evidence examining the mechanical properties of

various tissues across different species. It is only relatively recently that microstructural models have become a focus of attention. Phenomenological SEFs have the ability to fit mechanical test data well and provide a simple framework on which to perform tissue analyses. However, when examining the interaction of tissue microstructural constituents and their individual mechanical role these models fall short.

To date, bladder wall constituents and layers have not been quantified or modelled explicitly. With the recent advances in imaging technology, such as MPM, it is now possible to precisely observe and quantify the role of the microstructure during physiological loading. These advances mean microstructural models, such as those described previously, are now able to be validated and furthermore, obtain increased structural information from the tissue. Given the vastly different microstructure of each individual tissue, it is necessary for these models to be developed and validated specific to individual tissue types. Therefore, the aim of this chapter is to develop a bladder specific constitutive model using a microstructural description of the collagen in terms of recruitment, area density, orientation and dispersion.

4.2 Bladder Specific Constitutive Model Formulation: Passive Constituents

An overview of the modelling assumptions and model framework formulated in this section is given here. First, a background to the kinematic framework is given describing measures of deformation used for modelling. A multi-constituent, multi layered approach to modelling the wall structure of the bladder wall is taken with contribution from collagen and smooth muscle. The passive constituents are described with detailed illustration of the recruitment concept and application in the model. Using

imaging and mechanical testing data from previous work the microstructural model components are calculated and fitted to mechanical data obtained from young and aged specimens.

4.2.1 Passive Constituents

The new bladder model was built as follows. For modelling purposes it was assumed the bladder wall was a perfectly spherical thin-walled elastic membrane. In a study by [123], a spherical membrane assumption was tested against oblate and prolate spherical geometries. It was found that shape did not greatly influence compliance and was concluded that mechanical properties play a much larger role in determining intravesical pressure. A thin walled, membrane approximation has also been the assumed case in previous literature as the diameter of the bladder is much larger than the wall thickness [124].

The structural approach to passive constitutive modelling builds upon on early work by [125] and more recent developments specific to vascular tissue by [92] and [99] and bladder tissue by [15]. The passive model assumes the tissue level response is governed by bundles of fibres, dispersed about one or more common orientations with a cumulative mechanical response based on fibre recruitment, embedded within a ground matrix material. Individual collagen fibres are modelled using a simple linear material response; the non-linearity arises due to the collective recruitment that occurs with increasing stretch. This was represented with a triangular probability distribution function as previously defined by [120], and was characterised by minimum, modal and maximum recruitment stretches obtained directly from experimental observations of fibre recruitment. An exponential strain energy function was used to model the isotropic response.

4.2.2 Strain Energy Function of Passive Constituents

The following section describes the implementation of a microstructural collagen model and parameter estimation used to fit experimental data. All models in the following section were implemented in Matlab 2013a.

The bladder wall passive mechanical behaviour was modelled as an additive contribution of smooth muscle, and collagen. The detrusor layer was of mechanical interest in this case as it was assumed to be the main load bearing layer (See Figure 4.1). The detrusor was composed of two primary, perpendicular muscle bundles approximately aligned with the circumferential and longitudinal axes (θ_1 and θ_2 , respectively). Collagen fibres interwoven are closely aligned with the smooth muscle and are assumed to follow the same fibre direction.

Due to imaging limitations the second layer of muscle was assumed to lay perpendicular to that of the first. Dispersion was also assumed to be identical to that of the first observable layer.

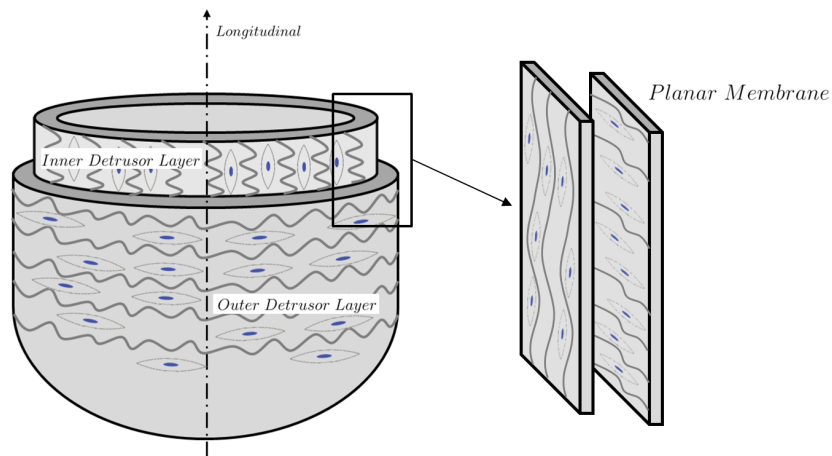


Figure 4.1: Illustration of the two detrusor layers modelled and the collagen and smooth muscle formation assumed in the model. A planar membrane was used in formulation of the model for biaxial testing (right).

To account for the relative proportion of the bladder wall that each constituent makes

up, area density fractions, ϕ_{sm} and ϕ_{col} ($0 < \phi_{sm}, \phi_{col} \leq 1$) are used to represent the proportion of the load borne by each constituent and giving the general strain energy function form,

$$\Psi = \phi_{sm} \Psi_{sm} + \phi_{col} \Psi_{col} \quad (4.12)$$

Each component is the additive contribution of 2 layers. Layer specific constitutive response is a function of stretch in the fibre directions θ_1 and θ_2 . The smooth muscle of the bladder wall was modelled using a cubic polynomial function.

$$\Psi_{sm} = \sum_{i=4,6} \frac{\mu_{sm}}{3} (\lambda_{f,i} - 1)^3 \quad (4.13)$$

μ_{sm} is a stress-like constant and defines stiffness of the smooth muscle component. Justification for use of a cubic function is given in the discussion.

Collagen was modelled using a modified recruitment approach, similar to [92] and [116]. A recruitment based constitutive model implements probability density functions to describe the statistical distribution over the range of stretch where collagen fibres are recruited into load bearing. Before considering the total collagen content of the tissue, initially an individual collagen fibre was described. The SEF of individual collagen fibres (Ψ_f) are assumed to have a simple quadratic form, giving a linear relationship between 1st Piola-Kirchoff stress and true fibre stretch, λ_t .

$$\Psi_f = \frac{\mu_c}{2} (\lambda_t - 1)^2 \quad (4.14)$$

Where μ_c is the collagen stiffness constant. λ_t is the 'true' fibre stretch along a given orientation. The assumption of collagen fibres having a different stretch regime to the

bulk tissue requires that the collagen fibres are defined in their own reference configuration. In this case, collagen fibres only begin stretching ($\lambda_t = 1$) at the recruitment stretch, λ_R . Therefore, the 'true' fibre stretch, λ_t , can be related to fibre stretch, λ_f by,

$$\lambda_t = \frac{\lambda_f}{\lambda_R} \quad (4.15)$$

Collagenous soft tissue have the characteristic strain stiffening effect at higher strains due to collagen recruitment. As each collagen fibre is considered linear, the non-linear response was obtained by assuming a statistical distribution of stretches over which collagen fibres are incrementally recruited. This concept is illustrated in Figure 4.2.

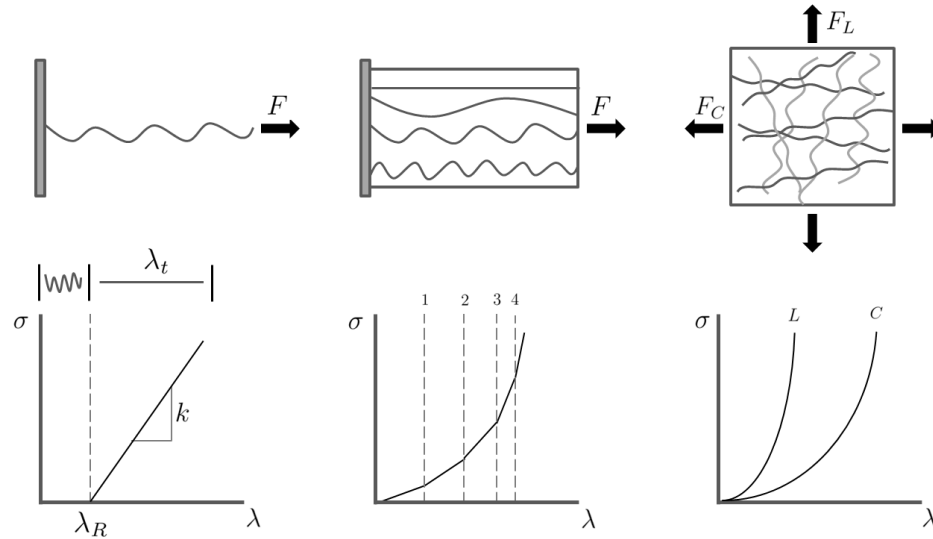


Figure 4.2: Illustration of collagen fibre recruitment concept of the collagen model. Individual collagen fibres are considered to have a linear response, defined by a stiffness modulus, 'k'. Gradual recruitment of these fibres (seen as discrete linear regions) creates a non-linear mechanical response. It can be seen that orientation of fibres in a biaxial sense can create a directional dependant response with recruitment a function of fibre stretch.

In this case a triangular probability distribution function (PDF), ρ_f was used to represent the range of stretches where collagen fibres may be recruited to bear load. Defining parameters to this distribution include minimum, modal and maximum recruitment stretches; a , c and b , respectively. It is noted that this approach has the distinct ad-

vantage over Beta and Gaussian distributions as parameters may be derived directly from imaging studies, as opposed to fitting. Additionally, skew of the distribution may be introduced and studied.

$$\rho_f(\lambda) = \begin{cases} 0 & \lambda_R < a \\ \frac{2(\lambda_R - a)}{(b-a)(c-a)} & a \leq \lambda_R < c \\ \frac{2(b - \lambda_R)}{(b-a)(b-c)} & c \leq \lambda_R < b \\ 0 & \lambda_R \geq b \end{cases} \quad (4.16)$$

An example illustration of the triangular PDF and its associated cumulative density function (integrated PDF) can be seen in Figure 4.3.

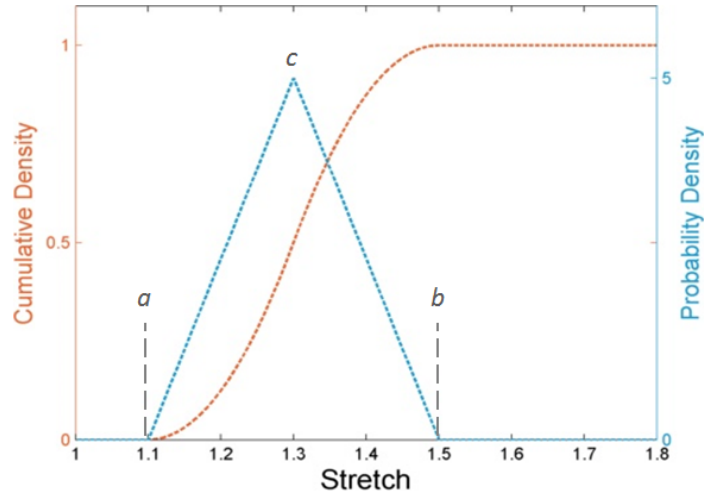


Figure 4.3: Illustration of triangular PDF defined by minimum (a), modal (c) and maximum (b) recruitment stretches. Collagen fibres are assumed to initiate recruitment at a stretch of 1.1 in this case, with all fibres recruited at a stretch of 1.5. Cumulative density function illustrates the additive effect of progressive collagen recruitment over the range of stretches and essentially defines the non-linear stress-stiffening response of the SEF.

The strain energy of the collective collagen fibres may be computed as the integral of the product of individual collagen response and the proportion of collagen fibres recruited at a given stretch.

$$\psi_{col,i} = \int_1^{\lambda_{f,i}} \Psi_f \left(\frac{\lambda_{f,i}}{\lambda_R} \right) \rho_f(\lambda_R) d\lambda_R \quad (4.17)$$

Here, the collagen fibre mechanical model was computed as a function of tissue stretch λ and recruitment stretch λ_R . As the PDF is a piecewise function, the resulting integral remains piecewise. This may be differentiated to obtain the 1st Piola-Kirchoff stress of the collective recruited collagen fibres at a given tissue stretch; yielding the following piecewise function,

$$P_{col} = \frac{d\psi_{col}(\lambda_f)}{d\lambda_f} = \mu_c \begin{cases} 0 & \lambda_f < a \\ f_1(\lambda_f) & a \leq \lambda_f < c \\ f_2(\lambda_f) & c \leq \lambda_f < b \\ f_3(\lambda_f) & \lambda_f \geq b \end{cases} \quad (4.18)$$

Where,

$$\begin{aligned} f_1(\lambda_f) &= \frac{1}{(b-a)(c-a)} \left[(\lambda_f + a) \ln \frac{\lambda_f}{a} + 2(a - \lambda_f) \right] \\ f_2(\lambda_f) &= \frac{(\lambda_f + a)}{(b-a)(c-a)} \ln \frac{c}{a} - (c-a)(\lambda_f + b) \ln \frac{\lambda_f}{c} + 2(c-a)(\lambda_f - b) \\ f_3(\lambda_f) &= \frac{(\lambda_f + c)}{(c-a)(b-c)} \ln \frac{c}{a} - (c-a)(\lambda_f + b) \ln \frac{b}{a} \end{aligned} \quad (4.19)$$

In Figure 4.4 an illustration of the stress-stretch relationship obtained by this model is given. The typical soft collagenous tissue stress-stiffening response was obtained by the additive effect of the recruited collagen fibres. It can be seen that at stretches above the maximum recruitment stretch, b , the response becomes linear with a gradient defined by the collagen modulus.

A brief parameter study is shown in Figure 4.5. Values of a , b and c and their effect

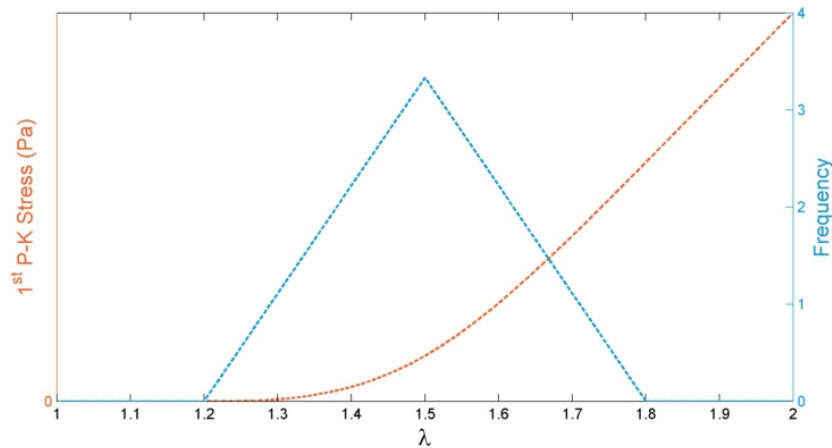


Figure 4.4: Illustration of the non-linear collagen response with associated recruitment stretch distributions. The integrated model of recruited collagen fibres provides the stress-stiffening. Note that stress remains zero until the first fibre is recruited and the curve becomes linear after all collagen fibres have been recruited.

on curve behaviour is shown. Changing the value of a shows the initiation of the heel region can be controlled whilst the gradient of the linear portion remains constant. c effects the initial gradient at the heel region and further influences the gradient through the linear portion of the curve, and reduces linearity with lower values of c . Maximum recruitment parameter, b , controls where all fibres have been recruited and the initiation of the linear portion of the curve at higher stretches. It also affects the initial gradient at the heel region and has influence over the linearity of the post-heel region of the curve.

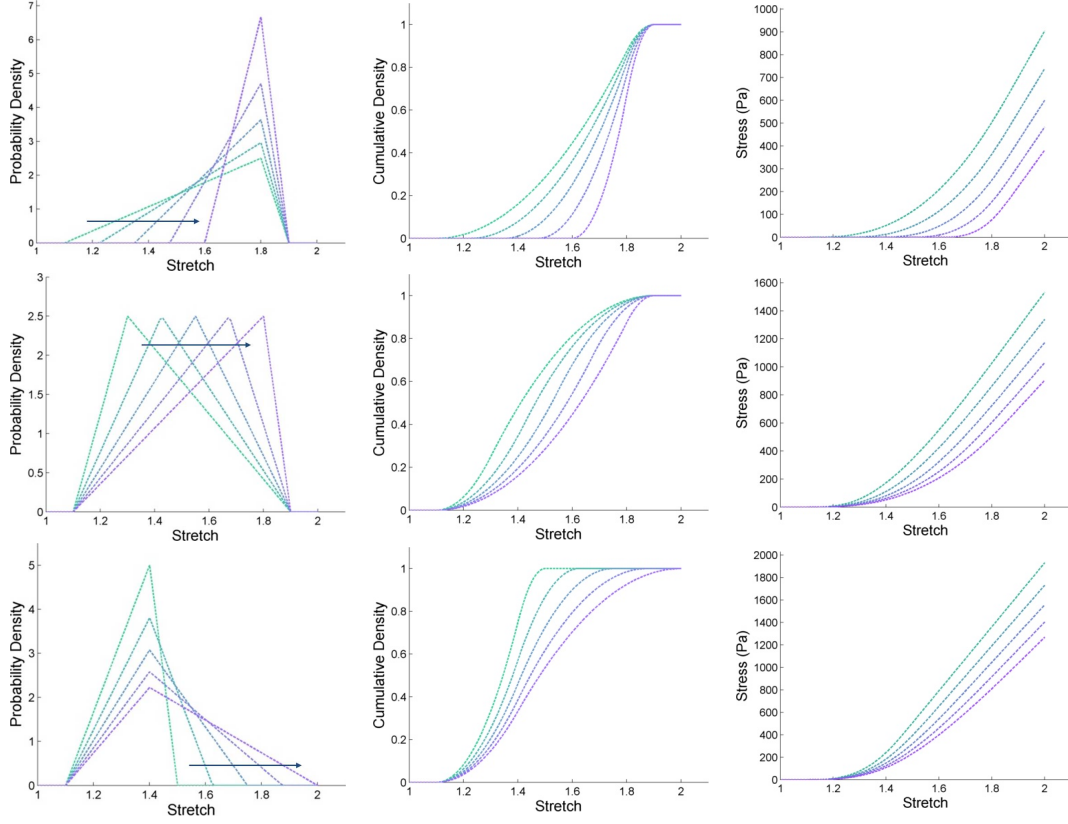


Figure 4.5: Parameter study of recruitment constants a , b and c showing PDF (left), CDF (centre) and 1st Piola-Kirchoff Stress (right) curves. (Top) Curves of model with a ranging from 1.1 to 1.6. (Middle) Curves of model with c ranging from 1.3 to 1.8. (Bottom) Curves of model with b ranging from 1.5 to 2. Arrows show direction of increasing values

Fibre Orientation

In collagenous tissues fibres do not align perfectly along a defined fibre direction. Rather, they tend to disperse about the mean orientation [126]. As such, this dispersion may be represented by a distribution across a planar splay, assuming the fibres lie within a single 2D plane [24]. Here, the fibre stress-stretch was integrated numerically across a semi-circle, representing the possible spread of fibres. A 2D orientation distribution ρ was defined as a function of orientation, θ , between ranges $[-\pi/2, \pi/2]$. Additionally, this distribution can be obtained by fitting imaging data, such as that presented in Section 2.4. Orientation distributions obtained previously from local imaging

data were used in all subsequent model fitting to biaxial testing data.

As described in Section 2.4 a modified *von Mises* distribution was fitted to fibre orientation data. To recap, the distribution across a single plane is defined as,

$$\rho(\theta_i) = 4 \left(\sqrt{\frac{b}{2\pi}} \right) \frac{\exp[b(\cos(2\theta_i) + 1)]}{\operatorname{erfi}(\sqrt{2b})}. \quad (4.20)$$

Where b is used as a fitting parameter. The distribution is defined under the normalisation condition,

$$1 = \frac{1}{\pi} \int_{-\pi/2}^{\pi/2} \rho(\theta_i) d\theta_i, \quad (4.21)$$

Following a similar approach to Lanir, 1994 the SEF response for the collagen can be expressed as the integrated response of fibres over the range of angles,

$$\Psi_f = \int_{-\pi/2}^{\pi/2} \rho(\theta_i) \psi_c(\lambda_{f,i}) d\theta_i \quad (4.22)$$

The strain energy of the collagen fibre ensemble was differentiated to compute the 1st Piola-Kirchoff anisotropic stress.

$$P_{f,i} = \int_{-\pi/2}^{\pi/2} \rho(\theta_i) P_c(\lambda_{f,i}) [\mathbf{m} \otimes \mathbf{m}] d\theta_i \quad (4.23)$$

The total SEF of the bladder was obtained by summing smooth muscle (Ψ_{sm}) and collagen (Ψ_{col}) components (See Appendix for derivation of stress function) and may

be expressed as,

$$\begin{aligned}
\Psi &= \phi_{sm} \Psi_{sm} + \phi_{col} \Psi_{col} \\
&= \phi_{sm} \sum_{i=4,6} \frac{\mu_{sm}}{3} (\lambda_{f,i} - 1)^3 + \phi_{col} \sum_{i=4,6} \int_{-\pi/2}^{\pi/2} \int_1^{\lambda_{f,i}} \Psi_f \left(\frac{\lambda_{f,i}}{\lambda_R} \right) \rho_f(\lambda_R) \rho(\theta_i) [\mathbf{m} \otimes \mathbf{m}] d\lambda_R d\theta_i
\end{aligned} \tag{4.24}$$

4.3 Results: Model Fitting to Biaxial Testing

The proposed constitutive model of the bladder wall in Section 4.2.2 was fitted to biaxial mechanical test data in young / aged bladders of rat with two individuals per group identified as FY01, FY03 (young) and FA03, FA04 (aged) (See Section 3 for protocols). Corresponding MPM imaging data of the bladder wall was introduced into the model to define PDF parameters and collagen fibre orientations (See Section 2 for details). Due to imaging depth limitations the second, deeper layer of muscle was assumed to lie perpendicular to the outer muscle layer, i.e. $\theta_2 = \theta_1 - 90$. This assumption is supported by literature [79] and observations presented in previous sections. Fibre dispersion about the second fibre family was assumed to be the same as the first fibre family. All fitting was performed in Matlab 2013a using the built-in function *nlinfit*. This function estimates model parameters using an iterative least squares estimation method given an initial coefficient 'guess' value. An overview of the model fitting process was as follows,

1. Collagen recruitment (fibre tracing) and orientation (2D FFT) quantified directly from imaging data,
2. Mechanical stress-stretch obtained from biaxial testing,
3. *von Mises* distribution fitted to orientation data centred about the mean fibre

orientation and used to obtain dispersion model,

4. CDF form of triangular PDF fitted to raw collagen recruitment data; minimum, maximum and modal parameters obtained,
5. Recruitment distribution parameters included in mechanical model,
6. Elastin modulus, μ_e and collagen modulus μ_c obtained by fitting of model to mechanical stress-stretch data.

