Examing Committee

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I would like to dedicate this thesis to the “giants upon whose shoulders I stand”:

James B. Metcalfe
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Also, with great thanks to the support and motivation from my incredible wife:

Andrea Metcalfe

Finally, I would like to thank all of my mentors at the Riley Hospital for Children; you set a standard to which I aspire every day.

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Abstract:

Introduction: Partial bladder outlet obstruction (pBOO) is a ubiquitous problem that results in renal damage. We hypothesize that change in the bladder progresses over time.

Methods: Fischer rats underwent surgical pBOO for 2, 4, 8, or 13 weeks and were compared to shams. Urodynamic measurements were taken, bladders weight and thickness recorded, and tissue analyzed with microscopy. RT-PCR was performed for inflammatory mediators and spectrometry used to quantify collagen.

Results: Urodynamics demonstrated an increased capacity and deterioration into high-pressure. H+E demonstrated an initial inflammatory response, and increased mRNA levels of TGF-β, CTGF, HIF-1α, and PDGF. Muscle hypertrophy was evident on H+E and increased bladder mass and thickness. Masson’s Trichrome and mass spectrometry showed an increase in collagen.

Conclusion: We believe that this represents distinct phases of bladder decompensation: inflammation, hypertrophy, and fibrosis. This could lead to improved preventative strategies, with respect to biochemical pathways and the time course of their initiation.
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<td>Partial bladder outlet obstruction</td>
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<td>PUV</td>
<td>Posterior urethral valves</td>
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<td>RT-PCR</td>
<td>Real-time reverse transcription-polymerase chain reaction</td>
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<td>Platelet derived growth factor</td>
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Chapter 1: Introduction

1.1 Scope of Problem

1.1.1. Definition

Partial bladder outlet obstruction (pBOO) is an extremely prevalent health concern, affecting the majority of aging males\(^1\)\(^-\)\(^2\) and is a life-long health risk for many children born with severe congenital anomalies of the urinary tracts\(^3\)\(^-\)\(^4\). pBOO can be defined as an increase in the bladder pressure required to void, secondary to a hypertrophied or overactive urinary sphincter, which usually results in an impaired rate of flow of urine\(^5\)\(^-\)\(^7\).

The lower urinary tract, in its simplest form, can be seen as a storage receptacle, whose role is to store urine, safely and without leakage, and empty completely, under voluntary command. pBOO affects both of these functions, as the increased urethral resistance makes it difficult to completely empty, placing the patient at risk for bladder infection, bladder calculi, and renal failure. The bladder undergoes progressive deterioration, whereby the increase in resistance results in detrusor muscle hypertrophy and instability. This results in a chronic elevation in bladder pressures, and may lead to urinary incontinence or renal damage.

The severity of the changes in bladder function is variable, depending on the etiology and its severity. Most aging males suffer primarily from lower urinary tract symptoms (LUTS), but with a real potential to significantly impact on quality of life\(^8\). pBOO in children, however, is due to severe congenital anomalies, and usually results in a more severe obstruction, with serious risks of renal failure\(^9\)\(^-\)\(^13\).

Although the etiology is the urinary sphincter, the bladder is the organ most affected and is the cause of morbidity. Primarily composed of smooth muscle, its initial response to an increase in outlet resistance is smooth muscle hypertrophy, and it initially is able to compensate. However, with prolonged mild high levels of resistance, the compensatory phase inevitably progresses to decompensation. The decompensation, clinically, progresses from a detrusor muscle that is able to generate sufficient pressures to void across the increased resistance, to a decreased ability to generate pressure, and finally, a fibrotic bladder. The marked tissue fibrosis severely decreases tissue compliance and results in a bladder unable to store urine safely, at low resting intravesical pressures.

1.1.2. Epidemiology
1.1.2.1. Adult Bladder Outlet Obstruction

The most common form of pBOO is benign prostatic hypertrophy (BPH), a progressive disease which has been found to affect 8% of 40 year old males and up to 82% of 80 year old males\textsuperscript{14}. This disease process results from an the enlarging male prostate, which gradually and insidiously increases resistance to voiding\textsuperscript{15, 16}. Although not usually a major health concern, it is primarily responsible for bothersome lower urinary tract symptoms (LUTS)\textsuperscript{17, 18}. LUTS account for significant morbidity in the ageing male, accounting for 4 billion dollars in the United States alone\textsuperscript{19}. Furthermore, more severe cases can result in urinary retention, recurrent urinary tract infections, bladder calculi, and acute renal failure\textsuperscript{20-23}. These cases usually mandate surgical intervention aimed at reducing pBOO\textsuperscript{21-23}, but they are not universally successful, as preexisting detrusor damage may have become irreversible.

