Published in final edited form as:

Neurol Urodyn. 2012 March ; 31(3): 293–299. doi:10.1002/nau.22195.

How does the urothelium affect bladder function in health and disease?

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Abstract

The urothelium is a multifunctional tissue that not only acts as a barrier between the vesical contents of the lower urinary tract and the underlying tissues but also acts as a sensory organ by transducing physical and chemical stresses to the attendant afferent nervous system and underlying smooth muscle. This review will consider the nature of the stresses that the urothelium can transduce; the transmitters that mediate the transduction process; and how lower urinary pathologies, including overactive bladder syndrome, painful bladder syndrome and bacterial infections, are associated with alterations to this sensory system. In particular, the role of muscarinic receptors and the TRPV channels system will be discussed in this context. The urothelium also influences the contractile state of detrusor smooth muscle, both through modifying its contractility and the extent of spontaneous activity; potential pathways are discussed. The potential role that the urothelium may play in bladder underactivity is introduced, as well as potential biomarkers for the condition that may cross the urothelium to the urine. Finally consideration is given to vesical administration of therapeutic agents that influence urinary tract function and how the properties of the urothelium may determine the effectiveness of this mode of delivery.

1. The response of the urothelium to ‘stress’

The urothelium is the lining of the lower urinary tract. It comprises multiple layers of cells (the exact number depends upon the species) that functions as a primary barrier as well as exhibiting signaling properties.^{1,2} Thus, the urothelium can be thought of as a first responder to different physiological interventions or stress (which can be defined as any stimulus or factor that alters homeostasis). These responses may in turn alter with age or the presence of disease-related factors.

Mechanical stresses include: changes to transmural bladder pressure; generation of lateral tension in the urothelium or bladder wall; torsion, movement of visceral organs and urine composition or tonicity. The transduction pathways whereby such stresses are converted to afferent signals are considered below. Biochemical stresses that impact on urothelial structure and function include changes to levels of trophic factors or steroid hormones. Altered levels of circulating estrogens have been linked to urinary bladder dysfunction, including overactive and underactive bladder syndromes.³ Some of the symptoms may be associated with alterations to urothelium structure; for example, epithelial shedding or mucosal atrophy as occurs in estrogen deficiency.⁴ Activation of the hypothalamic-pituitary-adrenal axis during altered physiological states results in increased production of corticotrophin releasing factor,⁵ one consequence of which is disruption of the urothelial barrier and increased prevalence of infection. For example, alterations to proteoglycan and bacterial defense molecule structures may lead to distinctive changes in urothelial structure and play a role in bacteria adherence.⁶ Recent findings reveal that during infection, uropathogenic *E. coli* reside in fusiform vesicles within urothelial cells.⁷ This permits bacteria to escape elimination during voiding and re-emerge into the urine during distension. When expelled into the urine during the storage phase, the urine may provide a nutrient-rich environment for bacterial survival.

The effect of the normal ageing process (including alterations to hormone levels) on urothelial function often goes unrecognized. Bladder complications occurring in the elderly are well documented, including changes of bladder capacity and incontinence which are due in part to an increased prevalence of detrusor overactivity.⁸ However, studies utilizing aged animals have also demonstrated significant alterations to the bladder mucosa such as urothelial cell degeneration and areas of mucosal denudation.^{9,10} In addition, the urothelium is sensitive to ischemia/hypoxia,¹¹ induced say by vascular pathologies, conditions associated with ageing.

Several bladder disorders (including overactive or underactive bladder, outlet obstruction, spinal cord injury, diabetes and painful bladder syndrome) all impact on urothelial structure and function. For example, findings in animal models used to elucidate complications of diabetes include a significant increase in urothelial proliferation.¹² Beside the impact of hyperglycemia and autonomic/neuroendocrine changes, diabetes is associated with oxidative stress and high levels of reactive oxygen species, which can lead to changes in urothelial permeability and ultrastructure.^{13,14} The impact of disease-related process on urothelial function will be also considered below.

Note on terminology. The term mucosa is used differently in various contexts. In this review mucosa refers to the layer of tissue that is removed from the detrusor muscle by blunt dissection, as this tissue is available for many *in vitro* studies using native tissue. It will include true urothelium, suburothelial cells (interstitial cells, blood vessels, nerves, etc) and even some smooth muscle (muscularis mucosa). Obvious deviations from this definition will be highlighted.