Area density fraction of smooth muscle, ϕ_{iso} and collagen ϕ_{col} were approximated from studies by [127]. Authors report smooth muscle in rat bladder accounts for approximately 60 - 70 % of the total bladder wall. A lower estimate in this range of 60 % was chosen and the remaining 40 % occupied by collagen. Note, the area fraction only accounts for tissue area that plays load bearing role.

Collagen orientation, fitting of fibre recruitment PDF and constitutive model to experimental results from collagen fibre tracing and mechanical tests in young rat bladder (Samples FY01 and FY03) are shown in Figure 4.6 and Figure 4.7.

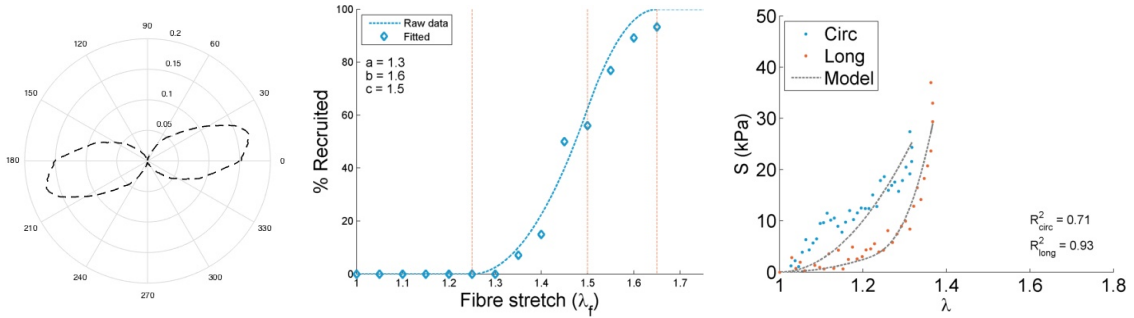


Figure 4.6: (Left) Polar plot of normalised orientation magnitude averaged across sample wall depth at $\lambda = 2$. (Centre) Collagen recruitment data with fitted PDF model with listed parameters in young mouse bladder (Sample FY01). (Right) Constitutive model fitted to mechanical stress-stretch curve data in circumferential and longitudinal axes of Sample FY01. Model has experimental data from recruitment, orientation and dispersion implemented for each specific specimen. Significant differences are seen between longitudinal and circumferential behaviour.

Normalised orientation magnitude plots of collagen orientation in samples FY01 and FY03 possess similar characteristics and mean direction (16° and 10°). Initiation of collagen recruitment occurs at $\lambda = 1.1$ and $\lambda = 1.3$ in samples FY01 and FY03, respectively. Modal recruitment was 1.2 and 1.5 in young samples, and means sample FY01 has a steeper recruitment curve gradient. Stretch at which all fibres have recruited occurs at $\lambda = 1.6$ in both samples. The recruitment PDF model fit data well with R^2 values of 0.71 and 0.93 for FY01 and 0.9 and 0.7 for FY03 (circumferential and longitudinal, respectively).

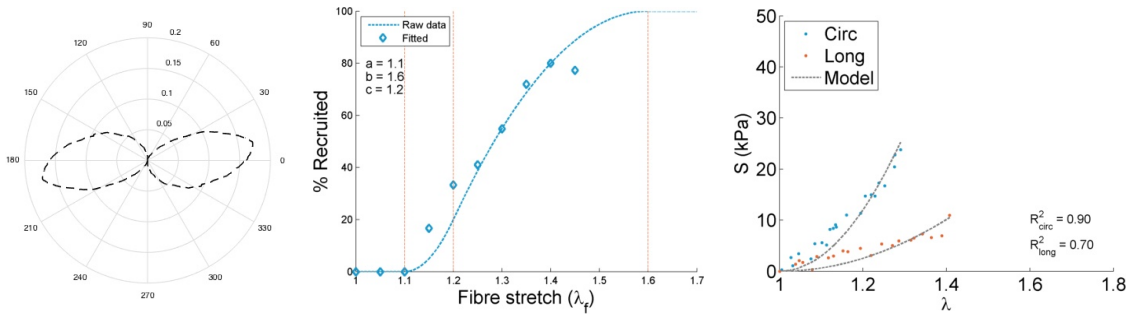


Figure 4.7: (Left) Polar plot of normalised orientation magnitude averaged across sample wall depth at $\lambda = 2$. (Centre) Collagen recruitment data with fitted PDF model with listed parameters in young mouse bladder (Sample FY03). (Right) Constitutive model fitted to mechanical stress-stretch curve data in circumferential and longitudinal axes of Sample FY03 with R^2 fit value.

Stress-stretch curves of FY01 show significant differences in mechanical response between longitudinal and circumferential directions. The toe region of the circumferential axis was much stiffer than seen in the longitudinal axis and spans a shorter range of stretch before a heel region was seen. The circumferential response has a steeper gradient than the longitudinal direction. Differences between circumferential and longitudinal mechanical responses are captured well by the model. Mechanical response of sample FY03 shows similar curves for longitudinal and circumferential axes, particularly in the toe region. A steeper gradient in the heel region was observed in the circumferential axis.

Orientation, recruitment and mechanical data of aged rat bladder, samples FA03 and FA04, biaxial testing with fitted models are given in Figure 4.8 and Figure 4.9.

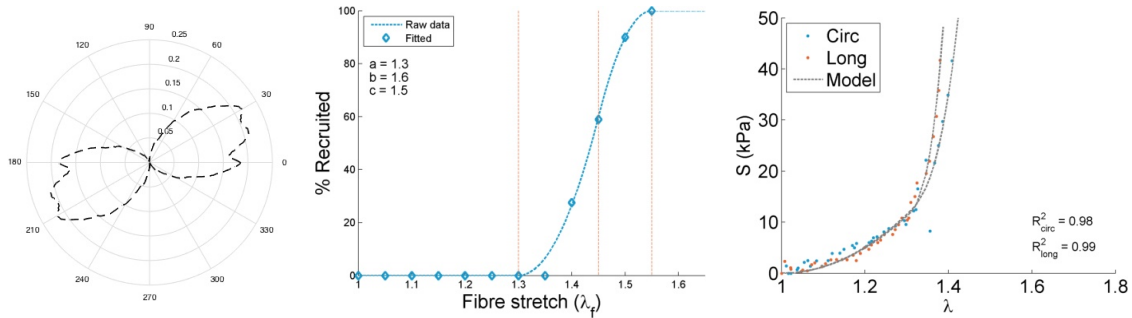


Figure 4.8: (Left) Polar plot of normalised orientation magnitude averaged across sample wall depth at $\lambda = 2$. (Centre) Collagen recruitment data with fitted PDF model with listed parameters in young mouse bladder (Sample FA03). (Right) Constitutive model fitted to mechanical stress-stretch curve data in circumferential and longitudinal axes of Sample FA03.

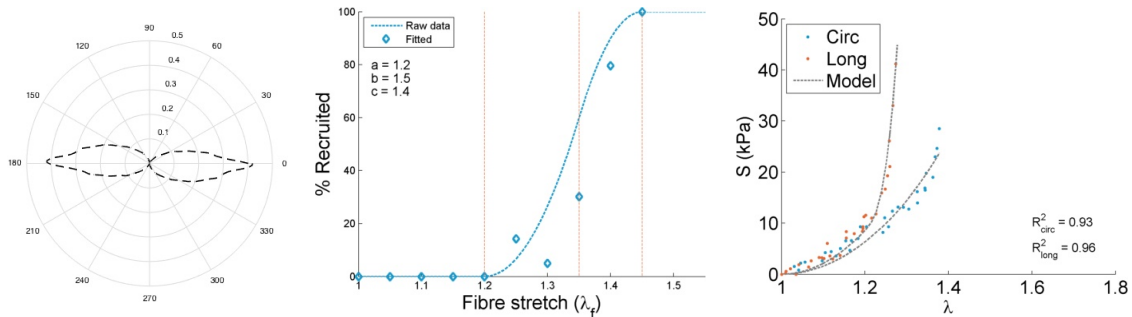


Figure 4.9: (Left) Polar plot of normalised orientation magnitude averaged across sample wall depth at $\lambda = 2$. (Centre) Collagen recruitment data with fitted PDF model with listed parameters in young mouse bladder (Sample FA04). (Right) Constitutive model fitted to mechanical stress-stretch curve data in circumferential and longitudinal axes of Sample FA04.

Figures of FA01 and FA03 show a stiffer mechanical response and a steep collagen phase of aged tissue. This resulted in greater collagen modulus values in both longitudinal and circumferential axis as would be expected (See Table 4.1). Model fitting to aged data also shows marginally higher recruitment stretch initiation of aged tissue; average a values of 1.2 and 1.275 in young and aged, respectively. Complete recruitment occurred similarly between samples at $\lambda = 1.6$. Fitting of the μ_{sm} reveals greater longitudinal stiffness in low stretch regions of aged specimens. Circumferential low strain stiffness

Table 4.1: Table of measured orientation parameters and fitted recruitment and stiffness parameters of model for representative samples. Note, orientation was angle from circumferential axis.

	Longitudinal		Circumferential					
	$\mu_{sm}(\text{kPa})$	$\mu_c(\text{MPa})$	$\mu_{sm}(\text{kPa})$	$\mu_c(\text{MPa})$	a	b	c	$\theta_1(^{\circ})$
FY01	51.4	6.34	20.9	0.13	1.3	1.6	1.5	16
FY03	54.6	0.16	251.0	0.19	1.1	1.6	1.2	10
FA03	103.4	17.4	106.5	6.19	1.3	1.6	1.5	31
FA04	178.9	14.9	132.1	0.56	1.25	1.5	1.35	1

was similar between aged samples. Differences between FY01 and FY03, were much larger and not consistent between the samples. Fitting of the model to aged mechanical data was more consistent and produced R^2 values near unity for FA03 (0.99 and 0.98 in circumferential and longitudinal, respectively). Fitting to FA04 produced similarly good fits to data (0.93 and 0.98).

Table 4.1 briefly summarises measured and fitted parameters for models fitted to each sample. Values were rounded to 2 significant figures.

4.4 Discussion

This study presents, for the first time, quantification of the collagen structure in terms of recruitment, orientation, and distribution of fibres of the bladder wall. Further, these findings are mathematically described using a novel, microstructural constitutive response. In the bladder wall, smooth muscle and collagen are hypothesised to be the main load bearing constituents with direction dependant mechanical properties.

Collagen is hypothesised to bear minimal load at low stretches and is responsible for the non-linear stress-stiffening seen at high stretch [4] [2]. To date the recruitment function has not been quantified in the bladder wall. In this study MPM imaging was used in conjunction with a custom biaxial testing machine to directly quantify collagen recruitment over a range of biaxial stretches in the same sample. A bladder specific microstructural model was developed with contribution from passive smooth muscle and collagen with microstructural representation of collagen recruitment, orientation, area fraction and dispersion. These parameters were quantified in aged and young adult murine bladder wall strips subjected biaxial stretch under MPM imaging. Subsequently, smooth muscle and collagen constants were fitted to circumferential and longitudinal biaxial data.

Collagen fibre tortuosity and orientation was measured in series of 2D MPM stacked images. Recruitment of fibres was observed to occur over a range of stretches, depending upon the initial waviness of fibres, with initiation of recruitment correlating with a sharp increase in stiffness of the tissue. Based on these observations a constitutive model was developed where collagen recruitment could be represented using a PDF over the measured range of stretches. Using this approach a single collagen fibre could be model using a simplistic linear model where incremental recruitment of these linear fibres resulted in a highly non-linear behaviour. This approach requires a single fitting constant for the collagen component.

To the best of the author's knowledge there has only been one attempt in the previous literature to model the bladder wall using a microstructural approach. In this case however, collagen parameters were set using theoretical assumptions and not quantified experimentally [15]. Quantified PDF parameters a, b and c were assumed to be consistent between longitudinal and circumferential axes, i.e. recruitment in terms fibre specific stretch. Initiation of collagen recruitment (i.e. a) in young samples was seen to

occur at marginally lower stretches compared with aged samples which corresponded to later stress-stiffening curves. b was consistently between 1.5 and 1.6 in all samples, suggesting all fibres had been recruited at approximately similar stretches. Modal recruitment, c , varied significantly between samples. Mid-portion of the curve gradient was controlled this parameter and shows the variation in the collagen portion of the stress-stretch curves obtained. Variation in the collagen portion of the curve was also underlined by the large differences seen between the collagen modulus constant. Intra-specimen variation is hypothesised to be the main contributor to differences in curve behaviour, although small differences in initial configuration may also be a factor.

The initial portion of the curve was found to be stiffer in the circumferential axis on average, represented by a higher smooth muscle modulus. Further, the toe region of aged bladder wall was significantly less compliant and was captured well by the smooth muscle component of the model. Heel region and stiffer portions of the stress-strain curve, i.e. $\lambda_f > a$, was modelled using the collagen component of the constitutive model. Collagen modulus μ_c , in most cases, was fitted < 1 MPa. Maximum stiffness modulus was estimated at 17.4 MPa. Assuming a linear stiffness, the experimentally derived stiffness of individual collagen fibrils is reported within the range of 100-1000 MPa [128]. Differences between values suggest that the model may preferentially conform to recruitment occurring at lower stretches of the heel region. Alternatively, there may be additional mechanisms involved during expansion that are not accounted for by the current model, i.e. interaction between smooth muscle and collagen.

Overall, the microstructural model used in this study was found to provide a range of fits to data with R^2 values ranging between 0.7 and near unity in both longitudinal and circumferential axes. Lower R^2 may be attributed to noise (biaxial has inherently more noise than uniaxial due to a measured displacement and force). This quality of fit was achieved using only two fitting parameters to describe collagen and smooth muscle stiff-

ness. As the higher stretch regions of the stress-stretch curve were attributed mainly to the collagen response, parameter fitting resulted in unique parameter values. The good fit to mechanical test data supports the ability of the model to capture the recruitment of collagen fibres over a distributed range of tissue stretches directly quantified from imaging data. Additionally, collagen orientation and dispersion characteristics could be attributed to differences seen between longitudinal and circumferential mechanical response.

Brief discussion of passive smooth muscle models

Several constitutive models were considered to model the low strain mechanical response, assumed to be primarily smooth muscle bearing load. A Neo-Hookean approach was found to be an inappropriate choice as the initial stress-stiffening fails to capture the long toe region of the bladder mechanical response. The linear region of the Neo-Hookean at higher strains was also a bad fit to bladder data. Fitting of the Neo-Hookean often resulted in a negative stiffness moduli or poorly fitted data. An exponential model appeared to fit the bladder data well in both low and high strain regimes. However, an exponential model was found to contribute disproportionately to the mechanical response at high strains where collagen is the main load bearing component. This resulted in low collagen modulus values and high exponential constants. A cubic polynomial model provided a compliant initial toe region and does not significantly stiffen at the heel region; allowing the collagen model to contribute mechanically to the stress-stiffening. Further investigation may look to modelling work by Wognum, 2010 (thesis). The author suggests a fibre recruitment model to represent smooth muscle in the bladder wall, assuming muscle cells to follow a similar recruitment mechanism as seen in collagen fibres. However, whilst this approach provides a novel insight into smooth muscle cell stretch, it has not yet been quantified, and to date there appears to be a lack of imaging protocols to accurately measure recruitment

of smooth muscle fibres.

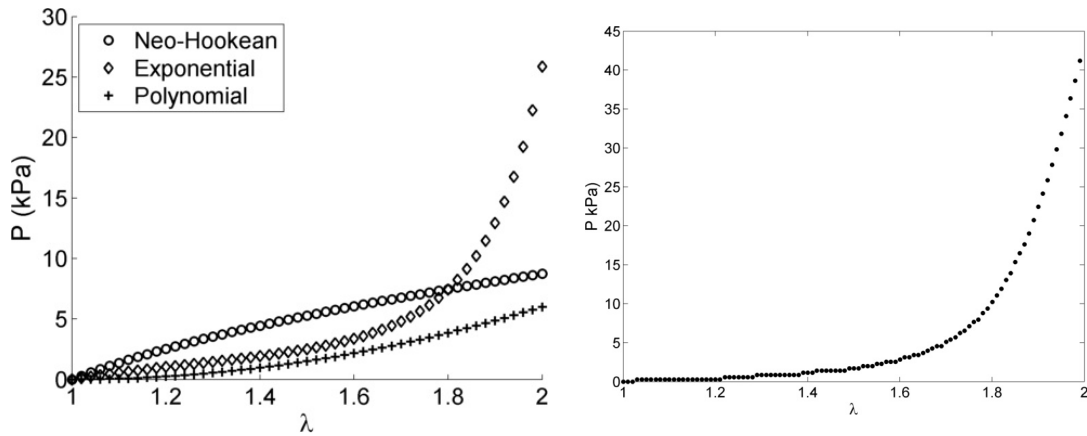


Figure 4.10: (Left) Plots showing comparison of Neo-Hookean, Exponential and Polynomial models to represent smooth muscle. (Right) Representative illustration of uniaxial bladder mechanical response for comparative purposes.

Limitations

Limitations of the model are tied to the current constraints in imaging modalities. Microstructural models are reliant upon accurate quantification of the key structural layers and constituents of the tissue. MPM has limited penetration of several hundred micrometres, and when compared to total tissue depth it is not sufficient to image the entire wall. This means quantification of the entire microstructure is currently not possible using MPM techniques. Therefore, simplifications or assumptions must be made. However, using high resolution micro-computed tomography it is possible to get full through wall thickness imaging data [129]. For example, in this study it was assumed muscle bundles lay perpendicular (which was observed), however could not be quantified in the same imaging state. The current model describes the smooth muscle passive component phenomenologically. Ideally, each component would be modelled using a microstructural description; however, to date the passive function of the smooth muscle has not yet been described in a way that has been quantified from imaging studies.

Experimental studies [23], [11] and [130]. suggest individual collagen fibres follow a nonlinear mechanical response to uniaxial tension that linear models cannot capture. A characteristic linear, low stress region at lower strains is followed by nonlinear stiffening at strains reaching 35% [131]. Exponential functions, much like the Fung model previously described, have been used extensively to model collagen, as have power-law functions [24]. Further investigations may look to improve the characterisation of the linear collagen model, particularly for higher strains, employed here.

In this study experimentally determined collagen recruitment, orientation and dispersion were integrated into a novel constitutive model and fitted to biaxial mechanical response. A triangular PDF was used to represent the range of stretches that recruitment occurs and was found to provide a good description of the stress stiffening seen at higher stretch regimes in bladder wall. The intended purpose of this approach to modelling was to gain understanding into how individual components that make up a tissue contribute to the overall macroscopic mechanical response and function. The current study provides a significant step forward to understanding of the bladder wall response in terms of the microstructure and its contribution to the macroscopic material response. Changes that occur to the functionality of the organ either through aging or pathology may be identified and attributed to specific changes in the microstructure. It is expected that further development of these models may provide increased information about the tissue (and microstructural) state to be obtained from clinical functionality testing, and further, be used a predictive tool for tissue remodelling and pathology progression.

Development of a Micturition Model to Simulate Normal Filling and Voiding in Patients

This chapter describes the development of a micturition mathematical framework to simulate filling and voiding. The purpose of this model will initially be to identify and define the key parameters of the lower urinary tract and nervous control involved in voiding and their influence on pressure and flow relationship. Normal patient data obtained from University College Hospital London (M. Duffy) was used to test hypotheses and validate aspects of the model, then further to fit the model.

Models such as the one described in this chapter may path the way for improved diagnostics for LUT disorders and optimise treatment choices selected. Three potential avenues for investigation are identified:

- In a case where a patient will present a certain history to a clinician describing their symptoms, it is quite possible that when undergoing diagnostic testing that the clinician may search subjectively for those specific symptoms; potentially missing other underlying disorders. Modelling may improve objectivity of the test by considering all the aspects of the LUT performance.

- Urodynamic studies are invasive, causing pain and discomfort to patients. Non-invasive alternatives such as uroflowmetry cannot be used for certain disorders as they lack the information required to make a reasonable diagnosis. Modelling may be used to fill in these gaps of information and may mean non-invasive alternatives become a viable option.
- Urodynamic studies produce a huge amount of data that is interpreted by highly skilled and experienced clinical staff. However, despite this there may be still be a large amount of additional information that can be gained by applying mathematical modelling that would otherwise be missed by a person. Data obtained in this way could lead to improved diagnostic measures, or compliment the clinician's interpretation of the pressure-flow data.

5.1 Introduction

The motivation for a clinically relevant micturition model is driven by current shortcomings of invasive techniques used in clinical diagnosis of LUT disorders [132]. The urinary tract is a complex and dynamic environment and a detailed and reliable model has not yet been developed for specific analysis of disorders. There is a tendency in clinical quantification of urodynamics to reduce the data to a series of minima, maxima and compliance values to distinguish normal from abnormal. Nomograms and normal range of values are often used to differentiate normal and pathological patients case, and can identify severity of certain disorders. However, clinicians will often seek the original urodynamic trace to make a diagnosis as simplified measures cannot yet accurately measure the state of the urinary tract. In an objective approach, the specific gradients, curvature and synchronisation of pressure and volume changes in the traces must be considered. Development of sophisticated computational models may provide

a tool to distinguish between normal and abnormal.

Potential applications include identification of masked secondary symptoms related to bladder impairment, and prediction of pathology progression to reduce frequency of follow-up procedures. Additionally, the development of micturition models has the appeal of reducing the need for invasive procedures, and increasing the ability of non-invasive tests to quantify dysfunction.

In the current literature, there is limited research involving modelling of the bladder when compared to topics such as cardiovascular modelling. Considering the vast population that is affected by LUT disorders, the potential benefits of producing a clinically relevant model are great. Therefore, this section aims to integrate previous modelling work and the microstructural description of the urinary bladder into a micturition modelling framework to emulate and evaluate voiding cycles of patients.

The primary aim of the work described in this chapter is to develop a micturition framework derived from existing flow-pressure mathematical laws, and implement novel microstructurally motivated constitutive models to describe the active and passive mechanical behaviour of the bladder. Secondly, patient urodynamic data are gathered (Megan Duffy, University College London Hospitals) and used to establish common physiological parameter values for the components of the model within normal clinical ranges.

5.2 Review: Micturition Models

Micturition models in existing literature encompass a large range of different approaches to modelling the LUT and the various systems that influence function [133], [134], [135], [136], [121]. This section will cover an overview of the various different aspects that are

broadly represented in the models to date, including; detrusor contractility, urethral function and resistance, elasticity, and nervous system control.

Mechanical properties, namely elasticity, visco-elasticity and contractility, of the detrusor layer govern the function of the bladder during filling and voiding. Mechanical response during filling is usually assumed to be attributed to the passive elastic response, or a combination of active relaxation and passive elasticity. Elastic models used in micturition frameworks (those that include the filling cycle) lack any microstructural description and have so far only employed non-linear lumped parameter models using power law or exponential responses (See Table 5.1). Not surprisingly, models with large numbers of parameters may give high quality fitting to data; however do not provide any relevant information into specific mechanisms or correspondence between individual model components and microstructure function [96]. Consequently, they are limited in their ability to stratify bladder wall changes due to dysfunction. Additionally, a high number of model parameters may result in overfitting of the data, as more parameters will essentially allow any curve to be fit, but may not necessarily give relevant conclusions.

Step-wise cystometry allows the bladder to be filled at different rates to examine pressure changes as a function of time and in doing so, obtain insight into viscoelastic properties of the bladder wall. Early authors of this technique, such as [137] and [18], note the value of evaluating viscoelastic properties and distinguishing between the active relaxation of the muscle and passive mechanisms. Whilst viscoelasticity has been suggested as an important measure in urodynamic studies by some authors and has been widely studied both experimentally and using lumped parameter models [137], [85], [18] it has not seen wide use in micturition frameworks [18], [138]. Authors tend to describe passive mechanics purely in terms of elasticity. [138] describe a delayed elasticity term to account for pressure changes seen at different fill rates, given

Table 5.1: Overview of micturition model bladder properties and nervous control methods used in published models.

Reference	Elasticity	Contractility	Nervous Excitation
Griffiths & Rollema, 1979	Exponent	Linear	Binary
Hubener & von Mastrigt, 1994	n/a	Parabola	Binary
Hosein & Griffiths, 1990	Parabola	Linear	Control System
Bastiaanssen et al., 1996	n/a	Parabola	Control System
Fletcher, 2004	n/a	Parabola	Exponential
Paya et al., 2013	Exponent	Exponent	Control System
Valentini et al., 1990	Filament	Parabola	Progressive
van Duin et al., 1998	Exponent	Parabola	Control System

as, $dL_{ve}/dt = (L_{as} - L_{ve})/\tau_{ve}$ (L_{ve} denotes the ratio between actual and minimum lengths of the viscoelastic elements and τ_{ve} the time constant). The authors note that addition of a viscoelastic element may highlight certain abnormal filling patterns.

The relation between volume, pressure and flow is typically assumed to be controlled by the urethra. This part of the system effectively splits the power generated by the detrusor into flow and pressure, i.e. increased flow means lower vesical pressure, and vice versa. The model typically chosen to represent this relation is usually described by urethral resistance relation (URR) and has been published in several formats (See Table 5.2). A linear relationship, as proposed by [31], provides a better approximation of low pressure-flow when compared to quadratic forms, such as those by [50] and [139].

The proper working of the bladder, sphincter and urethra is dependant upon synchronised nervous control. Experimental and modelling work by [140] quantify nervous efferent and afferent signal during voiding in rats and compare signal activity with

Table 5.2: Published models of the urethra resistance relation.

Reference	Model	Details
Griffiths & Rollema, 1979	$P_{det} = P_{op} + mQ$	Linear model based on wall compliance (m)
Schafer et al., 1985	$P_{det} = P_{op} + Q^2/2A$	Quadratic dependence on flow and sphincter area
Fletcher, 2004	$P_{det} = P_{op} + RQ^2$	Dependence on resistance (R), parabolic
Valentini et al., 1990	Flow derived from hydrodynamics of urethra	Resistance derived from flow and varies during void

mechanical function, i.e. detrusor pressure. Traces show a close correlation between nervous activity and ability of the detrusor to generate pressure (rise in pressure relates to increased nervous activity and vice versa, See Figure 5.1) [140]. Similar observations are made in mice by [141] during filling, as increased pressure results in higher nervous activity. Additionally, activity significantly increased in aged mice.

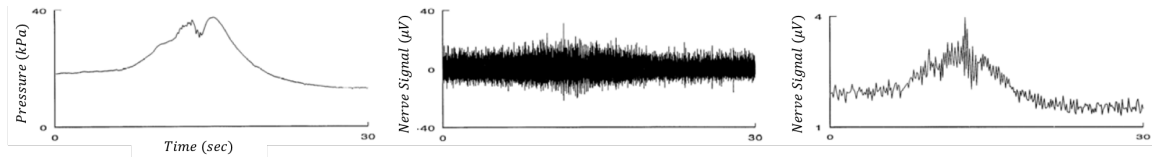


Figure 5.1: Illustration of nerve activity during voiding in rats shows almost analogous relationship between nerve activity and pressure. Sourced from [140].

Micturition models to date have simulated the efferent and afferent network in a variety of methods, from simplistic binary methods to complex neural control networks [142], [121]. For example, nervous control of the VBN model ([138]; named after the three authors) is controlled using a 'flip-flop' method as originally proposed by [139]. This model of the nervous system assumes normalised levels of stimulation of the detrusor

and urethra that ranges from 0 (no stimulation) to 1 (fully stimulated). This method allows the detrusor to be increasingly 'switched on' from 0 to 1 at initiation of voiding to contract and the urethra 'switched off' from 1 to 0 to allow relaxation and flow of urine. Whilst this approach to neural control modelling is simplistic, the flip-flop model remains a leading concept today and has been reproduced in bi-linear and exponential forms [133].

A more complex neural control network is given by [121] with input from 9 neural centres and 8 outputs acting on LUT muscles. Internal signals may be generated by a 'voluntary' system, whereby conscious factors such as voiding desire, or environment can be taken into account to initiate voiding (i.e. person will retain urine unless signal exceeds trigger threshold). Additionally, involuntary initiation is controlled by facilitators and inhibitors of voiding by what is known as the cortical diencephalic centre. These factors are associated with tension of the bladder and sensation of fullness. Again, voiding is activated by the internal signal exceeding a given threshold. This threshold approach is repeated by a pontine storage centre to inhibit voiding depending on whether the aim is to stop urinating or increasing urethral resistance during filling. Similarly, contraction and relaxation of the detrusor and sphincter are controlled by a threshold value to stimulate or relax the muscles in correspondence with storage and voiding. Ultimately, the nervous system model is largely binary in function, although certain functions may be partly activated or inhibited based on a various set of cases, allowing neural, obstructive and disruptive disorders to be studied in detail. The following section will give an overview of the mathematical modelling used to simulate nervous control.

5.3 Micturition Model

5.3.1 Nervous Control

The nervous feedback system of the bladder is extremely complex and extensive with input from both voluntary motor system and sympathetic and parasympathetic components of the visceral motor system (See Section 1.2.5). For simplicity, the afferent nervous control of detrusor contraction and urethral relaxation was modelled in a reduced fashion; namely two functions s_1 and s_2 , respectively. Efferent feedback was excluded from this model. Previously, the nervous signals controlling bladder contraction and urethra relaxation have been modelled previously as a bilinear response [143]. Angles α and β control rate of relaxation / contraction with C limiting relative urethral relaxation (See Figure 5.2).

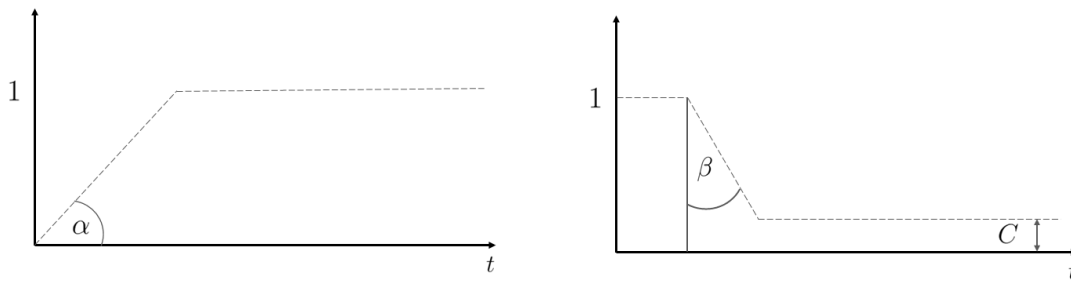


Figure 5.2: Illustration of bi-linear nervous signal as proposed by Hubener & van Mastrigt, 1994 (s_1 shown left and s_2 shown right). Angles α and β give a method of controlling rate of relative relaxation.

However, this bi-linear signal introduces discontinuities in the transition between linear portions of the signal; similarly transferring discontinuities to further parts of the model. Therefore, the new model presented here uses a continuous, exponential function which was chosen to smooth out discontinuities between segments and has been used in micturition models previously [133].

$$s_1(t) = 1 - e^{-k_1 t} \quad s_2(t) = (1 - C)e^{-k_2 t} + C \quad (5.1)$$

Where both signals are a function of time, t . The constants k_1 and k_2 control the rate of increasing or decreasing signal strength and can be considered analogous to the angles α and β (see Figure 5.3), respectively. C ($0 > C \leq 1$) was added to limit the relative relaxation of the urethra once fully relaxed. A delay parameter, δ was used with in s_2 signal and postpones the onset of the nervous response until $t > \text{delay}$. The addition of this parameter allows the onset of flow to be controlled effectively relative to detrusor pressure increase.

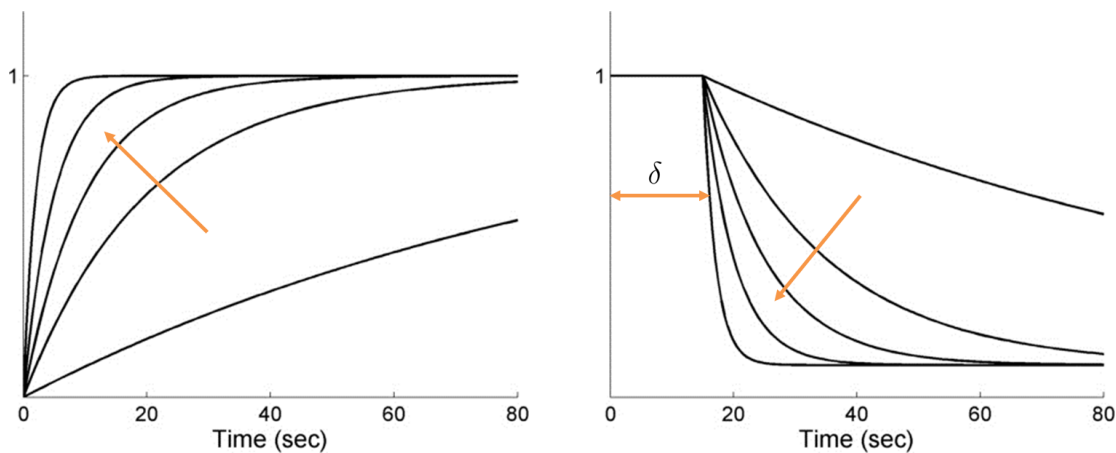


Figure 5.3: Illustration of exponential nervous signals, s_1 and s_2 with k_1 and k_2 ranging from 0.01 to 0.5. (Arrows show direction of increasing values). C was set to 0.1. Note the delay parameter, δ , seen in the s_2 signal response.

The form of s_1 signal was further modified to return to 'resting' nervous activity state towards the end of the filling phase using a similar exponent form, although gradient was described by parameter k_3 . Urethral relaxation was assumed to continue until voiding was complete, with s_2 remaining at minimum excitation once fully relaxed.

$$s_{1,relax}(t) = 1 - e^{-k_3(t_{max}-t)} \quad (5.2)$$

By switching between exponential functions it was possible to achieve a parabolic-like behaviour with individually controllable 'raising' and 'relaxing' activity portions (See Figure 5.4).

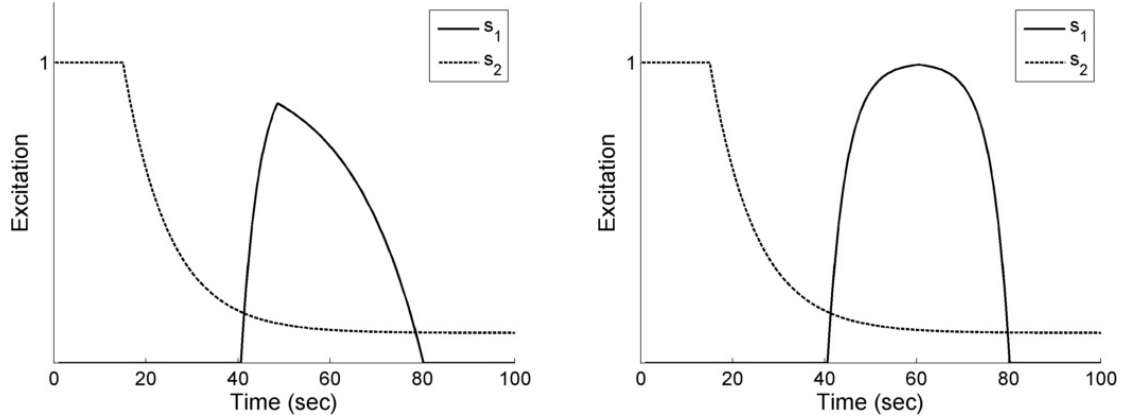


Figure 5.4: Example plots of total s_1 and s_2 signals, including 'excitation' and 'relaxation' portions of s_1 . (Left) shows a more rapid excitation and slower relaxation of signal ($k_1 > k_3$), compared to (Right) where the excitation and relaxation are equal ($k_1 = k_3$). A broad range of curve behaviours can be obtained in this manner.

5.3.2 Bladder

Modelling of the bladder was separated into filling and voiding phases; the properties of each governed by passive and active constituents. The two phases are discussed in the following sections.

Bladder Elastic and Contractile Elements

Filling of the bladder was modelled using contributions from passive constituents, i.e. collagen and elastin. Microstructural modelling of passive bladder behaviour outlined in Section 4 was modified to an isotropic formulation and implemented here for a spherical membrane.

Spherical Membrane

In this section we consider inflation of a spherical membrane to represent the bladder with radius R and wall thickness H in the unloaded configuration. A sphere subjected to uniform internal pressure p was assumed to retain its spherical symmetry during inflation. Work by [144] confirms the bladder's response is only related to the properties of the wall material and that a Laplace approximation of a sphere provides an adequate description of the wall tension and pressure relation. A thin-walled approximation has been used to describe the bladder previously by [31] and [108] amongst others as the bladder wall thickness is a great deal less than its radius (i.e. $H \ll R$) [108].

For simplicity we assume the bladder walls are isotropic and incompressible and that the sphere maintains a perfectly spherical geometry deformation under a uniform internal pressure. Following inflation of the spherical membrane, the principle stretches are,

$$\lambda = \lambda_1 = \lambda_2 = \frac{r}{R} \quad (5.3)$$

Here λ denotes radial stretch, and λ_1 and λ_2 the circumferential stretches of the membrane (considered equal due to symmetrical inflation). R and r denote the sphere radius in both the reference (stress-free) and current (deformed) configurations, respectively. Using the incompressibility constraint $\det \mathbf{F} = \lambda_1 \lambda_2 \lambda_3 = 1$, we can write,

$$\lambda_3 = \frac{1}{\lambda_1 \lambda_2} = \frac{1}{\lambda_1^2} \quad (5.4)$$

From the principle stretches the first invariant may be calculated for a spherical membrane (See Appendix A),

$$I_1 = 2\lambda_1^2 + \frac{1}{\lambda_1^4} \quad (5.5)$$

Where circumferential stretches are implied by assuming incompressibility of the material. For a thin spherical shell with loaded radius r , thickness t and subjected to an internal pressure $\bar{p}$, the Laplace Law is given as

$$\bar{p} = \frac{2\sigma t}{r} \quad (5.6)$$

where σ is true wall stress, or bladder wall tension. Substituting the strain energy function to describe wall tension gives the governing equation of an incompressible, spherical thin walled membrane,

$$\bar{p} = \frac{2\sigma t}{r} = \frac{2\sigma(H/\lambda^2)}{R\lambda} = \frac{2\lambda P(H/\lambda^2)}{R\lambda} = \frac{2PH}{R\lambda^2} \quad (5.7)$$

Where $P = \partial\Psi/\partial\lambda$ and R is the unloaded radius and H is the unloaded thickness. Here, $P = P_{col} + P_{act}$ (described later).

Here the total strain energy of the material (Ψ) can be written as the additive contribution of collagen (Ψ_{col}) and smooth muscle (Ψ_{sm}) constituents to obtain the internal pressure $\bar{p}$ acting normal to the mid-plane. H and R are the thickness and radius of the mid-plane in the unloaded configuration.

The microstructural recruitment model was modified to a single layer, isotropic form, i.e. collagen and smooth muscle orientation was assumed to be random. The modified strain energy function is in the form,

$$\Psi^* = \phi_{sm,iso} \Psi_{sm,iso} + \phi_{col,iso} \Psi_{col,iso} \quad (5.8)$$

Here, $\Psi_{sm,iso}$ is in the same form as the polynomial described previously, substituting

fibre stretch for I_1 ,

$$\Psi_{sm,iso} = \frac{\mu_{sm,iso}}{3}(I_1 - 3)^3 \quad (5.9)$$

And the resulting 1st Piola Kirchoff stress,

$$P_{sm,iso} = \frac{\partial \Psi_{sm,iso}}{\partial I_1} \frac{\partial I_1}{\partial \lambda} = \mu_{sm,iso} \left(4\lambda - \frac{4}{\lambda^5}\right) \left(2\lambda^2 + \frac{1}{\lambda^4} - 3\right)^2 \quad (5.10)$$

$\Psi_{col,iso}$ is an isotropic collagen SEF. True fibre stretch λ_t and recruitment stretch λ_R are related to first principle stretch, $\lambda_t = \frac{\lambda}{\lambda_R}$.

$$\Psi_{f,iso} = \frac{\mu_c}{2}(\lambda_t - 1)^2 \quad (5.11)$$

Fibre dispersion was removed from the model; the strain energy and integral over all fibres with recruitment function $\rho(\lambda_R)$ then becomes (See Section 4.2.2 for further details),

$$\Psi_{col,iso} = \int_1^\lambda \Psi_{f,iso} \left(\frac{\lambda}{\lambda_R}\right) \rho(\lambda_R) d\lambda_R \quad (5.12)$$

1st Piola-Kirchoff stress was then calculated,

$$P_{col,iso} = \frac{d\Psi_{col,iso}}{d\lambda} = \begin{cases} 0 & \lambda < a \\ f_1(\lambda) & a \leq \lambda < c \\ f_2(\lambda) & c \leq \lambda < b \\ f_3(\lambda) & \lambda \geq b \end{cases} \quad (5.13)$$

Where,

$$\begin{aligned}
 f_1(\lambda) &= \frac{\lambda + a}{(b - a)(c - a)} \left[\ln \frac{\lambda}{a} + 2(a - \lambda) \right] \\
 f_2(\lambda) &= \frac{(\lambda + a)}{(b - a)(c - a)} \ln \frac{c}{a} - (c - a)(\lambda + b) \ln \frac{\lambda}{c} + 2(c - a)(\lambda - b) \\
 f_3(\lambda) &= \frac{(\lambda + c)}{(c - a)(b - c)} \ln \frac{c}{a} - (c - a)(\lambda + b) \ln \frac{b}{a}
 \end{aligned} \tag{5.14}$$

When filling the mechanical response of the bladder was assumed to be governed by passive components, i.e., P_{col} . At the initiation of bladder contraction the stress of the bladder wall becomes the response of active components (P_{act}). This assumption was made as van Mastrigt, 1988 reports that shortening of smooth muscle by several percent is enough to almost render negligible the contribution of the elastic constituents.

Active contractile stress produced by the smooth muscle was modelled using a modified parabolic function as suggested by [145]. The active 1st Piola Kirchhoff stress is,

$$P_{act} = \begin{cases} \mu_{act} \lambda_{sm} (\lambda_{sm} - \lambda) (\lambda_{max} - \lambda) / \lambda, & \text{for } \lambda_{sm} \geq \lambda_a \text{ and } \lambda \leq \lambda_{max} \\ 0 & \text{for } \lambda_{sm} < \lambda_a \text{ and } \lambda > \lambda_{max} \end{cases}$$

Here, λ_{max} is the maximum stretch at which smooth muscle is able to produce a contractile force relative to the passive stretch configuration. The following relationship exists between activation stretch λ_a and smooth muscle stretch λ_{sm} ; $\lambda = \lambda_a \lambda_{sm}$. The smooth muscle modulus μ_{act} governs the magnitude of the parabola. The effect of varying different parameters on curve behaviour is seen in Figure 5.5.

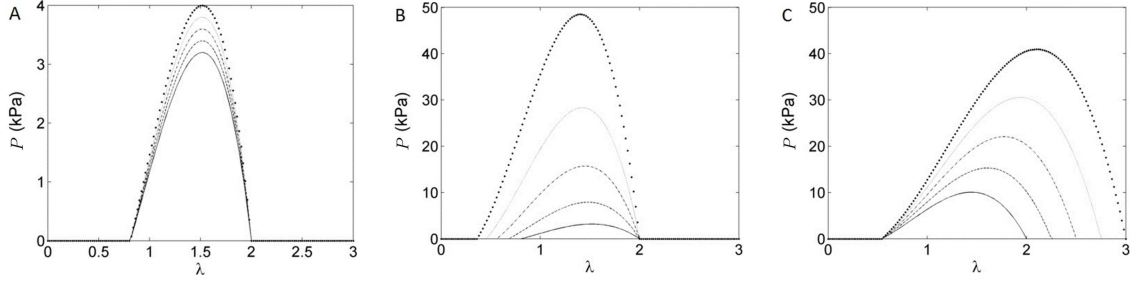


Figure 5.5: Plots of 1st Piola-Kirchoff stress-stretch curves of smooth muscle active model and the influence of parameters on curve behaviour. (A) Smooth muscle contraction modulus μ_{act} (20 - 25 kPa), (B) Smooth muscle minimum activation stretch λ_a (0.6 - 0.9), (C) Smooth muscle maximum contraction stretch λ_{max} (2 - 3).