2. Mechanosensitive properties and stretch-evoked ATP release

Urothelial cells express various receptors or ion channels that are responsive to external agents or mechanical or thermal changes, such as: receptors to bradykinin,¹⁵ trkA and p75,¹⁶ purines (P2X and P2Y),¹⁷⁻¹⁹ noradrenaline (α and β),^{20,21} acetylcholine (nicotinic and muscarinic);^{22,23} protease-activated receptors;²⁴ epithelial Na channels (ENaC),²⁵⁻³⁰ and the Deg/ENaC family³¹ and a number of transient receptor potential (TRP) channels (TRPV1, TRPV2, TRPV4, TRPM8, TRPA1),³²⁻³⁶ Stimulation of these urothelial sensor molecules in turn can control the release of chemicals such as ATP, prostaglandins (PG), NGF, ACh, and NO,^{20,37-40} which have excitatory and inhibitory actions on afferent nerves located close to or in the urothelium.^{31,41} - see Figure 1

ATP release has been particularly well-investigated. ATP is abundant in the cell cytoplasm and can be released to the extracellular space by several mechanisms including vesicular exocytosis, transporters such as a member of the ATP-binding cassette (ABC) transporter superfamily, or anion-selective channels such as maxi-anion channels.⁴² ATP released from bladder epithelial cells in response to distention acts on P2X₃ or heterometric P2X₂/P2X₃ receptors located in the subepithelial afferent nerve plexus.^{43,44}

Stretch-evoked ATP release is modulated by noradrenaline via α 1 receptors,⁴⁵ TRPV1 agonists,³³ and antimuscarinic agents via M2/M3 receptors.⁴³ In terms of mechanosensitive channels, basal ATP release from rabbit bladder epithelium was altered by amiloride, a blocker of ENaC.³⁸ Amiloride and Gd³⁺, a non-specific blocker of mechanosensitive channels, suppressed ATP release from cultured urothelial cells, using a hypotonic stimulus.⁴⁶ Amiloride also inhibited stretch-evoked ATP release from rat bladder epithelium.³⁰ Thus, some mechanosensitive channels may mediate stretch-evoked transmitter (ATP, ACh) release from epithelial cells. This mechanosensory transduction mechanism relies on interaction of chemical mediators (e.g. ATP) released by non-neuronal cells with neuronal ionotropic receptors (such as the P2X₃ receptor).

An additional mechanism may involve a role for TRP channels in mechanosensation. TRPV4 was originally postulated to serve as a mechano- or osmosensor,^{47,48} and is abundantly expressed in rodent bladder epithelium.^{33,49} Studies using mice lacking TRPV4 have revealed the involvement of this channel in sensing mechanical pressure, osmolarity, and warmth in vivo.^{50,51} TRPV4 knockout mice manifest an incontinence phenotype in displaying a spontaneous voiding pattern and exhibit a lower frequency of voiding contraction and increased bladder volume in continuous filling cystometry.⁵² With cultured rat bladder urothelial cells, TRPV4 agonists were shown to promote Ca²⁺ influx and enhance ATP release.³³ These findings indicate a critical role for TRPV4 in physiological bladder function. A recent study investigated the role of TRPV4 channels in a stretch sensing mechanism in mouse primary urothelial cell cultures using both wild-type (WT) and TRPV4-deficient mice. The results suggest that TRPV4 senses distension of the bladder urothelium, which is converted to an ATP signal in the micturition reflex pathway during urine storage.⁵³ Thus, TRPV4 may contribute to bladder function, especially mediating bladder distention signals to primary afferent nerves during urine storage.

More recent work has also highlighted the role of TRPM8 and TRPA1 in transducing bladder sensations.⁵⁴⁻⁵⁶ For example, TRPM8 may be involved in transduction of painful responses from the lower urinary tract. Up-regulation of these receptors is associated with raised pain and frequency scores, but not urgency.⁵⁷ Similarly, generation of an overactive bladder phenotype in an animal model of spinal cord injury is associated with upregulation of TRPA1 receptors, and bladder function is normalised with a TRPA1 receptor

antagonist.⁵⁸ The precise pathways these receptors may contribute to an overactive bladder is unclear, but they offer potentially interesting novel targets.