Bladder Outlet Relation

The model chosen for this study was derived from the well known Hill model describing the relationship between force and velocity of muscle [146]. This was used to obtain the force that the bladder can produce when contracting to void urine and was modified to describe the reciprocal relationship between intravesical pressure and rate of voiding [133], [139]. This framework is known as the bladder outlet relation (BOR). For derivation see Appendix C.

$$\left(p + \frac{p_0}{4}\right) \left(Q + \frac{Q_0}{4}\right) = \frac{5}{16} p_0 Q_0 \quad (5.15)$$

Here, p and Q are pressure and flow of the vesicle at a discrete time point, respectively. The relationship between these measures can be seen in Figure 5.6.

Q_0 and p_0 are used to define the slope of the curves and intersections with the axes, i.e. $Q_0 = Q$ when $p = 0$ and $p = p_0$ when $Q = 0$. The parameters p_0 and Q_0 are defined using physiological parameters, and are subject to nervous signal, s_1 , and current geometry; radius, r and stretch λ .

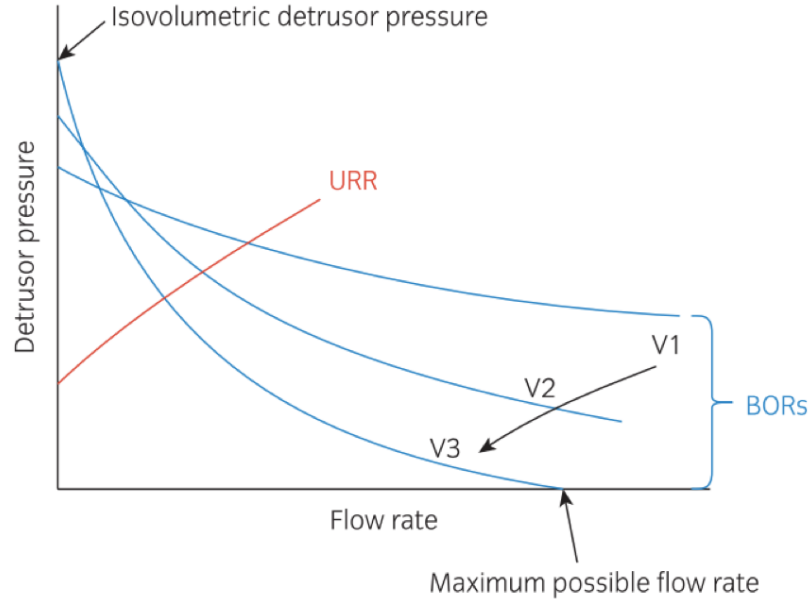


Figure 5.6: Plot showing relationship of detrusor contractility and resistance of the urethra during a micturition cycle. Image reproduced from Sekido, 2012, with permission from John Wiley and Sons.

$$p_0(\lambda, t) = s_1 p_m P_{act}(\lambda) \quad Q_0(r, t) = 2s_1 Q_m r^2 v_{max} \quad (5.16)$$

Parameters p_m and Q_m are intrinsic maximum levels of pressure and flow values, respectively. P_{act} is the contraction of the detrusor muscle. Further, v_{max} is the maximum contraction velocity of the detrusor and can be obtained from experimental data on muscle strips.

5.3.3 Urethra

A second model was then used to relate the flow through the urethra with detrusor pressure, namely urethral resistance relation (URR). The general form chosen for URR is originally proposed by [50] and relates pressure and flow in the urethra by two simple, physiological parameters, urethral opening pressure, p_{op} and urethral cross sectional

area, A . For the full derivation see [50].

Here, the bladder opening was simplified to a hole which has an idealised shape for hydrodynamic functioning. For these models, the effect of gravity was neglected, i.e. bladder is voided by active contraction, rather than weight of the fluid. Additionally, it was assumed fluid flowing through has infinitesimal viscosity, i.e. frictionless flow. This results in the assumption that there was no fluid energy loss flowing through the urethra. Figure 5.7 illustrates the dependence of the model upon the outlet diameter and how it impacts other variables in the model. The relationships between the variables are shown in Table 5.3.

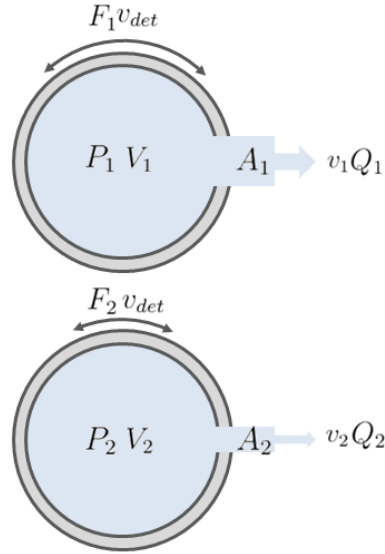


Figure 5.7: A reduced schematic of the model representing the bladder and its dependence upon outlet hole diameter. The detrusor contraction power is relative to its contraction force, F and contraction velocity v_{det} , and measured as a product of detrusor pressure and flow. Voiding volume influences contraction velocity, flow, detrusor force and intravesical pressure as state of smooth muscle stretch will determine contraction function. The area of the outlet hole, A determines how both pressure and flow characteristics are balanced and hence, the voiding velocity, v .

Here, the outlet was described as a distensible tube rather than a rigid pipe. The urethra, when relaxed, collapsed and inner lumen shut. The introduction of minimum

Table 5.3: Variables as illustrated in Figure 5.7 and their relationship with varying outlet hole cross sectional area. Arrow direction indicates increase or decrease of variable depending on case.

Case	A	Q	p	v	F	v_{det}
1	↑	↑	↓	↓	↓	↑
2	↓	↓	↑	↑	↑	↓

opening pressure, p_{op} requires that a certain pressure was generated before the outlet distends and flow commences. Similarly, the tube will collapse when pressure drops below the opening pressure at the end of voiding. This form takes into account the extended urethral cross sectional area, A to give the following relation;

- Narrow area gives high pressure and low flow
- Large area gives low pressure and high flow

The URR form used,

$$p = p_{op} + \frac{1}{2} \left(\frac{Q}{A} \right)^2 \quad (5.17)$$

It can be shown that a reduction in lumen area or an increase in opening pressure, p_{op} , results in a decrease in flow; allowing obstruction based disorders to be examined. The relationship described above typically results in a steep rise in initial flow rate with minimal increases in detrusor pressure (assuming a large urethral cross sectional area). Other studies have previously used values such as m_h , c and R , both describing urethral resistance [133] [51]. p_{op} is the urethral opening pressure at which urine will begin to void during detrusor contraction, i.e. $Q = 0$ when $p < p_{op}$. This simply reflects the pressure needed to overcome the urethral sphincter at void initiation and was computed by,

$$p_{op} = s_2 p_h \quad (5.18)$$

It can be seen that the opening pressure is subject to signal, s_2 , and as such will decrease over time as the urethra relaxes. p_h defines an intrinsic maximum pressure. Figure 5.8 shows an example of how the URR and BOR are solved at each time step as solutions are found at curve intersections. When no solution is found, $Q = 0$.

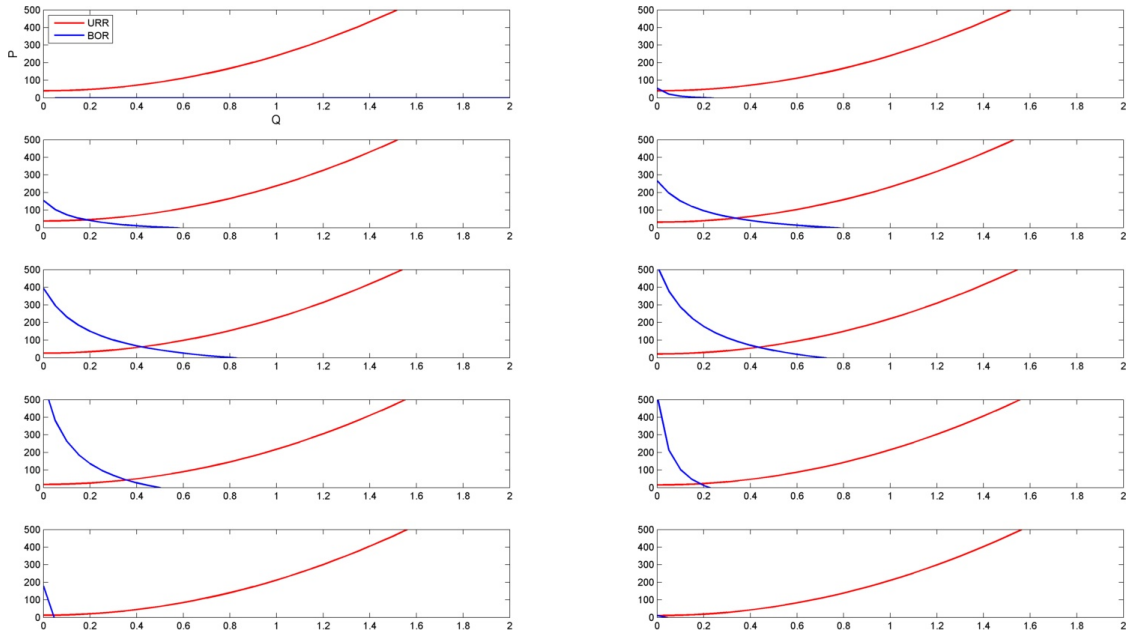


Figure 5.8: Plots of example BOR and URR curves at incremental time steps. Solutions for p and Q found at intersections of the curves. Variables p_0 , q_0 govern intersection of BOR curves with x and y axes. p_{op} defines the pressure at which the URR intersects with the y-axis.

Micturition Model Simulation Steps

The micturition simulation is described briefly below. Simulation time was chosen according to patient data (except in the case of parameter studies where an average time of 120 seconds was used).

1. Nervous control signal coefficients s_1 and s_2 calculated using initial $t_0 = 0$ value.
2. $p_0(t)$, $Q_0(t)$, p_{op} calculated from nervous signals.

3. Bladder radius r and stretch λ calculated from volume V_1 .
4. $P_{act}(\lambda)$ calculated from stretch.
5. $Q_0(r)$ and $p_0(\lambda)$ calculated from r and λ , respectively.
6. BOR and URR are solved at current time step to obtain Presure, p and flow, Q .
7. $dV/dt = -Q$ gives volume of urine voided at discrete time step. Subtracted from current volume to obtained new volume, $V_1 = V_0 - Qdt$.
8. Calculated parameters are stored.
9. Time step incremented $t_1 = t_0 + dt$.

5.4 Model Discussion and Parameter Study

To identify the effect of each parameter in the micturition model on pressure and flow characteristics, a parameter sensitivity study was performed. This type of study is particularly important when identifying parameters that may be exclusive to certain curve urodynamic characteristics, or specific to pathologies. Each parameter was studied within an extreme range of values (low to high) around normal literature values to identify all possible curve behaviours. Parameters tested were independent of each other, i.e. functions of only measured parameters; time and stretch.

Study Design

Initially, a literature review was carried out of clinical based studies to identify 'normal' parameters that are relevant to the model (See Table 5.5). These parameters are kept at an average physiological value when other parameters are varied to ensure consistency and reduce the occurrence of artefacts.

Table 5.4 outlines the clinically relevant parameters searched for in the literature and their corresponding values. Human studies were considered the most relevant. Some animal studies were included for comparison, and in some cases may be scaled appropriately. These values were used as a baseline for parameter studies whilst other parameters were modified.

The effects of each parameter were studied by varying the value from low and high extremes, whilst keeping all other parameters fixed at a baseline value. The fixed values chosen for each are as follows.

The parameters listed in Table 5.5 are those based on clinical values observed in existing literature. Parameters $k_{1,...,4}$ and C are not physiologically quantifiable. Nervous signal parameters were fitted to normalised detrusor pressure curve gradients (i.e. simulate relation between nerve activity and pressure). Table 5.6 illustrates the initial values chosen for the SMC model implemented in the micturition framework. Modifications to parameters were made on a patient specific basis.

It is known that the active force production of the detrusor is related to wall stretch, although the relationship between force and length is relatively broad [30]. Large physiological deformations of the bladder wall mean the detrusor is able to generate force over a large length range in humans [147] and animals [33] [148] [149]. [149] record tension-stretch curves in rat bladder between stretches of 0.5 and 1.5. Force contraction is optimal at approximately $\lambda = 1.2$ and dramatically decreases beyond these ranges, although active force is still produced. [147] report very similar values in human detrusor muscle and through extrapolation show force production to cease at approximately $\lambda = 0.2$. Optimal contraction force of 200 mN/mm² was seen close to $\lambda = 1$ and upper range of force production dropped significantly at stretches above 1.2. Extrapolation of the force production curve suggests force production is possible until much higher stretches. For the model presented here, minimum and maximum contraction stretches

Table 5.4: Clinical parameter values relevant to micturition model obtain from existing literature. Parameters were obtained from 'normal' or control patients in studies. Note some parameters included in the model are not typically recorded clinically.

Parameter	Value	Model	Reference
vol_1	25-50 ml	Human	Bravo et al., 2000
	<50 ml	Human	Digesu et al., 2003
	50-100 ml	Human	Jeffery et al., 2015
	<50 ml	Human	DuBeau et al., 2006
vol_0	393 ml	Human (m)	Maderbascher et al., 1998
	329 ml	Human (m)	Schmidt et al., 2002
	176-447 ml	Human	Chou et al., 2005
v_{max}	10-20 mm/s	n/a	Griffiths et al., 1979
	16 mm/s	Human	Cucchi et al., 2006
Q_{max}	25-50 ml/s	Human	Bravo et al., 2000
	<50 ml/s	Human	Digesu et al., 2003
	<50 ml/s	Human	DuBeau et al., 2006
p_{op}	25-50 cmH_2O	Human	Schmidt et al., 2002
	<50 cmH_2O	Human	Cucchi et al., 1990
	36-74 cmH_2O	Human	Chou et al., 2005
R_{ure}	10-19 mm	Dog	Feeney et al., 1984
	<50 mm	Human	(text) Lierse, 1987
H	1.5-5.5 mm	Human	Hakenberg et al., 2000

Table 5.5: Baseline, minimum and maximum parameter values chosen for study based on literature. Parameters without clinical quantification are assigned a mid-range value as a baseline, then modifications between minimum and maximum ranges made to fit patient data. Parameters with no baseline values are measured directly from patient data and are considered within the bounds of the min / max ranges.

Parameter	Baseline Value	Min	Max
vol_0	-	25	150
vol_1	-	300	800
v_{max}	20 mm/s	10	30
Q_{max}	25 ml/s	10	50
p_{op}	-	20	70
R_{ure}	20 mm	10	30
H	3.5 mm	1.5 mm	5.5 mm
k_1	0.1	0.01	0.5
k_2	0.1	0.01	0.5
k_3	0.1	0.01	0.5
C	0.3	0.1	0.9

Table 5.6: SMC model parameters for active contraction modulus and minimum, maximum stretches over which the smooth muscle is able to generate force. Upper and lower ranges are given.

Parameter	Lower	Upper
λ_{min}	0.3	0.5
λ_{max}	1.8	3
λ_a	0.7	0.9
μ_{act}	10 cmH_2O	150 cmH_2O

were approximated within ranges based on experimental detrusor contractility studies. Active modulus μ_{act} was given a large range of values as increasing or decreasing minimum and maximum stretches altered maximum contraction force.

Modelling

URR and BOR curve behaviours were studied before fitting to patient data. Parameters were varied between extreme minimum and maximum values to assess the full range of curve behaviours. For this study k_3 was set to zero for simplicity, (assumes smooth muscle was under maximum potential nervous excitation).

Effect of Nervous Control

The nervous control detailed in the model describes the rate of 'stimulation' that the detrusor muscle undergoes; while simultaneously controlling the rate of relaxation of the sphincter. Figure 5.9 shows the effect of changing the nervous parameter k_1 , responsible for muscular stimulation. Decreasing values of k_1 decreases detrusor pressure and delays the maximum detrusor pressure value until later into micturition. Voiding time increases due to lower maximum flow rate of urine. Reduced detrusor stimulation results in a more relaxed urethra at a time point in micturition. Interestingly, at the lowest rates of stimulation it was apparent that the detrusor reaches a limit where it was no longer able to overcome the opening pressure of the urethra; delaying micturition until the urethra has relaxed sufficiently. Physiologically, it is unlikely pressure would decrease to such an extent before the sphincter has opened without some variation of detrusor / sphincter disorder. A higher active modulus or increased maximum contraction stretch would remedy a decreased pressure, while simultaneously overcoming the sphincter opening pressure.

The parameter k_2 describes the rate of urethra opening or relaxation at initiation of

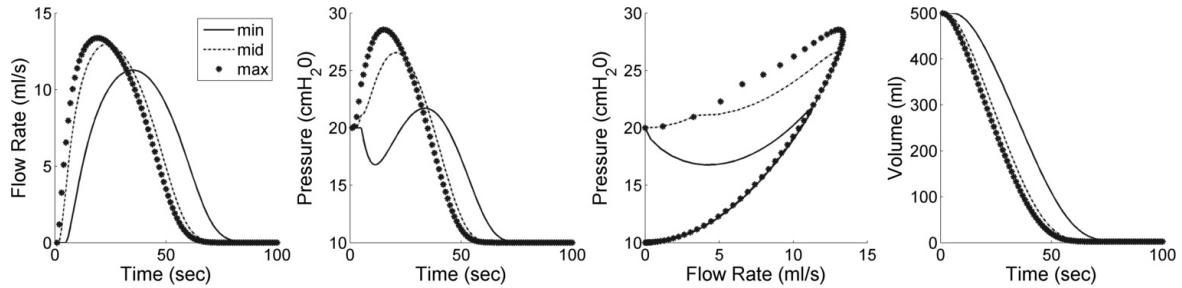


Figure 5.9: Parameter study of nervous signal parameter, k_1 with values ranging from 0.05 to 0.2.

micturition and can be seen in Figure 5.10. Increased relaxation of the urethra, denoted by lower s_2 values can be seen to increase maximum flow rate as the resistance to urine flow was decreased. This results in lower flow pressures as urethral resistance was decreased earlier in the micturition cycle (also meaning the detrusor pressure will drop faster). In the case that urethral stimulation was decreased, resistance to flow increases; meaning pressure in the detrusor will remain higher in order to overcome the initial resistance before flow can initiate. At very low sphincter stimulation it was clear the voiding time increases dramatically (lowest simulation results in incomplete void within the given time) as flow was significantly reduced. Physiologically, this may be typical of obstructive disorders and will often result in post-void residual volume; similarly increasing detrusor pressure which was also observed in the model.

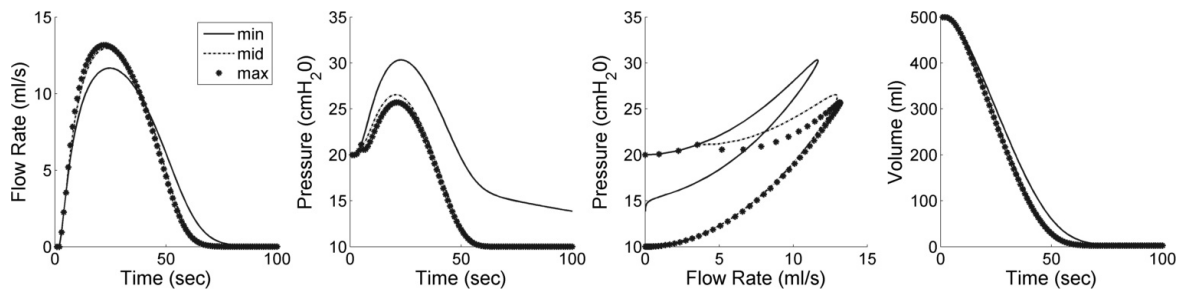


Figure 5.10: Parameter study of nervous signal parameter, k_2 with values ranging from 0.01 to 0.2.

The effect on voiding of controlling relative maximum urethral relaxation, C is shown in Figure 5.11. The main function of the C parameter is to define the final intravesical

pressure relative to the initial voiding pressure, i.e. the pressure required to overcome initial urethral opening pressure. It may also be used to tailor the point of maximum urethral relaxation. Unfortunately, C has yet to be described physiologically. The range given in this study can be defined by the relative change in detrusor pressure between p_{op} and final pressure when flow stops. Typically, only p_{op} is recorded as part of normal clinical practice, however C can be approximated readily from pressure traces ($C \approx p_{final}/p_{op}$, where p_{final} is pressure when flow ends).

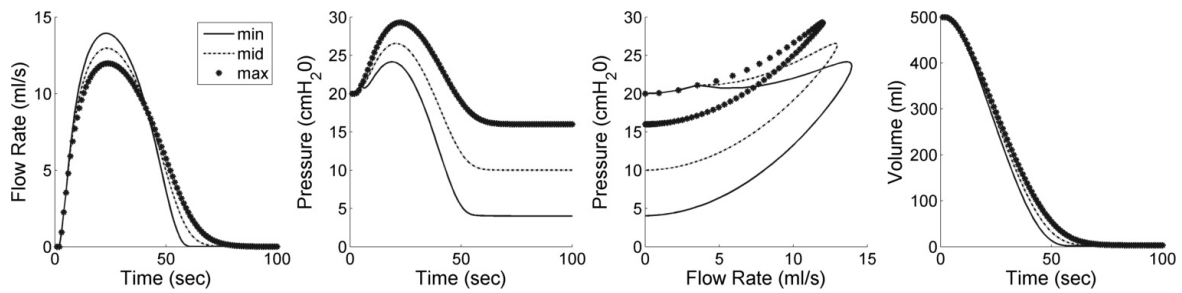


Figure 5.11: Parameter study of nervous signal parameter, C with values ranging from 0.2 to 0.8.

Effect of Bladder and Urethra Geometry

Initial geometry of the bladder was defined as the volume at which voiding was initiated after filling (see Figure 5.12). Importantly, the size of bladder alters the stretch configuration of the smooth muscle, changing the bladder stretch with respect to the smooth muscle peak contraction stretch. A geometry that closely matches peak contraction stretch of the smooth muscle will be capable of producing increased contractile strength and hence, intravesical pressure. However, in extreme cases as illustrated in the plots the volume may be low enough that the stretch was not sufficient to achieve contraction to overcome opening pressure until further stimulation. This behaviour is similar to that of detrusor underactivity, meaning these voiding conditions are unlikely to be seen in normal voiding cases. Larger volumes also significantly increase flow rate as a stronger contraction can be achieved, i.e. a stretch closer to the peak SMC con-

traction parabola. Despite a faster flow, larger volume typically means a longer void duration.

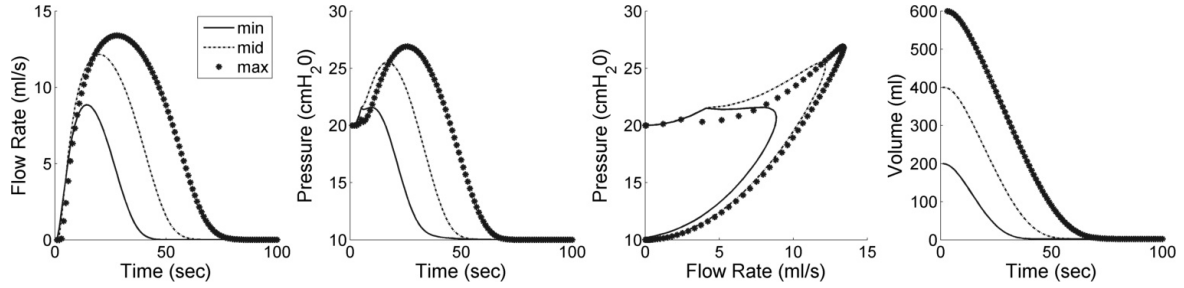


Figure 5.12: Parameter study of voiding volume, vol_0 with volumes ranging from 200 to 600 mL.

The cross sectional area of the urethra plays an important role in the pressure / flow relationship (see Figure 5.13). By decreasing cross sectional area the gradient of the URR was increased, having the effect of reducing flow through the urethra, whilst simultaneously increasing pressure. A smaller area also has the effect of prolonging voiding due to the lower achievable flow, i.e. volume decreases more slowly. The quadratic form of the URR also means small changes to area can have a relatively large impact to the pressure / flow curve behaviour. [150] reveal obstructive disorders in men may decrease maximum flow by over half (9.0 ± 6.2 obstructed vs. 20.1 ± 10 unobstructed), and double detrusor pressure at maximum flow ($P_{det}@Q_{max}$) (42.8 ± 22.8 obstructed vs. 22.1 ± 11.3 unobstructed) [150]. Modelling shows this range can easily be obtained by simply tuning urethral area; the primary physiological parameters responsible.

Effect of Intrinsic Maximum Pressure / Flow Parameters

Opening pressure of the urethra was controlled using the p_{op} parameter and indicates the pressure at which flow will initiate. Note, for cases where $p_0 < p_{op}$ there will be no solution and hence no flow until p_0 increases with further excitation from nervous signals. Typically this will only happen in pathologies such as obstructive disorders,

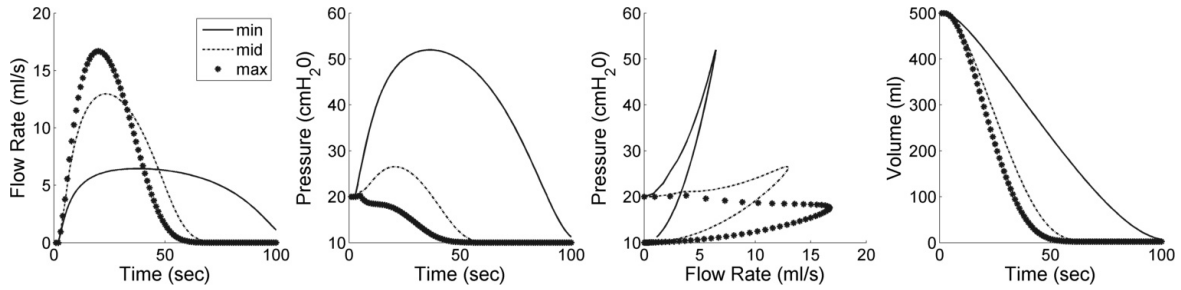


Figure 5.13: Parameter study of urethral cross-sectional area, $Area$ with urethral radius ranging from 0.1 to 0.3 mm.

however the characteristics of such a delay on flow are illustrated in Figure 5.14. Increased opening pressure increases voiding time and means intravesical pressure must increase to overcome the additional resistance to flow. Typically an increased opening pressure to flow will cause an increased detrusor pressure for flow to initiate. A decrease in pressure at higher opening pressures seen is unlikely to be physiological, however increase smooth muscle modulus would result in an increase in pressure; bringing behaviour more in-line with normal (pathological) conditions.

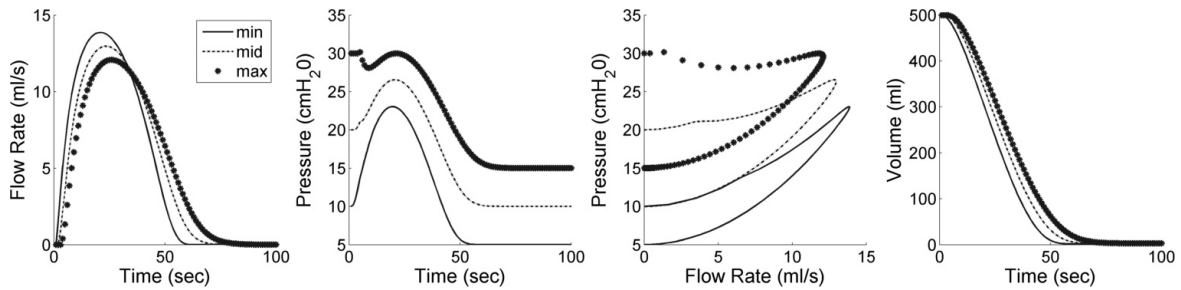


Figure 5.14: Parameter study of urethral opening pressure, P_{op} with pressure ranging from 10 to 30 cmH₂O.

The effect on voiding curves of altering parameters Q_{max} and v_{max} was broadly similar as seen in Figure 5.15 and Figure 5.16, respectively. Increasing values shifts q_0 to the right along 'flow' axis, meaning the intersection of URR and BOR curves occurs at higher flow and pressure. The result of this was a shorter voiding duration as flow was required to increase due to the higher detrusor pressure as a result of increased muscle contraction velocity.

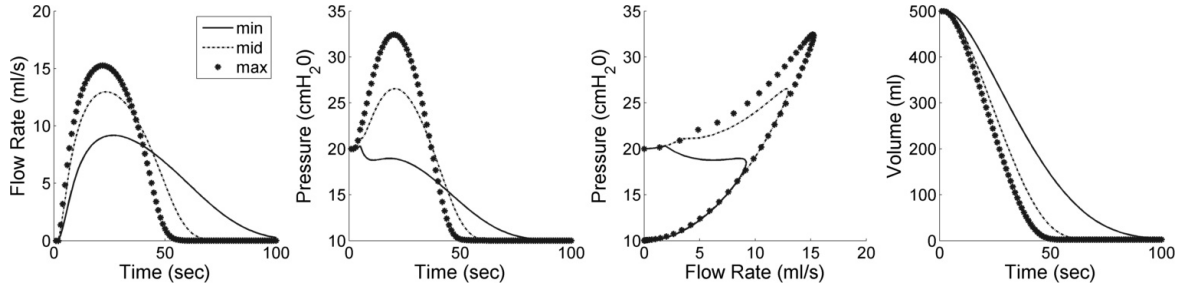


Figure 5.15: Parameter study of maximum flow rate, Q_{max} with flow values ranging from 0.5 to 1.5 ml/s.

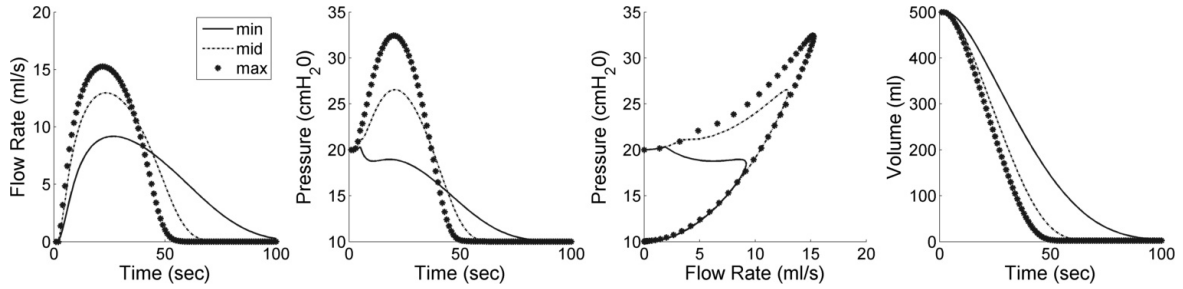


Figure 5.16: Parameter study of detrusor contraction velocity, v_{max} , velocity ranging from 3 to 20 mm/s.

Effects of Smooth Muscle Model Parameters

The active component of the smooth muscle model was defined by minimum and maximum contraction stretch ranges, λ_{min} and λ_{max} and an active modulus, μ_{act} . Effect on curve behaviour of altering the smooth muscle contraction modulus is seen in Figure 5.17. Increasing this parameter raises the intersection of p_0 with the pressure axis and steepens the BOR curve. Decreased flow rate with lower SMC modulus results in increased voiding time, with higher overall detrusor pressure for longer periods of time. This parameter shows similar characteristics to observations of muscle fatigue or impaired detrusor function, i.e. low pressure and increased voiding duration [151] [64].

Parameter studies of maximum contraction stretch, i.e. the stretch at which smooth muscle elongates past its ability to contract (See Figure 5.18). Reducing the maximum

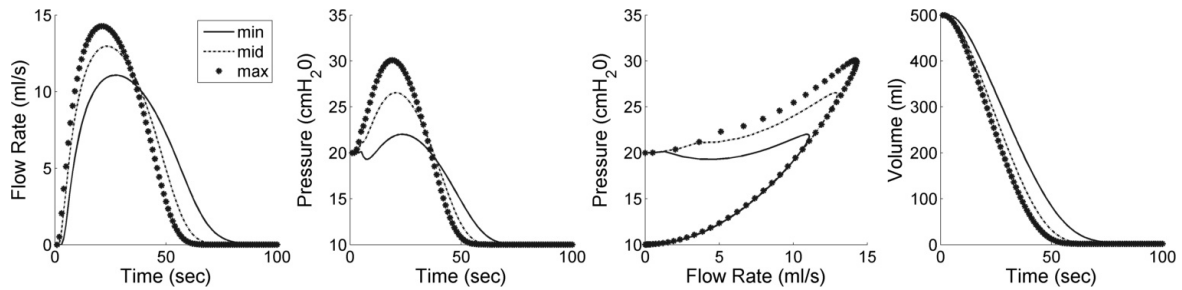


Figure 5.17: Parameter study of smooth muscle contraction modulus, μ_{act} , modulus ranging from 50 to 100 cmH₂O.

smooth muscle stretch has the simultaneous effect of decreasing the amplitude of the contraction, and reducing p_0 . In extreme cases, i.e. low maximum contraction strength the pressure generated may be insufficient to overcome the urethral opening pressure until further nervous excitation; delaying flow and prolonging void duration. A low λ_{max} may also result in a significant drop in pressure following the initiation of voiding, followed by a gradual peak in pressure. The duration of this type of simulated void is unlikely to occur clinically, typically lasting under 40 seconds [47]. Small changes to curve behaviour may also be tuned by λ_{max} as the peak contraction force can be shifted relative to stretch depending upon desired output.

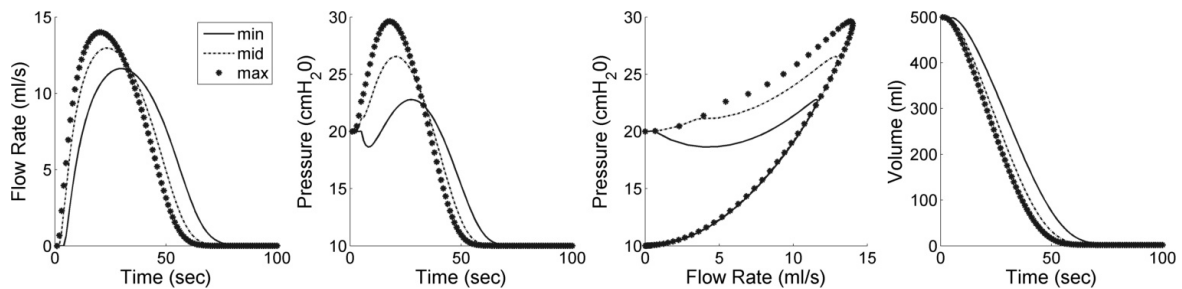


Figure 5.18: Parameter study of smooth muscle maximum contraction stretch, λ_{max} , stretches ranging from 2.5 to 3.4.

Figure 5.19 shows effect on curve behaviours with minimum contraction stretches ranging from 0.3 to 0.8. Changing this parameter has two main functions; altering minimum stretch at which smooth muscle is able to produce force, and contraction force. Reducing λ_{min} greatly reduces contractility, and subsequently reduces flow and intravesical

pressure. Additionally, if λ_{min} is set low (i.e. too low to overcome opening pressure) flow will either be delayed until pressure raises enough to exceed p_{op} or no flow will occur. This closely resembles the symptoms of detrusor underactivity. Low flow, or delayed flow results in increased voiding time with the potential for increased residual volume. Increased residual volume may also occur if λ_{min} is not small enough to maintain pressure towards the end of voiding. Importantly, this value is explicitly related to the initial reference configuration given. The extent of residual volume is therefore dependant on resting state of the bladder.

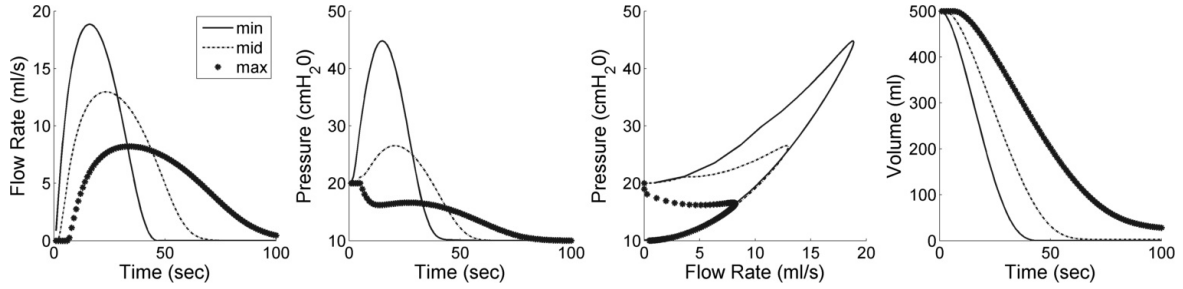


Figure 5.19: Parameter study of smooth muscle minimum contraction stretch, λ_{min} , stretches ranging from 0.3 to 0.8.

5.5 Patient Specific Cases

The micturition model was fitted to patient specific voiding data of 6 male and 6 female cases. For the first time, the introduction of a microstructural constitutive material model and modified smooth muscle representation into a micturition framework on human specific cases was undertaken. Clinically measurable parameters were used where possible to reduce overfitting and non-physical values, and physiological ranges suggested for those not measurable. X-ray data was used in conjunction with volume traces to measure bladder geometry and a novel approach to defining stretch configurations given. No ethical approval is required for this retrospective analysis of anonymised data given with consent to a database run by our partners.

5.5.1 Image Measurements and Stretch

Urodynamic traces provided volume and pressure information, however models are specified in terms of stretch and stress. Volume-in recordings provided accurate volume of filling fluid, however do not consider residual volume in the bladder. X-ray images accompanying urodynamic data were used to approximate current bladder volume and to establish consistent stretch measures.

Prior to measurement of the bladder, calibration for individual patient images was required. Patient x-ray images had no reference scale, meaning an approximation was needed for calibration purposes. Clinically, the femoral head can be used to approximate scaling, and was used in this study as a reference. A study by [152] gathered femoral head measurements from 200 cadavers. Average femoral head size was 46.1 mm with a SD of 4.8 mm. Accuracy was measured to within ± 7.6 mm, giving an error margin of $\pm 17\%$ [152]. These measurements were used in calibration of all x-ray images (See Figure 5.20).

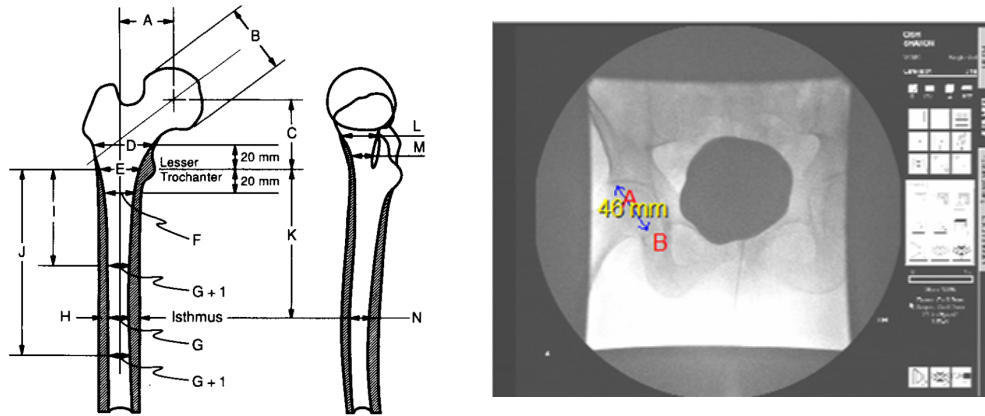


Figure 5.20: (Left) Schematic of femoral head measurement used to calibrate x-ray images. **B** Indicates location of measurements taken (Sourced from [152]). (Right) X-ray image of abdomen during urodynamic test. Measurement shown is femoral head used to calibrate scale for further measurements.

Bladder volume was approximated after image calibration for every recorded time point

(See Figure 5.21). Bladders were assumed to occupy the region of dark contrast. A custom automated script implemented in Matlab was used to distinguish bladder area by intensity thresholding. A mid point in each bladder was 'chosen' and nearby pixels of similar intensity were labelled as 'bladder'. All other pixels values were labelled 'not-bladder' and excluded from analysis. Remaining islands were filled using binary dilation, then original area restored using erosion with the same parameters.

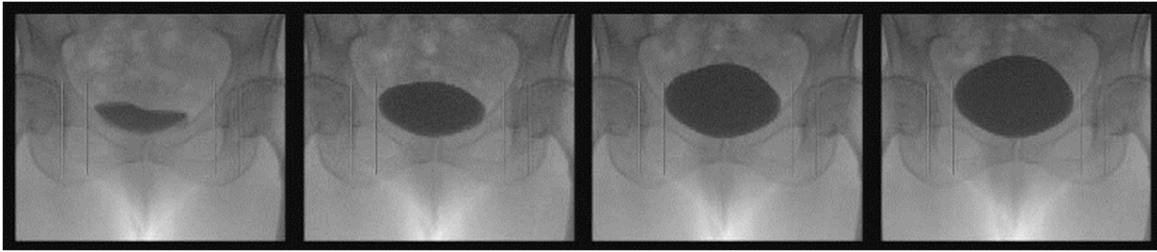


Figure 5.21: X-ray images of bladder filling during urodynamic procedure. (From left-to-right) Volumes shown are 20 mL, 110 mL, 215 mL and 285 mL.

Area in terms of pixels was converted to mm^2 using values from calibration. The area calculated was assumed to be an idealised circle from which a radius could be calculated. This radius was inferred on an idealised sphere and volume in terms of mL calculated (vol_{x-ray}).

Calculated sphere volumes were compared with fluid volume-in (vol_{in}). Volumes from successive x-rays at intervals in filling were averaged. vol_{in} was subtracted from measured volume to calculate residual volume. i.e. $vol_{res} = vol_{x-ray} - vol_{in}$.

Bladder Stretch

Defining the initial configuration of an in-vivo pressurised sphere is a challenge due to the dynamic nature of the bladder and the many possible configurations that could be considered to be the reference state. For example, the bladder may be considered to be under no stretch when completely empty. However, this means volume is exceptionally low, from which defining reference state is unlikely to give an accurate representation

of the stretch. Therefore, an alternative state must be defined. An illustration of the possible stretch configurations that could be obtained from available patient data in an ideal case is shown in Figure 5.22.

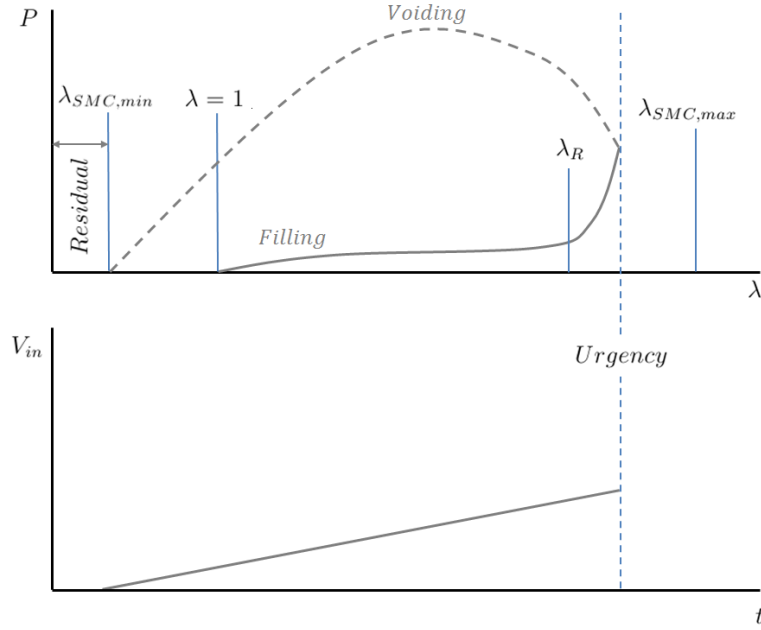


Figure 5.22: Schematic of ideal filling and voiding stretch configurations; minimum SMC contraction stretch $\lambda_{SMC,min}$, maximum SMC contraction stretch $\lambda_{SMC,max}$, and collagen recruitment stretch λ_R . Ideally $\lambda = 1$ should define stretch at which the pressure rises above the baseline pressure and passive constituents are bearing load.