3. The influence of the mucosa over detrusor spontaneous contractile activity

The presence of an intact mucosa is associated with an increase of spontaneous contractile activity in whole bladder preparations.^{59,60} This spontaneous activity changes in character from small amplitude, high frequency contractions to larger, more prolonged and less frequent phenomena in bladders with say an overactive phenotype resulting from spinal cord injury.⁶¹ Two origins of this activity have been proposed: i) the mucosa itself generates spontaneous contractions; ii) the mucosa, through either cell-to-cell interaction or release of diffusible agents, influences detrusor function.

Evidence exists for both origins. *In vitro* preparations of mucosa stripped from the detrusor layer themselves generate small spontaneous contractions,⁶² these have a frequency similar to those from detrusor strips with an attached mucosa. These contractions may arise from small detrusor muscle bundles present at the base of the mucosa,⁶² or contraction of myofibroblasts (interstitial cells) in the suburothelial layer. Myofibroblasts have a contractile phenotype and contain smooth muscle actin⁶³; in the suburothelium interstitial cells label for smooth muscle actin⁶⁴ as well as vimentin.⁶⁵ However, there are no detailed studies demonstrating contraction of bladder interstitial cells despite the fact that they can generate large intracellular Ca^{2+} transients on exposure to exogenous agents, including P2Y-receptor agonists such as ADP and extracellular acidosis.⁶⁶ Overall it is unlikely that contraction of the mucosa can account solely for the increase of spontaneous contractile activity of isolated mucosa-intact detrusor preparations, as contractions of isolated mucosa are proportionately small, however they may contribute to this increase of contractile function.

Signal transfer between the suburothelium and detrusor has been demonstrated directly by optical imaging experiments measuring propagation of intracellular Ca^{2+} and membrane potential waves. These waves originate in the suburothelium and propagate firstly throughout this layer, and then after a delay of several hundred milliseconds to the detrusor.⁶⁷ Propagation is enhanced by mechanical stretch and exogenous agents, such as very low concentrations of carbachol and P2Y agonists such as ADP and UTP. The extent and velocity of wave propagation is also enhanced in bladder wall preparations from spinal cord injured rats. Enhancement of wave propagation by these interventions and the above phenotype is mirrored in an increase of spontaneous contractile activity further suggesting a link between mucosa-to-detrusor signaling and spontaneous contractile activity. A role for interstitial cells in signaling is suggested by several observations:

- interstitial cells are the major site for the gap junction protein Cx43,⁶⁵ which would allow propagation of electrical and Ca^{2+} signals across a functional syncytium;
- P2Y agonists increase spontaneous contractile activity and generate large excitatory responses in interstitial cells, whereas they reduce the contractility of detrusor muscle *per se*;⁶⁸
- cable calculations using parameters derived from optically-imaged membrane potential signals and interstitial electrical properties yield a value of conduction velocity similar to that experimentally measured both in the absence and presence of ADP;
- interstitial cell number, the intensity of Cx43 labelling and Ca^{2+} and membrane potential wave propagation are all increased in overactive bladders.⁶⁹

It remains to be shown if there is a significant diffusion of excitatory agents between the mucosa and detrusor. Certainly the increase of stretch-induced mucosal ATP release, coupled with reduced extracellular ectoATPase activity in idiopathic detrusor overactivity^{70,71} would provide such a substrate, but definitive experiments remain to be done.

4. Urothelial-derived relaxing factors

The bladder mucosa from several different species, including human, releases a number of substances that have depressant effects on smooth muscle contractility and include nitric oxide, prostaglandins and adenine nucleotides. However, there is no clear understanding of the role that these substances play in physiological or pathophysiological control of bladder contractility. In muscle bath studies of several different animal species, surgical removal of the mucosal layer increases the contractile response to several different agonists. This indicates that the mucosa; constitutively releases agents that depress muscle contractility; metabolises or otherwise inactivates these agonists; acts as a barrier to diffusion and penetration of agonists into the muscle; or responds to the agonists by release of substances that reduce the contraction of the underlying muscle.