Stretch of 1 was defined as the point at which the detrusor pressure raises above the baseline value, assuming the bladder had voided completely prior to urodynamic testing. Patients with incomplete voids and large volumes of residual urine were excluded from the analysis. Current bladder volume was calculated as sum of residual volume and current filling volume, i.e. $vol_{cur} = vol_{res} + vol_{in}$.

In summary, the following steps were taken,

1. Femoral head measured (pixels) and converted (mm)
2. Contrasted bladder segmented and area calculated
3. Area converted to ideal circle and radius calculated. Sphere inferred from radius

and volume in mL calculated

4. First three steps repeated for all x-ray images in patient dataset and volume averaged
5. Calculated volume matched to corresponding filling vol_{in} from urodynamic traces
6. vol_{in} at matched points subtracted from vol_{x-ray} to calculate vol_{res}
7. Current volume calculated as sum of vol_{res} and current vol_{in}
8. Stretch approximated using state at which pressure rises above baseline as reference stretch.

5.5.2 Patient Information

Patient details from urodynamic traces were anonymised by clinicians / medical scientists working at University College London before being made available for processing. A summary of patient clinical diagnosis, age and gender was provided. A total of 6 male (aged 21 to 77 years) and 6 female patients (aged 26 to 70 years) were used in this study. All patient data were considered to be absent of abnormal bladder functionality according to medical records. Table 5.7 shows patient details. All traces were smoothed before processing using a moving average filter '*smooth*' in Matlab with span of 11 (approximately 1 second).

5.6 Results

Models outlined in previous sections for filling and voiding were fitted to patient data. The filling phase was determined between the time at which $vol_{in} > 0$ and where filling

Table 5.7: Table of patient information for cases used in this study.

Patient Number	Gender (M/F)	Age	Residual vol (ml)
01127701	F	59	40
10628802	F	28	78
00505201	F	26	56
01031201	F	28	45
01033601	F	48	22
30050301	F	70	25
30060801	M	54	80
30064401	M	50	79
30064502	M	77	17
01342301	M	67	40
40034001	M	21	19
00551603	M	33	40

ends. Voiding phase was defined between the point in time at which $vol_{out} > 0$ and flow ends.

Passive model fitting to the filling phase was performed using Matlab's built-in fitting function '*nlinfit*'. Stress moduli were fitted in this fashion. Constants of the triangular PDF were defined before fitting on a patient specific basis. Due to the number of parameters associated with the voiding model it was fitted manually. As previously mentioned in Section 5.4 parameters with a clinical definition were kept within a 'normal' range. Parameters that were unable to be measured from patient data or had 'normal' ranges were manually adjusted to achieve fits to data. Significance was tested for using Student T-test with built in Matlab function *Ttest2*.

Filling

Urodynamic traces showing pressure vs. time and volume in for all male and female patient data are shown in Figure 5.23. Individual traces show a high level of variability between pressures during filling with peak filling pressures as low as 5 cmH₂O and exceeding 40 cmH₂O in some cases. Average pressure traces indicate females tended to have higher detrusor pressures during filling; however, peak pressure obtained at full capacity was comparable in males and female traces (16.5 and 17.8 cmH₂O, respectively). Filling volume achieved before urgency ranged between 372 and 575 ml in males, compared to 370 to 637 ml in females; however, this does not take into account residual urine. Significant differences in filling time may be accounted for by different filling rates or pausing during filling. Generally, filling times were higher for males, despite smaller bladder volume.

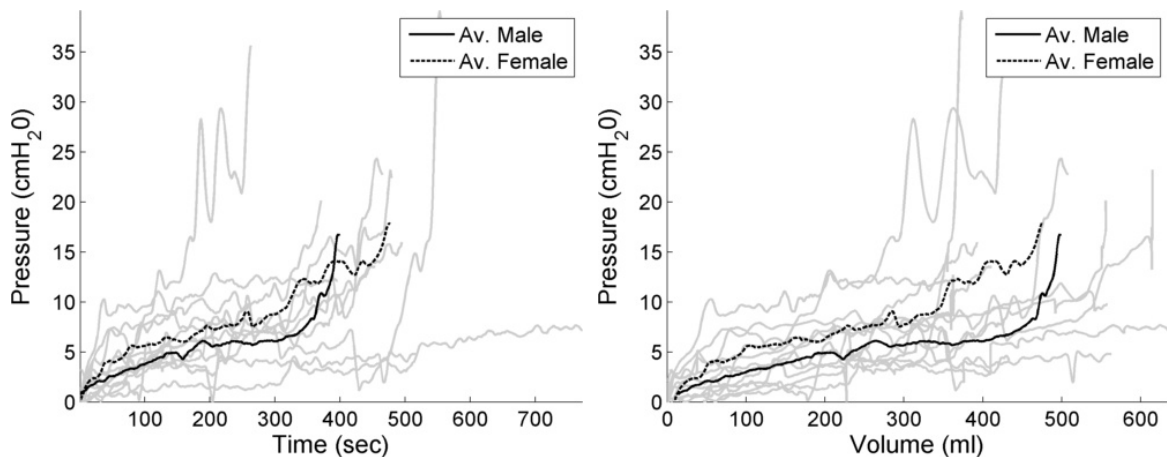


Figure 5.23: Plots of average filling pressure vs. time (left) and pressure vs. volume for all patient samples. Male and female individual and average cases shown in grey and black curves, respectively.

Voiding

Pressure and flow curves vs. time of voiding from patient data with average curves of male and female cases are shown in Figure 5.24. Average voiding times were 58 and 50 seconds for male and female, respectively. Maximum average flow during voiding for females was lower (11.3 ml/s) when compared to 14.3 ml/s in men.

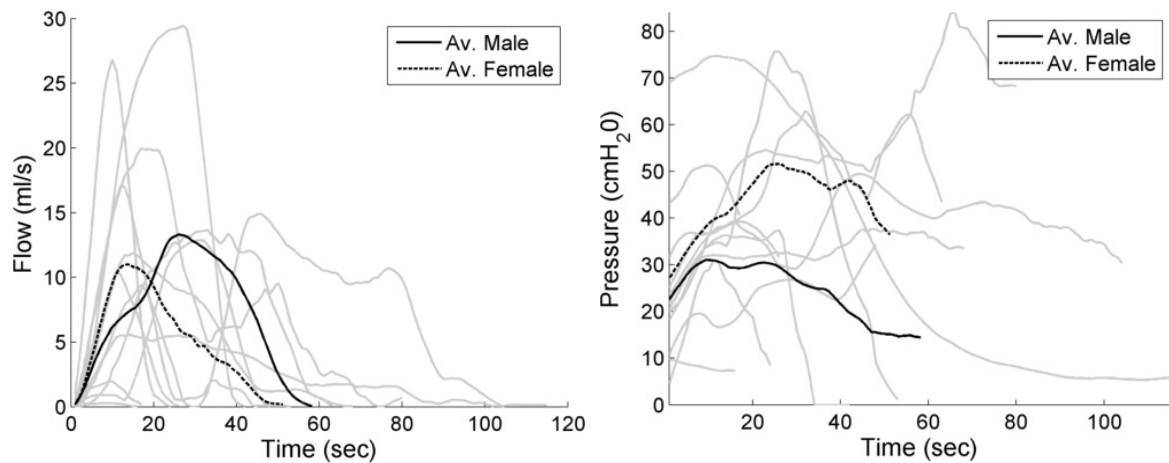


Figure 5.24: Average flow and pressure curves from urodynamic voiding phases of male and female patients (black solid and dotted lines). Individual patient data shown in grey.

Figure 5.25 shows flow vs. pressure in all 12 male and female patient urodynamic tests. Average curves show minimum urethral opening pressure, i.e. detrusor pressure at initiation of void, show similar values of 27.1 and 23.2 cmH₂O in females and males, respectively. Male detrusor pressure was substantially lower than females throughout voiding, reaching maximum average pressures of 32 and 53.8 cmH₂O, respectively.

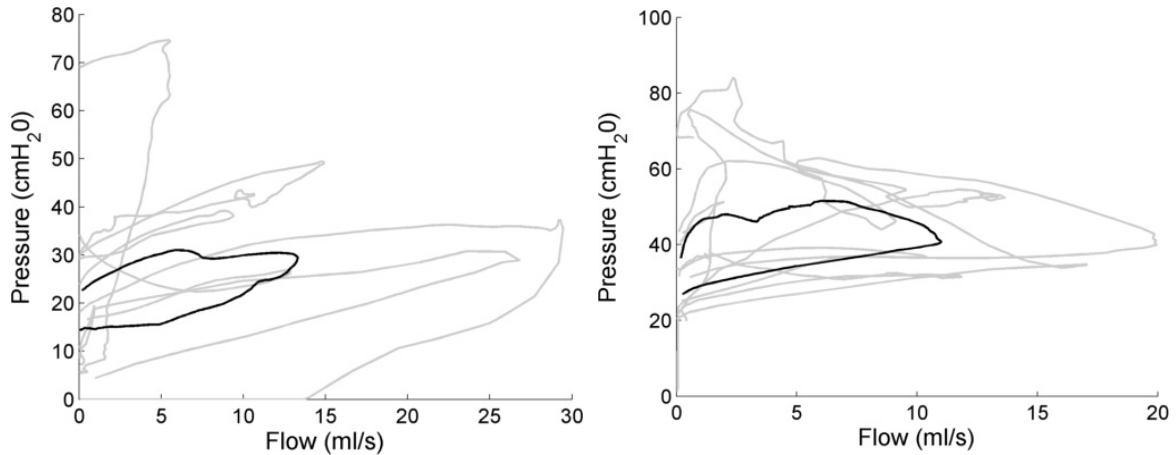


Figure 5.25: Average flow vs. pressure plots of all patient voiding data (black line) with individual's voiding data (grey). Male patients (left) and female (right) shown.

5.6.1 Model Fitting

Models were fitted independently to filling and voiding phases, manually segmented from urodynamic traces. Constants with clinical values were set within normal ranges. Others were constrained within a defined range and manually (voiding phase) or automatically (filling phase) fitted.

Filling

Models were fitted with the assumption of $\lambda = 1$ for any residual volume calculated from x-ray images. Stress moduli, $\mu_{sm,iso}$ and μ_c were fitted using the Matlab built in function '*nlinfit*', with goodness of fit measured using least squares method. Triangular PDF constants, a , b and c were set using a custom iterative script where all variations (rounded to the nearest 0.5) of constants are tested, then the best fit chosen. Table 5.8 shows all fitted constants to filling urodynamic traces. All plots of model fitting to filling pressure-volume curves can be found in Appendix D.

Significant variations in detrusor pressure are observed in patient traces; female patient

Table 5.8: Model fitting constants for all patient filling urodynamic traces. p-values indicate significance between male and female fitting parameters (5 % significance).

Patient No.	Sex (M/F)	$\mu_{sm,iso}$ (kPa)	μ_c (kPa)	a	c	b	R ²
01127701	F	3.1	84.2	1.8	1.9	2.4	0.76
10628802	F	6.7	73.4	1.7	1.9	2.2	0.16
00505201	F	0.98	50.0	1.1	1.2	1.4	0.89
01031201	F	19.7	37.8	1.2	1.3	1.5	0.32
01033601	F	18.7	57.2	1.4	1.7	1.8	0.82
30050301	F	1.5	53.2	1.45	1.7	1.8	0.72
30060801	M	140.9	1589.4	1.3	1.6	1.9	0.80
30064401	M	-	-	-	-	-	-
30064502	M	7.6	3562.5	1.8	1.9	2.2	0.9
01342301	M	18.2	1.3×10^6	2	2.1	2.5	0.63
40034001	M	13.5	4.2	1.3	1.9	2.1	0.39
00551603	M	26.4	2299	1.6	1.8	2.1	0.98
<i>p</i>		0.18	0.01	0.27	0.14	0.14	

traces show higher detrusor pressure throughout voiding than males, although pressure at maximum capacity between genders was comparable. Variation of PDF constants; a, b and c was seen in female cases and was largely attributed to the large differences in reference ($\lambda = 1$) volumes. *a* ranged from 1.1 to 1.8, *b* from 1.4 to 2.4, and *c* from 1.2 to 1.8. Quality of fit varied significantly across female patient traces with R² ranging from 0.16 to 0.89, and was largely due to varying degrees amount of noise, despite smoothing. Fitting to male data also revealed large variations in the onset of recruitment. *a* ranged from 1.3 to 2 whilst values of *b* and *c* were more consistent between samples. Quality of fit ranged from near unity (0.98) to poorly fitted 0.39, largely due to artefacts caused by noise or periodic cough tests. A large range of collagen modulus values were seen when fitting collagen modulus with several magnitudes between highest (3562.5 kPa)

and lowest (1.3 kPa), largely attributed to later recruitment stretch and thus a more rapid stiffening. In comparison, female data was highly consistent between collagen modulus values, all within a similar magnitude.

Voiding

Voiding phases of patients were cropped from urodynamic traces initiating at $Q > 0$ and ending at $Q = 0$. BOR and URR components of the model were fitted manually by assigning clinically measurable parameters relevant values from literature or the urodynamic traces themselves. Other constants were assigned a 'normal' range as discussed in Section 5.4. Voiding model parameters fitted on a patient specific basis are listed in Table 5.9.

Representative ($n = 2$) urodynamic female patient data and fitted model examples are shown in Figures 5.26 and 5.27. Volume measurements of patient no. 00505201 (Figure 5.26) show a relatively low volume voided (approx. 130ml) compared with the 450 ml approximate total volume, suggesting an incomplete void. Modelling predicts a voiding volume of 150 ml, slightly higher than actual. Pressure increase during void was captured well by the model during urine flow. When flow ceases pressure remains high, a behaviour the model was not able to reproduce. Peak detrusor pressure was 39 cmH₂O compared with model prediction of 38 cmH₂O. Nervous signal s_1 had almost equal stimulation and relaxation rates ($k_1 = 0.15$, $k_3 = 0.19$), producing a near symmetric flow rate curve. Final pressure after void in the detrusor was 29 cmH₂O, approximately 6 cmH₂O greater than initial opening pressure. C was set to 0.9 to give a final detrusor pressure of 22.8 cmH₂O.

Table 5.9: Fitted model parameters to all 12 patients for voiding phase of urodynamic study. Units given: μ_{act} (cmH₂O), v_{max} (mm/s), Q_{max} (ml/s), A (cm²). p-values indicate significance of parameters between males and female (5 % significance). Note, patient number 30064401 does not have values due to incomplete void.

Patient	M/F	μ_{act}	k_1	k_2	k_3	C	v_{max}	Q_{max}	A
01127701	F	12	0.07	0.01	0.25	0.9	12	10	0.15
10628802	F	19	0.2	0.3	0.2	0.1	12	18	0.17
00505201	F	18	0.15	0.01	0.19	0.9	11	5	0.18
01031201	F	115	0.05	0.2	0.2	0.2	18	12	0.15
01033601	F	28	0.28	0.01	0.3	0.2	8	3	0.04
30050301	F	28	0.08	0.05	0.03	0.7	10	9	0.21
30060801	M	75	0.02	0.01	0.5	0.2	12	4	0.17
30064401	M	-	-	-	-	-	-	-	-
30064502	M	16	0.25	0.01	0.05	0.1	12	10	0.13
01342301	M	50	0.06	0.01	0.05	0.7	18	19	0.36
40034001	M	15	0.25	0.1	0.15	0.3	18	12	0.17
00551603	M	48	0.1	0.05	0.1	0.1	17	19	0.37
p		0.8	0.96	0.32	0.33	0.08	0.1	0.37	0.12

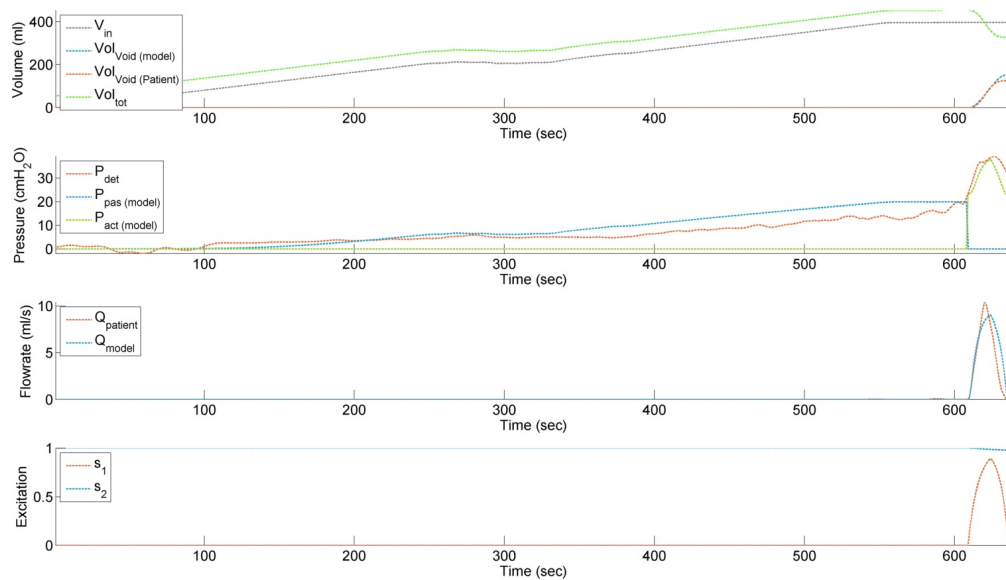


Figure 5.26: Complete urodynamic traces of volume, pressure, flow with simulated nervous signals of female patient (no. 00505201). Passive and voiding models fitted to data.

Figure 5.27 plots urodynamic traces and model fitting from the second female patient (no. 01033601). Maximum capacity was approximately 510 ml including 22 ml residual volume. The void was incomplete as only 17 ml was voided with a maximum flow rate of 2 ml s^{-1} . Oscillations in pressure are seen throughout filling and a small increase in pressure occurs during void with a maximum detrusor pressure of $52 \text{ cmH}_2\text{O}$. Despite a small incomplete void, lasting approximately 17 seconds, the model was able to capture the behaviour of the pressure and flow curves. Low A and v_{max} values are used to achieve this response, although are unlikely to be the cause of the low flow rate. Excitation and relaxation of s_1 was similar with k_1 and k_3 values of 0.3 and 0.28, respectively.

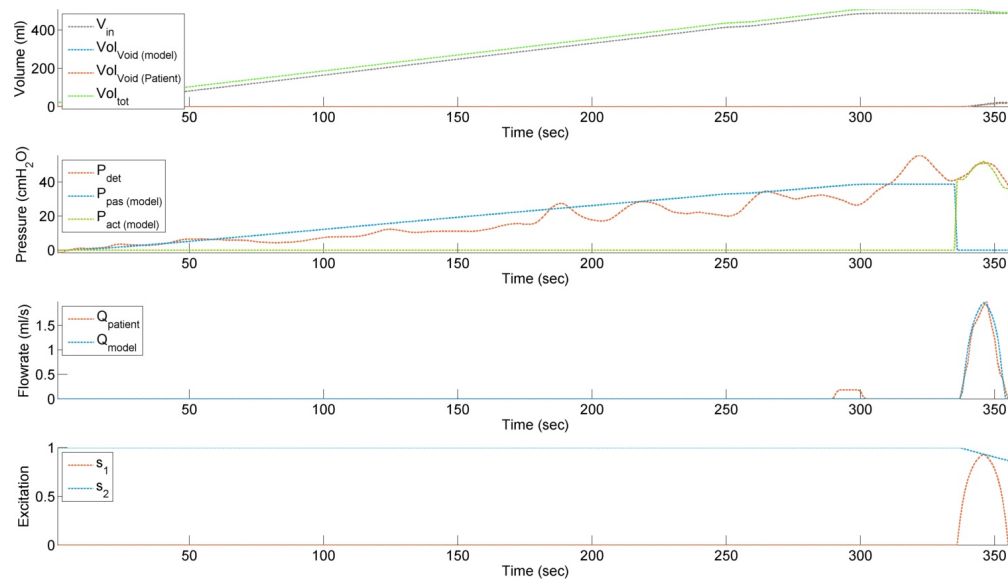


Figure 5.27: Complete urodynamic traces of volume, pressure, flow with simulated nervous signals of female patient (no. 01033601). Passive and voiding models fitted to data.

Representative ($n = 2$) urodynamic male patient data and fitted model examples are shown in Figures 5.28 and 5.29. Male patient (no. 30060801) volume trace indicates maximum capacity of 800 ml; considerably higher than the average of 461 ml in male and 484 ml in female patients in this study. Residual urine of approximately 220 ml was seen as a result of incomplete voiding pre-study. Complete bladder emptying

was achieved during the study. Peak detrusor pressure reaches 50 cmH₂O compared with 45 cmH₂O predicted by the model. Pressure was maintained through patient voiding, whilst the model drops in pressure throughout the second half of voiding. Approximately 30 seconds into voiding flow abruptly weakens, with a corresponding drop in pressure. Flow restarts immediately and increases to a peak flow rate of 15 ml/s. Disruption to flow was not captured by the model using the current nervous system model; rather it achieves a smooth curve that fits gradients of increasing and decreasing flow rate curves, and achieves similar total voiding volume in the time span. Nervous signal s_1 was set with a much lower value of k_1 relative to k_3 to achieve a gradual increase in pressure and flow and obtain a longer void, as observed in the patient trace.

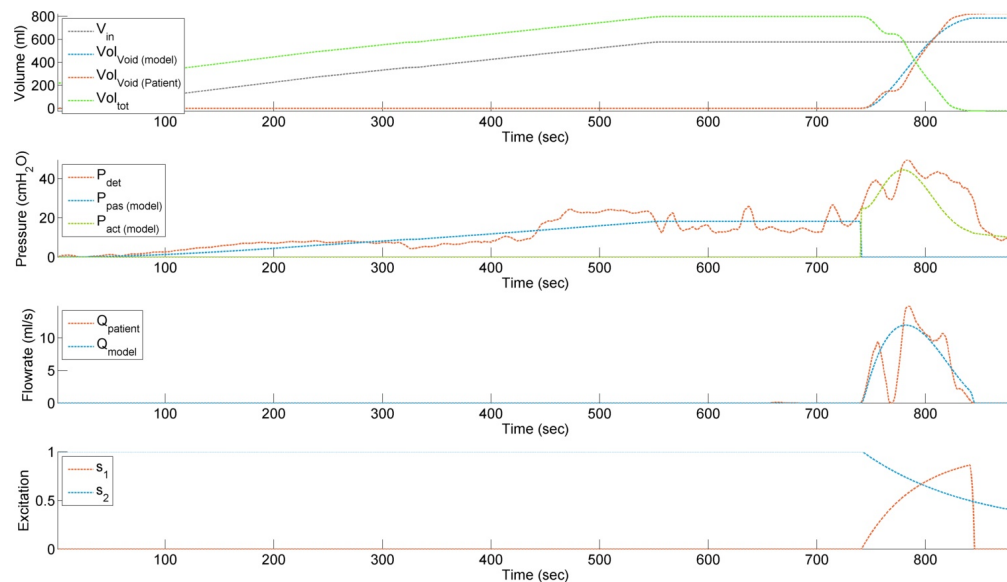


Figure 5.28: Complete urodynamic traces of volume, pressure, flow with simulated nervous signals of male patient (no. 30060801). Passive and voiding models fitted to data.

Urodynamic traces of patient no. 40034001 are shown in Figure 5.29. Maximum filling volume reaches 550 ml, and with residual volume gives a maximum capacity of approximately 570 ml. During voiding detrusor pressure peaks at 42 cmH₂O and increases further to 52 cmH₂O after voiding. The model closely fits pressure curve

during voiding, however as modelled pressure drops and second increase in pressure was seen after voiding. It was indicated by clinicians that the only abnormality (in the trace) was detrusor contraction after void. The model was unable to capture the increase in detrusor pressure after voiding was complete. A maximum flow rate of 14 ml/s was seen, and close symmetry around peak flow was fitted well by the model using similar values for k_1 , k_2 and k_3 (0.25, 0.2 and 0.15, respectively). Flow rate curves indicate small voids (leakages) occur during the start of filling. Additionally, volume and flow measurements from the trace are not matched (i.e. integral of flow does not equal volume voided). This was attributed to a recording error.

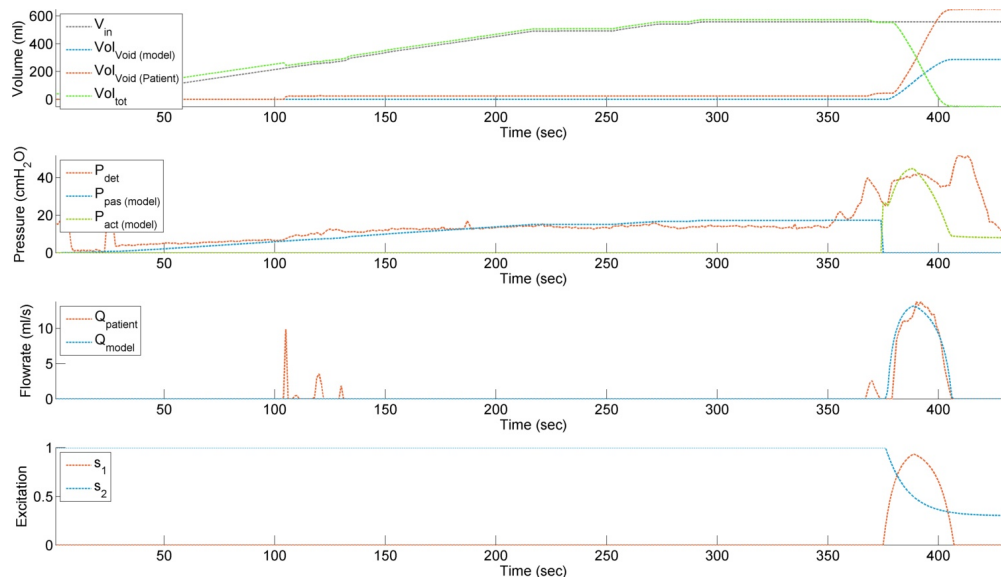


Figure 5.29: Complete urodynamic traces of volume, pressure, flow with simulated nervous signals of female patient (no. 40034001). Passive and voiding models fitted to data.

5.7 Discussion

A micturition model to simulate human bladder filling and voiding was developed by implementing a novel microstructural model of collagen recruitment and smooth muscle contraction with nervous signalling response into a corollary micturition framework. For the first time this type of model was applied on a patient specific basis with clinically measured parameters where possible. By using established relationships between flow, pressure and power generated by the detrusor it was possible to simulate patient micturition using a relatively uncomplicated framework. This gives the distinct advantage of maximising clinically relevant parameters and reducing computational load; meaning this framework has potential for eventual clinical translation with further development.

Detrusor pressure during filling was modelled using a passive microstructural constitutive model. The bladder was assumed to be an idealised sphere with an isotropic dispersion of collagen fibres and a passive smooth muscle component. Fit quality varied considerably (R^2 between 0.16 and 0.98). Fit was largely influenced by noise and artefacts as live signals will be influenced by breathing, talking, movement or abdominal contraction during tests [153]. Underlying gradients of the filling pressure curves could be captured well by the model. Passive smooth muscle modulus $\mu_{sm,iso}$ was assumed to be the main loading bearing component in physiological ranges with collagen playing a load bearing role at stretches of 1.2 to 2 depending upon the patient. Average stretch at collagen recruitment was 1.44 in females compared with 1.6 in males. Collagen is hypothesised to mainly play a load bearing role towards higher peak physiological loads. The low compliance of collagen increases intravesical pressure (usually seen after the heel region), which at low stretches may lead to upper urinary tract damage. Examples of this are seen in obstructive disorders, where secondary fibrous accumulation reduces

compliance, and damage occurs to the upper urinary tract [39].

When not contracting, stress in the detrusor is dependant upon its elastic properties and viscoelastic properties. Viscoelastic properties were not included in the current model. Urodynamic filling typically occurs over a time span of several minutes, where in reality the bladder takes several hours to fill. [122] illustrate at a stretch of 1.5 stress is approximately 60 % lower at a strain rate of $500\text{ }\%s^{-1}$ compared with $10\text{ }\%s^{-1}$ [122]. Therefore, time constants used to fit urodynamic data have little relevance in normal physiological filling. Subsequently, viscoelasticity was disregarded from the analysis, while also keeping fitting parameters to a minimum. With further biomechanical investigation of viscoelastic properties it would be beneficial to incorporate a model supported by experimental data into the micturition model framework.

Broadly speaking there have been two approaches to modelling the LUT in the literature; explicit representation of the different components, sometimes with detailed fluid dynamics [154], [138], and modelling the relationship between pressure and flow in the urethra and bladder (BOR and URR type models) [50] [121]. There are arguments for either approach depending upon the question being asked and application. Explicit representation may improve accuracy of the analysis given sufficient measurement of patient LUT dimensions. However, explicitly representing every component of the lower urinary tract is somewhat limited in a clinical setting. The vast amount of dimensions and parameters required to define the LUT is practically impossible to obtain from current clinical urodynamic protocols on a patient specific basis. However, a BOR / URR approach, as used in this study, requires much fewer dimensions and parameters; most of which can be directly obtained from urodynamic studies (may even be obtained automatically during tests). Clinics with coupled x-ray imaging and urodynamics give valuable dimensional information of the LUT, within reasonable error, that may be used to acquire the few dimensions needed. With current clinical

technology, or at least until imaging modalities improve to the point where accurate, rapid measurements of the LUT can be obtained, URR and BOR frameworks arguably provide the best opportunity to improve interpretation of urodynamic data.

This study attempts to measure dimensions of the bladder during filling from x-ray images taken throughout urodynamic studies. X-ray images are dimensionless and require calibration. The femoral head was chosen as it is present in all x-ray images and deviations in size are relatively small [152]. Measurements were taken from 2D images of the bladder and an approximation of the volume made by assuming an idealised sphere. Whilst a rough measure of volume could be obtained, accuracy could be improved by 3D imaging or multiple 2D slices at varying depths. Additionally, variations in dorsal and ventral geometry could be identified. However, this data would not be practical to collect in normal clinical practice due to time constraints and x-ray exposure time. Individual case studies may provide more insight into 3D geometry and give potential application for finite element analysis modelling.

Discussion of Nervous System Modelling

The nervous system constitutes an essential part of bladder control and function. It is one of the most challenging aspects of the LUT to model as the control system is not yet fully understood. The main aim of the nervous control model was to provide a basic model to aid understanding of normal mechanical function of the LUT and urodynamics of micturition. The current study uses a continuous exponential model with functions that allow contraction and relaxation of detrusor and urethra to be controlled, including the ability to delay or offset the timing of the bladder contraction relative to urethral relaxation. The function of the LUT, including mechanical properties and control, has been modelled in several studies [138] [142] [155] [121].

Different approaches to modelling the nervous system are taken in the literature. There

exists several sophisticated nervous control models that aim to simulate both efferent and afferent nervous systems [156] [139] [121]. Practically, it is almost impossible to model all the neural reflexes involved in bladder function so simplification is necessary with a number of assumptions. Modelling work by [121] identifies 9 simplified neural centres to model male normal and dysfunctional bladder micturition, i.e. obstruction, involuntary voiding and suprasacral lesions. This study provides the most comprehensive insight into modelling the nervous system, considering many previous studies employ a single nervous signal for regulation of the LUT [157] [136]. However, representation of the nervous signal uses a discretised binary response leading to discontinuities in the nervous signal. Authors note that a continuous signal is preferable and more physiologically relevant [121] .

As well as stimulating the urethra and bladder to relax and contract, another challenge to nervous system modelling is efferent signalling from the bladder and incorporating physiological feedback loops. This raises the question of how the bladder recognises or 'feels' its current state of fullness, whether this be through pressure, volume or stretch. [121] use a tension threshold to initiate voiding and simulate a full sensation based on wall stress. Similarly, efferent signals proposed in the nervous system model by [156] employs detrusor pressure to signal fullness. Previous iterations of the model in [142] had considered output from the bladder in terms of pressure, tension and volume depending upon the neural centre feedback being signalled. Interestingly, the model assumes intravesical pressure does not trigger micturition with the assumption that a rise in abdominal pressure would cause voiding. Identifying the correct mechanisms is key in development of nervous control models of the LUT, particularly in a patient specific application where irregularities in capacity or intravesical pressure may play a role in nervous system related pathologies.

The nervous system model used in this study allows the bladder wall mechanics and

relationship between flow and pressure to be studied in normal bladder function. However, the lack of a sophisticated neural control model limits the ability to model neural-specific pathologies. Additionally, certain voiding features seen in normal patients could not be captured using the current neural model. For example, isolated incidences of leakages and voiding disruptions were observed, however, cannot be simulated using the current nervous control model. Future iterations of the model framework may look to integrate more sophisticated nervous control systems to better understand coordination of the neural system and study pathology of the LUT. Further, the function of the neural system is only partly known; necessitating thorough experimental studies to quantify nervous activity in both normal and dysfunctional human to better define and validate such models.

Differences in Male and Female Urodynamics

The functional and anatomical differences between male and female LUT must be addressed in urodynamic studies and modelling. Anatomically, males and females have considerable differences in the urethra and ideally a urodynamic model would differentiate between the two anatomies. In female the urethra is relatively short (2 -4 cm); resulting in minimal frictional losses - estimated at approximately 10 % (comparable with error expected in urodynamic measurements) [50]. It is therefore a fair assumption that frictional losses are negligible except those at the flow rate controlling region (in this case, the distal outlet), and normal female voiding can be fit to the 'lossless' model. Male urethra, in comparison, is approximately 22 cm in length [158]. The significantly greater length means frictional losses are more prominent. However, this only becomes the case if the flow controlling zone, i.e. the outlet, lies further from the bladder opening. In reality, the portion of the urethra that requires the highest static pressure to distend is located at the proximal urethra, near the pelvic floor, much alike the female urethra. The implication of this is that only the proximal region of the

urethra must be considered in the pressure / flow relation with respect to frictional energy loss (frictional losses in the distal region due to geometry and elasticity impacts fluid exit velocity, not flow rate). As this is relatively short, similar to female urethra, the frictional losses created in the urethra may be neglected for the purpose of this model [50].

Average pressure curves obtained in this study show male bladder was more compliant throughout filling, despite final pressure at maximum capacity being comparable between gender. To date there have been very few studies examining the differences between male and female filling compliance. An animal study examining LUT function of male and female rats by [159] reveals differences in compliance. Females show greater pressure plateaus compared to males in immature and mature animals. Interestingly, Wyndaele et al., 1998 reports sensations of fullness differ significantly between human male and females during bladder filling. Despite large SD in results, average male filling volumes were significantly greater than females for first sensation, first desire and strong desire to void. Similar differences between human male and females were observed in another study [160]. Bladder compliance of males was also reported to be higher than females ($9.8 \text{ ml/cmH}_2\text{O}$ compared with $4.9 \text{ ml/cmH}_2\text{O}$, respectively) [160]. As a side-note, clinical metrics such as compliance must be interpreted with caution as with such non-linear material properties small changes in volumes can lead to large changes in pressures. Care must therefore be taken to ensure the region of the curve chosen to represent overall gradient (i.e. compliance) is consistent.

Voiding flow and pressure curves reveal higher detrusor pressure in female patients throughout voiding following higher average opening pressure when voiding initiates. Average female flow rate was slightly lower than men, although peak flow rate occurs much earlier giving a steeper rise in flow rate after the urethra has distended. From a modelling point of view this may imply that the cross sectional area of the urethra

in female patients is narrower, i.e. narrow opening results in low flow rate with high pressure, however imaging data could not be used to confirm. Contrary to these results, [161] report higher maximum flow rate in 183 women compared with 253 men aged 40 and over. The same study also observed significantly higher detrusor pressure at maximum flow in men, opposing observations in this study. Differences seen between studies may be related to age, as authors of [161] observe significant differences in urodynamic parameters with age. Average female age was 43 years compared with 50 years in male sample groups. This may offer an explanation to the differences seen between studies. Future studies would benefit from a larger sample size within a narrower age range to improve representation of gender differences.

Study Limitations

Limitations to this study exist with respect to patient data, particularly in male patients. Young, normal male urodynamic data is relatively rare as men are typically less inclined to apply for clinical tests unless it is required. Future studies would benefit from increasing sample size within a narrower, younger age range to acquire data as close to normal functionality as possible. This may be used as a basis for establishing model parameter definitions for abnormal cases when modelling bladder function.

The primary drawback using clinical urodynamic data is the high amount of noise and artefacts present, and often indication of abdominal contractions due to coughing, talking, moving, etc. Additionally, the position of the patient relative to the transducer during testing can greatly impact baseline pressure, i.e. normal supine resting pressure is expected to be 5 - 20 cmH₂O whereas seated resting pressure is typically 15 - 40 cmH₂O [153]. Whilst the baseline pressure was normalised to zero when fitting models, seating position is an important factor in pressure calculation and must be considered in both a clinical setting and in theoretical modelling, i.e. consistent measures for comparison. This also becomes an issue when comparing genders during voiding as

men are able to urinate when standing, and women in a seated position. Currently there are no protocols in place to record patient position or movement during studies, nor post-processing methods to account for this.

Where stretch or strain are used as measures of deformation in models there must be consistency in the definition of the initial 'reference' configuration of the tissue. Whilst several authors establish a resting state in biaxial mechanical testing [15], [93] and inflation testing [19], there is currently no definition for clinical urodynamic testing. This becomes particularly important as different definitions dramatically impacts the stress-strain behaviour observed. The method proposed in this study utilises x-ray imaging in conjunction with urodynamic testing to visualise bladder geometry at various stages of the study. Using the patient's 'natural' resting state after urination (pre-testing) gives a patient specific resting configuration and is clinically measurable (and may be automated in future). The disadvantage to this definition is that it is dependent upon good voiding pre-urodynamic testing which may not be applicable in certain cases of disorder, i.e. detrusor underactivity or obstruction. Future works may look to improve the definition of resting state of the bladder as a function of maximum capacity, or detrusor pressure.

Brief Discussion of Pathology

Examples of urodynamic traces given in this study are based on 'normal' patient data with parameters that are considered healthy (those that are clinically quantifiable). Certain pathologies, such as; detrusor underactivity and obstruction may be studied with the micturition model presented here. Obstructive disorders in men typically occur due to prostate enlargement, reducing the cross sectional area of the urethra, or in the case of strictures, increase urethral rigidity. These changes increase pressure required to distend the urethra and initiate flow in compressive obstruction, or reduce effective outlet area in constrictive obstruction [150]. This may be studied by parameters P_{op} and

A , respectively. Additionally, the urethral opening pressure must be generated by contraction power of the detrusor. However, the power supplied cannot be used to increase fluid velocity at the outlet with an elevated P_{op} . Subsequently, when the lumen eventually distends the maximum possible flow will be lower; proportional to reduced fluid velocity. Detrusor underactivity is defined as "contraction of reduced strength and/or duration resulting in prolonged bladder emptying and/or failure to achieve complete bladder emptying within a normal time span". Simulation of this can be achieved by combination of nervous signals s_1 and s_2 and the detrusor contraction modulus μ_{act} . Additionally, detrusor overactivity may be possible to emulate with further study of nervous signals and increased complexity of nervous system modelling.

In this study the filling and voiding functions of the LUT were modelled by integrating constitutive passive and active models of collagen and smooth muscle into an existing, and proven micturition relationship. The relatively simplistic model presented here was able to capture main voiding curve characteristics using parameters that are able to be measured clinically. Whilst able to successfully simulate normal voiding function, sophistication of the nervous system model, perhaps by correlating recorded EMG signals (which are not currently standard clinical practice), would greatly improve the ability to model normal LUT function and differentiate pathology. The LUT is a highly complex system and with an equally complex function that is yet to be fully understood. Additionally, urodynamic studies are largely unphysiologic. Studies report large ranges of reference values, and coupled with differences in urodynamic protocol differences between clinical centres, makes modelling of the LUT function a challenging task. In future there must be a coordinated multidisciplinary effort between clinical, experimental and modelling disciplines where novel modelling systems can be evaluated clinically and developed in conjunction with the newest understanding of the underlying biology to aid translation of models to clinical computational tools.

Final Discussion and Future Work

The overall aim of this project was to gain in depth insight into murine bladder wall morphology and relate individual components of the microstructure to the mechanical behaviour. After identifying and modelling these key components using a novel microstructural model on murine bladder, the model was translated into a micturition framework to be fitted to 'healthy' clinical patient urodynamics data. This was achieved by taking a multi-disciplinary approach to bring together experimental, modelling and clinical aspects of interpreting urinary bladder microstructure. Extensive microscopy studies combined with uniaxial, biaxial and inflation testing of young and aged murine bladder gave a great deal of information of the microstructural response of the bladder wall to loading. Collagen microstructural parameters including; orientation, dispersion, recruitment, and area fraction, from these studies were quantified and implemented in a novel microstructural constitutive model. A modified version of this model was integrated into a micturition framework and, combined with a smooth muscle model, used to simulate filling and voiding function of the bladder in normal patient urodynamics.

The comprehensive MPM studies of bladder wall at rest and under loading show the expansion mechanisms and interaction between layers in bladder wall may be more

complicated than originally believed. A complex multi-hierarchy of mechanisms is revealed including the gradual unfurling of the lamina propria followed by the uncoiling of collagen fibres. Although the interaction that occurs at the interface between the lamina propria and detrusor is hypothesised to involve a capacitance role of collagen, the exact mechanism and collagen-smooth muscle interaction is yet to be elucidated.

For the first time the combination of MPM and a range of mechanical testing was used extensively on bladder wall tissue of young and aged murine tissue. Although only a small number of samples were examined, this thesis reports striking morphological differences at a tissue and microstructural level between young and aged bladder wall. Uniaxial testing showed there was a clear distinction between the compliance of young and aged tissue, and differences in collagen area fraction, recruitment and orientation observed are hypothesised to play a role in the changes to macroscopic tissue mechanical behaviour. A novel bladder specific microstructural model with contribution from passive smooth muscle and collagen was used to correlate these aspects and successfully fit to experimentally measured imaging parameters and mechanical data.

The micturition framework used in this study takes a relatively simple approach to a complex problem. Whilst there exists various models that attempt to capture a great deal of the different mechanisms that occur during voiding many of the parameters required to complete such a model are currently impossible to quantify clinically on a patient specific basis. The model employed here was therefore kept as simple as possible to produce adequate pressure / flow relations whilst also attempting to maximise the number of parameters able to be measured during standard urodynamic studies.

Inclusion of microstructural constitutive models into micturition frameworks is a novel approach and brings the additional benefit of being able to break down the individual aspects of the mechanical response into contribution from constitutive components of the bladder wall. This gives a great deal more information than the traditional

compliance measures currently used. In future, with further experimental validation, they may be used to identify damage or remodelling that may have occurred to the tissue microstructure as a result of disease or aging. This may be then used as a predictive tool for disease progression or intervention outcome.

To date there exist many of the modelling tools and experimental knowledge required to develop an accurate, yet simplified, in-silico model of the lower urinary tract. However, individual aspects have yet to be fully explored and developed to the point where they can become of relevant clinical use. In future work there must be a multi-disciplinary effort to bring together the collective knowledge of the LUT to develop clinically relevant and useful modelling frameworks that can accurately capture the mechanical and neurological functionality of bladder filling and voiding. The end-goal outcome of this would be a clinical tool that can be used to distinguish or improve interpretation between pathologies and the severity and progression of these disorders. Models may also look to give guidance and monitoring for the most efficient treatment modality on a patient specific basis with indication, or quantification of intervention outcome.

6.1 Future Work

Potential future directions related to this project are discussed in the following section. In some cases, initial studies were performed or test apparatus constructed for implementation in future studies.