Removal of the mucosa from rat detrusor preparations increased contractile responses to the cholinergic agonist carbachol but had no effect on electrical field-stimulated responses.⁷² Pre-incubation with ATP increased the contractile response to carbachol of urothelium-intact rat detrusor strips, to match responses of urothelium-denuded strips, thus indicating a role for purinergic receptors in this process.⁷³ Gentle removal of the urothelium by longitudinal sweeps with a cotton wool applicator (which preserves the underlying suburothelial layers) caused a decreased response to electrical field-stimulation, carbachol and ATP in rat preparations and also resulted in decreased carbachol-evoked release of ATP and nitric oxide.⁷⁴ These observations may suggest that the urothelial layer exerts an excitatory effect on the underlying muscle, whilst the suburothelial layer causes an inhibitory effect.

An increase of contractions elicited by NK-1, NK-2 and NK-3 receptor agonists substance P, neurokinin A and neurokinin B occurred when the mucosa was removed from canine preparations of the bladder body.⁷⁵ With the pig bladder, removal increased carbachol-induced contractions which were in turn reduced upon co-incubation with a mucosa-intact muscle strip. This indicates that a diffusible factor mediates the inhibitory effect. This inhibitory effect of the pig mucosa could not be reversed by inhibiting nitric oxide synthase (NOS) with L-NOARG or methylene blue, inhibiting cyclooxygenase with indomethacin, blocking purinergic receptors with suramin, blocking β -adrenergic receptors with propranolol or blocking potassium channels with TEA or apamin.⁷⁶ With human bladder strips obtained from cancer cystectomy specimens, mucosa removal increased the contractile response to carbachol and electrical field stimulation, but not neurokinin A or membrane depolarization with raised potassium. Furthermore, the inhibitory effect of the mucosa could not be reversed by inhibition of NOS with L-NOARG, soluble guanylyl cyclase with ODQ, cyclooxygenase with indomethacin, or β adrenergic receptors with propranolol.⁷⁷ Thus, urothelial-derived inhibition is not mediated by any of these known inhibitory pathways.

The particular role of mucosal muscarinic receptors in the modulation of detrusor contractility has been investigated. The density of mucosal M₃ receptors positively correlated with the maximal contractile response to carbachol in intact human bladder specimens. It was proposed that mucosal M₃ receptors induce the release of a contractile agonist or suppress the release of an inhibitory agent.⁷⁸

5. Urothelial structure and function associated with disease

The structure of bladder and urethra urothelium undergoes changes associated with different types of disease, such as spinal cord injury or during a bacterial urinary tract infection,⁷⁹ and during treatment of different conditions. The barrier function against pathological bacteria and urine contents is maintained by surface glycans, membrane lipids, tight junction proteins and uroplakins.⁸⁰ Uroplakins may play a major role in protection, bacterial adhesion and even cellular differentiation.¹ For example, infection with uropathogenic *E. coli* in rats stimulates a nitric oxide-dependent pathway in the urothelium that increases bladder contractility.⁸¹ Alternatively the same bacterium inhibits ureteric contractility via host-urothelium interactions thus increasing the possibility for vesico-ureteral reflux.⁸² Furthermore, bacteria can be taken up by the urothelium and be presented to the immune system during successive filling and emptying of the bladder.⁸³ As a result, cystic structures in the urothelial layer can occur during infections and if bacteria are not eliminated, the infection can become chronic.⁸⁴

With interstitial cystitis (painful bladder syndrome), the urothelium undergoes several changes including increased permeability. Ultrastructurally, an altered vascular supply is observed in its ulcerative form with locations of moderate to severe redness, interspersed among a whitish discoloration. A decreased E-cadherin content and an increased number of apoptotic urothelial cells have been shown in interstitial cystitis patients,⁸⁵ as well as an altered purinergic receptor expression in cat.¹⁷

The sensory function of the urothelium is implicated in several other conditions. Stones in the bladder, ureters and even kidneys cause changes to the urothelium resulting in bladder overactivity and urgency incontinence. Neoplasms of urothelium also cause urgency and irritative symptoms that can even lead to their discovery. An intact urothelium has been proposed to prevent detrusor overactivity and may be mediated in part by mechanisms involving TRPV1 receptors and release of nitric oxide.²¹ In addition, studies in patients with symptoms of OAB have shown increased pelvic floor activity in the presence of intravesical potassium, suggesting possible urothelial dysfunction (including changes to urothelial barrier function).⁸⁶