Clinical Application

The long term aim of micturition modelling is progression towards a clinical application, in this case, to aid interpretation of the traces obtained from urodynamic studies. For a micturition framework to progress towards clinical use, it would firstly need validation on a large number of patients (upwards of one hundred per group) to establish baseline parameters; for different age groups and genders. Large databases of patient data would be required for this and fitting of the model would need to be matched with diagnoses obtained from several blinded experts to validate the model and assess specificity and sensitivity. From this it would also be possible to identify whether the model captures features or diagnostic parameters that would otherwise be missed in certain cases. A project involving data from centres with currently established collaborations would be possible, although increasing the number of centres involved would allow increased patient participation from different demographics and younger age groups that are typically harder to acquire.

Imaging modalities

MPM imaging was extensively used throughout this work. Whilst it provides many benefits over other imaging technologies its main drawback is imaging penetration depth. This meant that information throughout the entire wall could not be captured. To overcome this in future, alternative imaging modalities, such as micro-CT scanning were investigated. The main benefit of this being high resolution, 3D imaging of the

entire intact bladder. Initial studies indicated that it was possible to obtain good contrast when the tissue is dehydrated and fixed in paraffin wax (See Figure 6.1). The entire bladder wall could be observed even at relatively low magnifications (0.4x and 4x were trialled). These magnifications allowed discrimination of individual layers and larger features in the wall. Further work may look to increase magnification and optimise methods of obtaining contrast. The expected benefits of this would be more accurate fibre orientation and recruitment information as they may be quantified in the entire wall in 3D. Additionally, a more physiologically relevant method of deformation, such as inflation can be used to load the tissue.

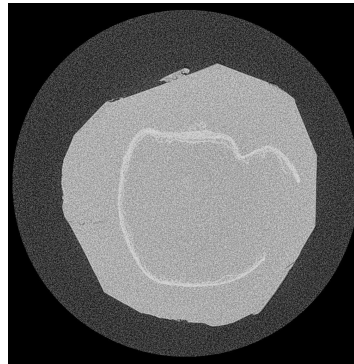


Figure 6.1: 2D CT scan image of mouse bladder cross section embedded in wax obtained from preliminary scanning study. Protocols and initial scanning parameters were investigated. Future work would look to optimise protocols to obtain highest quality images and wall-layer information.

Nervous system modelling

A critical component of passive and active functions of the model is nervous control and how it governs relaxation and contraction of the bladder and urethra. In the current model a relatively simplistic afferent nervous model is used to simulate active contraction of the bladder and relaxation of the urethra. Whilst this allows basic function to be studied, in its current form it does not allow nervous control related disorders to be studied; such as detrusor overactivity. To date, there have been various studies

that model the nervous system with more complex neural centres [156] [139] [124]. Future studies would look to integrate a more complete nervous control model into the current framework as to study nervous-related disorders in more depth. Additionally, modelling studies would benefit from interaction with experimental imaging and mechanical testing to understand local wall response to nervous signal, i.e. stimulation of local regions of tissue and visualise response using MPM. Together, this would give a more complete picture of how the bladder contracts and sophisticate models based on experimental findings to translate into clinical applications.

Standardised measures

One of the current outstanding issues with bringing forward mathematical modelling into a clinical application, or even modelling for purely biomechanic purposes, is the lack of consistency across measures and methodology. For example, protocols to describe an initial configuration of tissue during mechanical testing or urodynamics either differ across studies or are undefined. Models, such as those used in this work, typically relate stress in the tissue to stretch or strain based measures. Inconsistent definitions of initial configuration may result in misleading stress-stretch relationships and incomparable models. Additionally, future studies need to establish a controlled and reproducible methodology for defining different deformation states in urodynamic studies for stretch-based constitutive models to become more clinically useful. Approaches to this may look to x-ray imaging data, or use experimental pressure from traces to define states.

Mobile Technology

Current clinical practice is heavily reliant upon paper based reports, and even computational technology has limited integration with databases or third party software. This severely limits the amount of intra and inter-clinical data that can be accumu-

lated for research purposes, or impedes the ability to refer patients to other clinics. It is proposed that mobile technology may in the future allow digitalisation of various aspects of urology, for example; digital voiding diaries or digital uroflowmetry at home for patients. These would all enable data to be conveniently collected and accessed when required, also allowing for large scale processing of data. These combined would greatly improve clinical efficiency and diagnosis quality, while reducing the reliance on awkward, and outdated paper based data acquisition and storage.

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A Non-linear Elasticity

Kinematics and Definitions

Let the term χ denote the vector that describes the position of point P in the reference configuration and the vector $\mathbf{x}$ describe the position of that material point in the current, deformed configuration. The local deformation of this material point between the initial configuration $\chi \in \Omega_0$ and current configuration $\mathbf{x} \in \Omega$ can be described using a deformation gradient tensor, denoted as $\mathbf{F} = \delta \mathbf{x} / \delta \chi$. The deformation gradient may define both rigid body rotation and stretch for a given transformation. Any changes in volume of an infinitesimal element dV between the initial configuration and the loaded configuration can be characterised by the dilation ratio, J .

$$J = \frac{dV}{dV_0} = \det[\mathbf{F}] \quad (1)$$

As the deformation tensor contains information related to rigid body motion, the right Cauchy Green tensor $\mathbf{C}$ and left Cauchy Green tensor $\mathbf{B}$ are used as an alternative

means of describing a rotation independent measure of deformation.

$$\begin{aligned}\mathbf{C} &= \mathbf{F}^T \mathbf{F}, \\ \mathbf{B} &= \mathbf{F} \mathbf{F}^T.\end{aligned}\tag{2}$$

These tensors are symmetric have the same eigenvalues, meaning they have the same invariants. Elastic material properties can be described in terms of a strain energy function, denoted in this case as Ψ , which is typically a function of $\mathbf{F}$, expressed in terms of $\mathbf{C}$ and $\mathbf{B}$, i.e. $\Psi = \Psi(\mathbf{C})$. Strain energy functions are invariant to the reference system. Therefore they may be expressed in terms of principal stretches or invariants of $\mathbf{C}$ and $\mathbf{B}$, i.e.

$$\Psi = \Psi(I_1, I_2, I_3, \dots, I_i)\tag{3}$$

For an isotropic material, the strain energy can be expressed through the first three principal invariants which are defined by,

$$\begin{aligned}I_1 &= \text{tr}[\mathbf{C}], \\ I_2 &= \frac{1}{2}[(\text{tr}[\mathbf{C}])^2 - \text{tr}(\mathbf{C}^2)], \\ I_3 &= \det[\mathbf{C}].\end{aligned}\tag{4}$$

Soft biological tissues are typically assumed to be incompressible when modelling which arises due to the high water volume fraction of soft tissue, resulting in isochoric deformation behaviour, i.e. no change in volume during deformation ($dV = dV_0$). Studies such as [20] provide a basis for this assumption and gives a good approximation of biological tissue in physiological ranges [20]. This leads to the material constraint of $J = 1$, and therefore $I_3 = \det[\mathbf{C}] = \mathbf{1}$. Isotropic constitutive models cannot always

be used to adequately describe many soft biological tissues due to natural anisotropy, i.e. material properties are direction dependent. Locally aligned collagen structures embedded in an isotropic matrix material give soft tissues a preferential direction along the direction of the collagen fibres, denoted by unit vector $\mathbf{m}_i$. The structure tensor $\mathbf{M}_i$ can then be denoted as the tensor product $\mathbf{m}_i \otimes \mathbf{m}_i$ ($i = 1, 2$).

$$\mathbf{m}_i = \begin{pmatrix} 0 \\ \cos \theta_i \\ \sin \theta_i \end{pmatrix} \quad (5)$$

And for a 2D membrane,

$$\mathbf{m}_i = \begin{pmatrix} \cos \theta_i \\ \sin \theta_i \end{pmatrix} \quad (6)$$

A new set of anisotropic variants are then introduced,

$$\begin{aligned} I_4 &= \mathbf{m}_1 \cdot (\mathbf{C} \cdot \mathbf{m}_1), \\ I_5 &= \mathbf{m}_1 \cdot (\mathbf{C}^2 \mathbf{m}_1). \end{aligned} \quad (7)$$

I_4 can be interpreted as the square of the stretch in the fibre direction, i.e. $I_4 = \lambda_{f,1}^2$. Unlike I_4 , I_5 doesn't have a physical description. In the case of a material where two families of fibres can be identified, the unit vector $\mathbf{m}_2$ is defined to describe the second direction of fibres and introduces several additional invariants.

$$\begin{aligned}
I_6 &= \mathbf{m}_2 \cdot (\mathbf{C} \cdot \mathbf{m}_2), \\
I_7 &= \mathbf{m}_2 \cdot (\mathbf{C}^2 \mathbf{m}_2), \\
I_8 &= [\mathbf{m}_1 \cdot (\mathbf{C}^2 \mathbf{m}_2)](\mathbf{m}_1 \cdot \mathbf{m}_2).
\end{aligned} \tag{8}$$

Similar to I_4 , I_6 can be physically interpreted as the square of stretches in a second preferential direction, i.e. $I_6 = \lambda_{f,2}^2$. I_7 and I_8 do not have direct translation to physical interpretation.

Stress and strain (or stretch) can be used to measure a material's behaviour when subjected to loading. Stress is defined as the force divided by the area on which the force is acting and strain or stretch measures the change in dimension a material undergoes under load. The stress acting on a material may be described in terms of the configuration. For example, the Piola-Kirchoff stress measures relate stress to the current, or reference, state. 1st Piola-Kirchoff stress ($\mathbf{P}$) relates forces in a deformed state to the reference configuration. 2nd Piola-Kirchoff stress ($\mathbf{S}$) may be used to relate forces acting on a material in the reference configuration relative to the reference state, i.e. original cross-sectional area. Cauchy stress ($\boldsymbol{\sigma}$) is a 'push-forward' of the material state, giving current stress relative to its current configuration. Briefly, the relationship between each stress measure is as follows,

$$\mathbf{P} = \mathbf{J}\mathbf{F}^{-1} \cdot \boldsymbol{\sigma} = \mathbf{S} \cdot \mathbf{F}^T \tag{9}$$

Similarly, various strain measures may be used to describe deformation of a solid body. Engineering strain is defined as $\epsilon = l/L - 1$; where l is deformed length and L is reference length. Additionally, stretch is calculated as the ratio for deformed to reference dimension ($\lambda = l/L$). For larger deformations Lagrange strain E defines gradients in terms of the reference configuration and may be more convenient to give strain in terms

of changes in squared length,

$$E = \frac{1}{2}(\mathbf{F}^T \mathbf{F} - \mathbf{I}) \quad (10)$$

B Derivation of Passive SEF

The SEF described in Section 4.2.2 is as follows,

$$\begin{aligned} \Psi &= \phi_{sm} \Psi_{sm} + \phi_{col} \Psi_{col} \\ &= \phi_{sm} \sum_{i=4,6} \frac{\mu_{sm}}{3} (\lambda_{f,i} - 1)^3 + \phi_{col} \sum_{i=4,6} \int_{-\pi/2}^{\pi/2} \int_1^{\lambda_{f,i}} \Psi_f \left(\frac{\lambda_{f,i}}{\lambda_R} \right) \rho_f(\lambda_R) \rho(\theta_i) [\mathbf{m} \otimes \mathbf{m}] d\lambda_R d\theta_i \end{aligned} \quad (11)$$

For a 2D thin-walled membrane 1st Piola-Kirchoff stress is given by,

$$P = \phi_{sm} \sum_{i=4,6} P_{sm,i} + \phi_{col} \sum_{i=4,6} P_{f,i} = \phi_{sm} \sum_{i=4,6} \frac{\partial \Psi_{sm}}{\partial \lambda_{f,i}} + \phi_{col} \sum_{i=4,6} \frac{\partial \Psi_{col}}{\partial \lambda_{f,i}} \quad (12)$$

Where collagen fibre component 1st Piola-Kirchoff stress is,

$$P_{f,i} = \int_{-\pi/2}^{\pi/2} \rho(\theta_i) P_c(\lambda_{f,i}) [\mathbf{m} \otimes \mathbf{m}] d\theta_i \quad (13)$$

And smooth muscle 1st Piola-Kirchoff stress,

$$P_{sm,i} = \mu_{sm} (\lambda_{f,i} - 1)^2 \quad (14)$$

C Derivation of BOR

The basis of the framework references work of Hill in 1938, giving the well known force-velocity relationship of striated muscle.

$$(F + a)(v + b) = (F_0 + a)b \quad (15)$$

Here F is force and F_0 is isometric force. v is the shortening velocity of the muscle and a and b are constants. It can be seen $F = F_0$ when $v = 0$. Experimental studies by Hill found $a/F_0 \approx 0.25$ (and subsequently $a/p_0 \approx 0.25$) for urinary bladder and also reported by [162]. As maximum shortening velocity v_{max} occurs when $F = 0$, and when F_0 is maximal $v = 0$, the following is true; $(F_{max} + a)b = (v_{max} + b)a$ or $a/F_{max} = b/v_{max}$. The Hill equation can be written in a normalised form,

$$\left(\frac{F}{F_0} + \frac{a}{F_0} \right) (v + b) = (F_0 + a)b \quad (16)$$

This equation is also known as the Bladder Working Function, however only describes the force-velocity behaviour of strips of muscle. For a useful description of bladder function it must be re-written to describe pressure-flow relation. An approach previously used is to relate pressure p and force by considering F as total force about the circumference of the bladder wall, assumed to be a thin walled spherical membrane with radius r .

$$p = \frac{F}{\pi r^2}, \quad p_0 = \frac{F_0}{\pi r^2}, \quad \text{giving} \quad \frac{F}{F_0} = \frac{p}{p_0} \quad (17)$$

Meaning the Hill equation can be written as,

$$\frac{5}{4}b = \left(\frac{p}{p_0} + 0.25 \right) (v + b) \quad (18)$$

Then, by comparing with the relationship between v and p in terms of p_0 , v_{max} and a/p_0 (a parameter that defines the curvature of the parabola) [12],

$$v_{max} \left(\frac{p}{p_0} + \frac{a}{p_0} \right) \left(v + \frac{a}{p_0} \right) = \left(1 + \frac{a}{p_0} \right) \frac{a}{p_0} v_{max} \quad (19)$$

Then, by substituting $a/F_0 \approx 0.25$ we can write,

$$\left(p + \frac{p_0}{4} \right) \left(v + \frac{v_{max}}{4} \right) = \frac{5}{16} p_0 v_{max} \quad (20)$$

This equation can be further modified to describe the bladder contraction in terms of volume V and flow Q . Flow-velocity relationship can be derived by,

$$Q = -\frac{dV}{dt} = -\frac{d}{dt} \left(\frac{4\pi}{3} r^3 \right) = -4\pi r^2 \frac{dr}{dt} \quad \text{and} \quad v = -\frac{d}{dt} (2\pi r) = -2\pi \frac{dr}{dt} \quad (21)$$

Yielding the bladder outlet relation, or BOR used in this study, relating pressure to flow.

$$\left(p + \frac{p_0}{4} \right) \left(Q + \frac{Q_0}{4} \right) = \frac{5}{16} p_0 Q_0 \quad (22)$$

D Passive Model Fitting Plotting

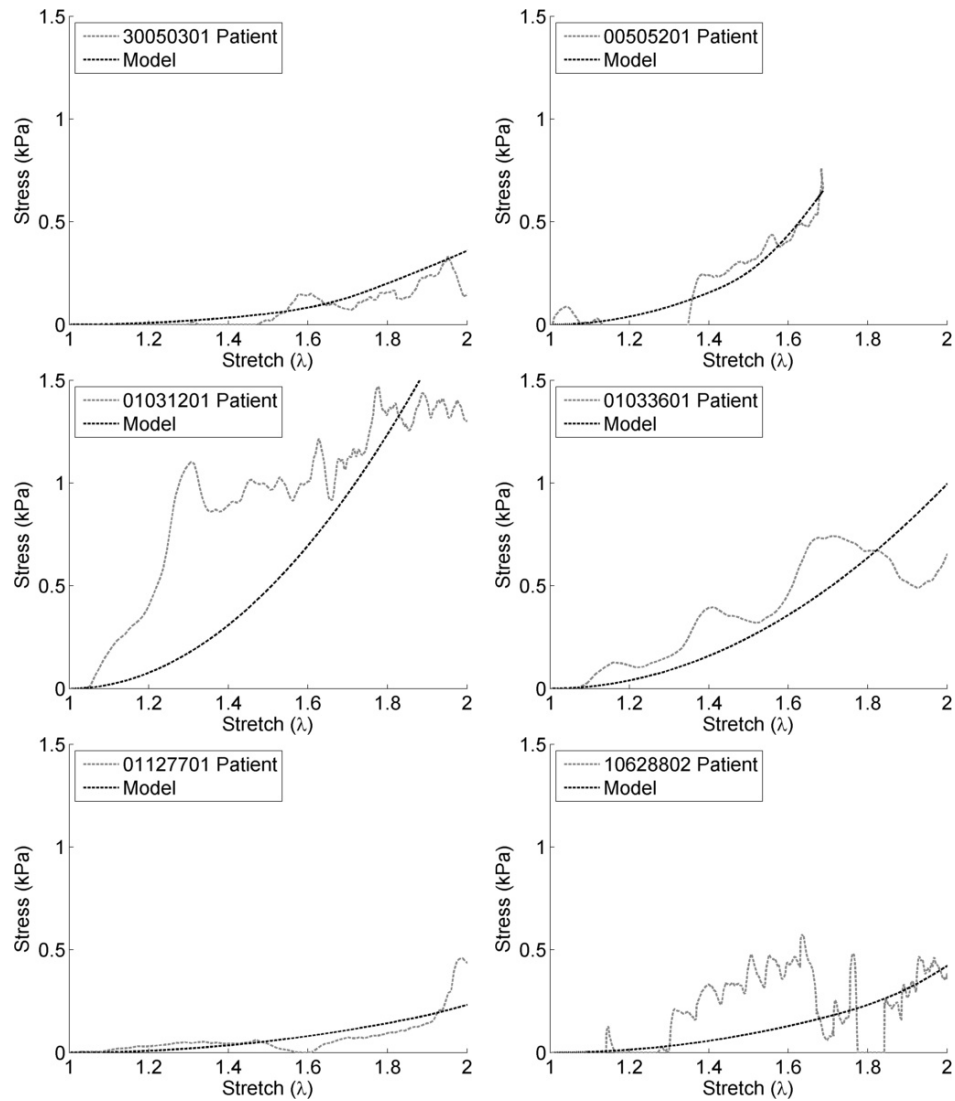


Figure 2: All female patient bladder filling traces with fitted passive model. Note the large differences in detrusor pressure behaviour between patients and high amount of noise.

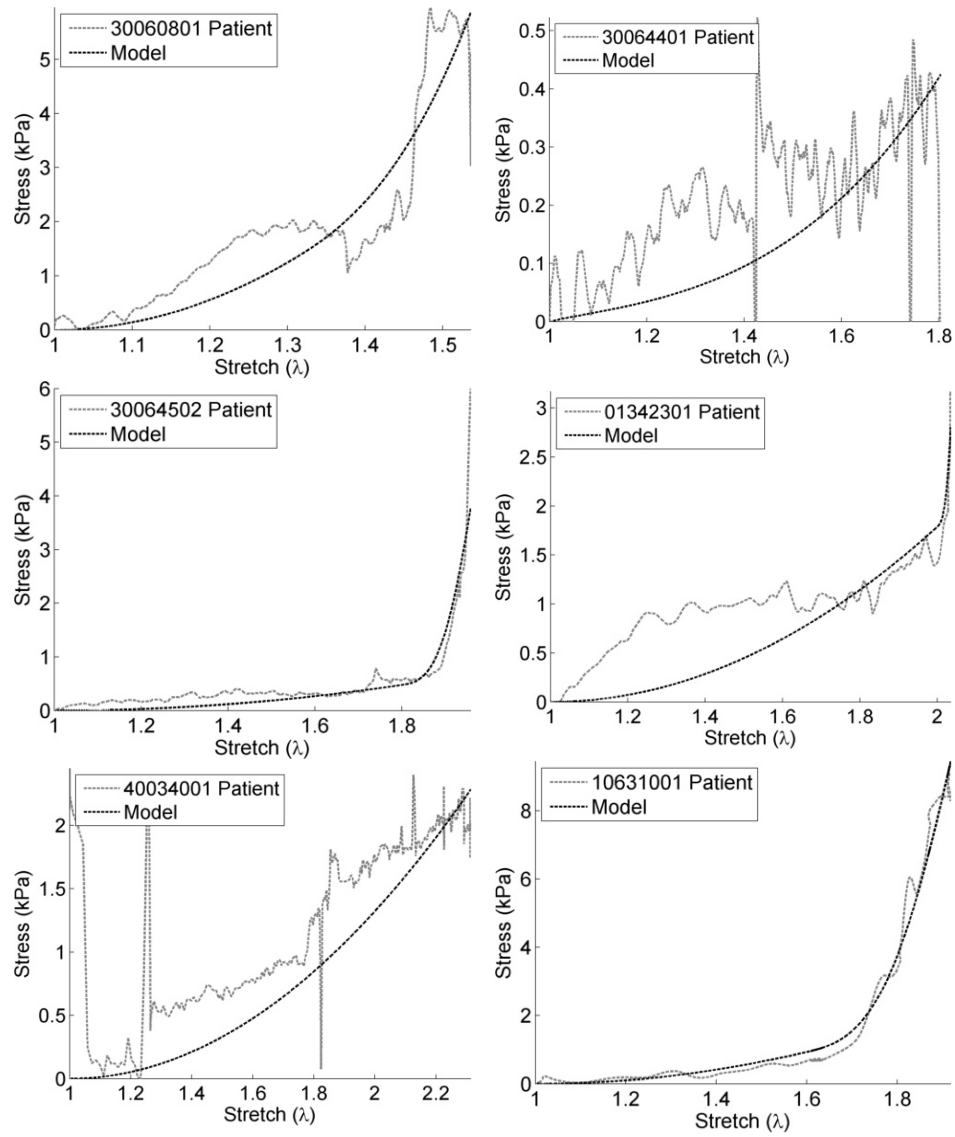


Figure 3: All male patient filling traces with fitted constitutive models. Quality of fit varies considerably with the amount of noise.