The molecular basis of these changes are unknown. However, changes to urothelial-muscarinic receptor expression have been reported in rats with detrusor overactivity (induced by bladder outlet obstruction) as well as in patients with neurogenic and idiopathic overactive bladder.^{87,98} In addition, it has been proposed that close apposition of TRPV4 and adherence junctions in the urothelium might be associated with bladder sensation.⁸⁹, but these observation require further study.

Regional variation in sensory innervation to the urothelium and suburothelium may also be relevant to normal and abnormal micturition patterns. A difference in innervation of the bladder dome compared to bladder neck and urethra has been shown.⁹⁰ Sensation in the bladder and urethra is essential for initiation and continuation of a micturition and an alteration to this innervation pattern has been proposed to contribute to bladder pathologies such as bladder underactivity.⁹¹

Different forms of treatment can influence the urothelium leading to either a dysfunction or a beneficial effect. Removal of diseased urothelium for example during the treatment of the ulcerative form of interstitial cystitis by laser treatment can be beneficial to symptoms of bladder or pelvic pain.⁹² The formation of a fresh urothelial lining immediately after treatment is associated with non-recurrence of pain for as long as six to 12 months after therapy and may be related to the very long turnover time of urothelial cells of approximately 200 days. During transurethral resection of the prostate the urethral urothelial

lining of the prostate interior surface is removed. Little is known about the effect of this with respect to recurrence of obstructive and urgency-frequency symptoms. Radiation therapy for neoplasms also causes distinct changes to the bladder urothelium, but whether this is related to functional disorders is unknown.

6. Underactive bladder: potential biomarkers, treatments and the role of the urothelium

Underactive bladder (UAB) is a condition whereby bladder contraction is either of reduced strength or duration to complete voiding within a normal time-span. The etiology of UAB may involve ageing, diabetes, bladder outlet obstruction (e.g. BPH), as well as neurological disorders such as Parkinson's disease and multiple sclerosis. UAB is clinically characterized by incomplete and/or protracted/delayed bladder emptying (urine retention), which may lead to urinary frequency and potentially renal damage. To produce pharmacological treatments, it is important to understand the pathology of the disease, develop robust diagnostic tools and validate potential urinary biomarkers. For the latter, the ability of biomarkers to cross the urothelium will be important to ensure adequate levels can be measured. For all these stages it will be important to differentiate patients with UAB from those with other bladder disorders and from healthy subjects.

In healthy subjects invasive cystometry has also been tested with contraction of the external urethral sphincter measured using a two-channel microtip pressure transducer catheter in the rectum and urethra.⁹³ Non-invasive diagnostic tools used in early clinical trials are measurement of: a) bladder capacity with ultrasound; b) maximum flow rate with uroflowmetry; c) post-voided residual; d) voiding efficiency; e) urethral opening and closing pressures and elastance in women using urethral pressure reflectometry (UPR) that demonstrates greater sensitivity and less variability than conventional urethral pressure profile approaches.⁹⁴⁻⁹⁶ With UPR the pharmacological effect of noradrenaline-reuptake inhibitors has been demonstrated and translated into improvements in stress urinary incontinence (SUI) diary symptoms.⁹⁶

Potential biomarkers that may traverse the urothelium include cGMP, NO, tachykinins, and prostaglandin E₂ (PGE₂).⁹⁷ For example, platelet cGMP has already been shown to be a relatively straightforward and reliable biomarker of PDE5 inhibitory activity in patients with erectile dysfunction.⁹⁸ During the voiding phase, the nitric oxide (NO)-cGMP signaling pathway in men is activated, accommodating efficient voiding through the relaxation of urethra and prostate. Systemic NO augmentation lowers functional bladder outlet resistance very rapidly in men and the NO-cGMP pathway may be a target as well as a biomarker target for medical evaluation and treatment of lower urinary tract symptoms.⁹⁹ Tachykinin-induced activation of neurokinin-2 (NK₂) receptors in the human bladder leads to contraction of detrusor muscle.^{100,101} PGE₂ increases detrusor contraction and urethral relaxation and in patients with brain disease PGE₂ was shown to be increased in urine and associated with the presence of OAB.¹⁰² Whether it is useful in demonstration of UAB requires evaluation. However, current small studies involving urinary biomarkers show high variability and may require more standardization in sampling conditions and robust bioanalytical method validation.

7. Delivery of therapeutic agents across the urothelium

The ability of the urothelium to modulate afferent nerve activity and allow some agents to cross the barrier has increased interest in it as a target for therapeutic intervention. This is exemplified by: intravesical instillation of vanilloids (capsaicin, resiniferatoxin) to achieve bladder deafferentation, which in some patients with hypersensitivity disorders improves

urodynamic parameters and reduces bladder pain; as well as the use of antimuscarinics such as oxybutynin to treat intractable detrusor overactivity.¹⁰³⁻¹⁰⁶ However, some drugs, such as cyclophosphamide and methotrexate, are secreted into the urine and can have profound effects on bladder sensation and function and in the case of cyclophosphamide even cause urothelial neoplasms.¹⁰⁷

Intravesical administration of drugs aims to maximize the therapeutic doses reaching the bladder wall whilst minimizing associated systemic side-effects. However, intravesical drug delivery can be limited by the low permeability of the urothelial layer and the fact that instilled drug solutions become diluted by urine and get washed out during voiding.¹⁰⁸ Approaches to augment the amount of drug reaching the bladder include permeation enhancers that improve transport across the bladder wall or mucoadhesive drug carriers that strongly adhere to the urothelium and ensure persistence of the therapeutic agent near the site of action.

Translating the use of polymeric hydrogels as intravesical drug depots from animal models to humans is hampered by the higher capacity of the human bladder, and consequently this approach has yet to be used in patients.¹⁰⁸ More success has been derived from attempts to increase urothelial permeability, despite the fact that transepithelial electrical resistance of the urothelium, a measure of ion permeability, is the highest of all epithelia.¹⁰⁹ Techniques include the use of electromotive force, drugs that temporarily disrupt urothelial integrity and the use of nanocarriers such as liposomes to augment drug delivery. Several studies have shown that electromotive drug administration using an electric field significantly enhances the transport of hydrophilic drugs into the bladder.^{110,111} Agents such as chitosan and protamine sulfate disrupt urothelial permeability barrier whereas dimethyl sulfoxide, a solvent with anti-inflammatory and bacteriostatic properties, is capable of penetrating living tissue without causing significant damage.¹⁰⁵ The latter has therefore been approved by the US Food and Drug administration for the treatment of interstitial cystitis. Liposomes are versatile drug delivery systems consisting of an aqueous core enclosed in one of more phospholipid bilayers and can be used to transport both hydrophobic and hydrophilic drug molecules.¹⁰⁵ Alternatively, empty liposomes might enhance the barrier function of a dysfunctional urothelium and reduce the penetration of irritants.

The lower urinary tract is ideally suited for minimally invasive intravesical treatments. Thus, continued research efforts are needed not only to improve our understanding of the pathophysiological mechanisms that underlie bladder dysfunction, but also to improve our knowledge of the chemical and physical properties of the bladder wall and the processes that regulate drug transport across it.

Acknowledgments

This review was supported by grants from EU FP7 InComb and NIH (R37 DK54824).

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Figure 1.

A diagram of the bladder wall and some of the external factors that influence urothelial function. The mucosa consists of a true urothelium and suburothelium and is in contact with an external proteoglycan layer facing the urine. The urothelium includes an outer layer of umbrella cells facing the urine and coupled by tight junctions thus reducing the ability of solutes to penetrate the urothelium. The suburothelium contains afferent nerve fibres, blood vessels and interstitial cells and at the inner face some muscle cells. Upon exposure to external stresses the urothelium releases a number of transmitters including that may influence afferent nerve activity or diffuse to the detrusor layer and alter contractile function, these include: ATP, acetylcholine (ACh), nitric oxide (NO) and prostaglandins (PG). External factors affecting transmitter release include physical factors, such as change of transmural pressure, ΔP , or lateral or tortional strain, or changes to the chemical composition of urine or the blood.