1.1.2.2. Posterior Urethral Valves

Although less common, pBOO in the pediatric population represents an equally significant health concern\textsuperscript{4, 24}. Instead of a ubiquitous physiologic process, it occurs secondary to a variety of major congenital anomalies, including posterior urethral valves (PUV)\textsuperscript{25, 26}, myelomeningocele\textsuperscript{27}, other forms of spinal dysraphisms\textsuperscript{28, 29}, and indirectly, bladder and cloacal exstrophy\textsuperscript{30, 31}. These represent much more severe forms of obstruction, and the potential for serious health risks. This includes renal failure, permanent bladder dysfunction, and major reconstructive surgery which are very common\textsuperscript{9, 24, 32, 33}. Again, although the primary pathology occurs at the bladder outlet / sphincter region, the morbidity occurs through an irreversibly damaged detrusor muscle and bladder\textsuperscript{34, 35}.

The most common congenital form of pBOO is PUV; which occurs in approximately 1/5000 to 1/8000 births\textsuperscript{36}, with many instances now detected antenata\textsuperscript{37}. The pathophysiology is secondary to an embryologic malformation, whereby a thin, obstructing membrane in the posterior urethra severely compromises the ability of the fetal bladder to empty\textsuperscript{26}. Therefore, the developing bladder is exposed to extraordinarily high voiding pressures, and is incapable of normal development. The natural history of less severe cases is becoming understood, and highlights the role and pathology of the decompensated detrusor\textsuperscript{12, 38-40}. The “valve-obstructed” bladder may appear normal in early childhood, but if not carefully monitored, insidious detrusor decompensation results in a poorly contractile bladder that is unable to overcome the outlet resistance. The subsequent chronic urinary retention progresses to a hypercontractile bladder and eventually to an end stage bladder with high storage pressures, and risk of renal failure.
due to subsequent reflux and increased pressures in the upper urinary tract including the ureters and kidney collecting system.

Despite increasingly early and aggressive intervention, including fetal surgery\textsuperscript{10},\textsuperscript{37, 41, 42}, outcomes remain poor. Fetal death rates, historically reported to be as high as 50\%\textsuperscript{11}, have improved, but still occur in 2-3\% of cases\textsuperscript{43}. The incidence of renal failure remains high\textsuperscript{13}, and progress with improvements in quality of life have occurred primarily though renal transplantation\textsuperscript{44, 45}.

1.1.2.3. Neuropathic Bladder

BOO may also occur secondary to aberrant innervation of the urinary sphincter, including congenital maldevelopment of the spinal cord. Spinal dysraphism refers to a spectrum of abnormalities, and is commonly referred to as spina bifida. The most common form of this is myelomeningocele, where the spinal cord and meninges are everted and exposed on the skin at birth. However, any spinal cord abnormality, including trauma, tumors, and the “occult tethered cord” are capable of producing a functional pBOO. Despite the absence of a true anatomic obstruction, patients with a neuropathic pBOO often suffer the most severe consequences, including marked hydronephrosis and renal failure. This is often felt to be due to the progressive, but silent obstruction, leading to pathologic changes.

In these cases the pathophysiology involves the innervation of the urinary sphincter, and results in abnormal sphincter resistance, either with respect to a permanent fixed tone, or an inappropriate contraction at the time of voiding (detrusor sphincter dyssynergia [DSD]. The increase in bladder pressures results in several problems, including vesicoureteral reflux (VUR). The progressive nature of the pathology is seen with VUR being diagnosed in 3-5\% of newborns\textsuperscript{46} to and increasing to 30-40\% of untreated 5 year olds\textsuperscript{47}. Historically, the neuropathic bladder carried a significant risk of renal failure\textsuperscript{48, 49}. Contemporary literature supports that early and aggressive treatment is required to prevent this life-threatening problem.\textsuperscript{50} However, due to incomplete knowledge of the molecular pathophysiology, we are not yet entirely successful with management, with renal damage and incontinence remaining a persistent problem.

1.1.3. Anatomy and Physiology

1.1.3.1. Anatomy
The normal healthy lower urinary tract must be able to store urine at low pressures and empty when desired\textsuperscript{51}. However, this involves a complex interaction of bladder and sphincter, which is enabled by the central nervous system and effected by intricate molecular pathways\textsuperscript{52-54}. Detrusor contractions are primarily innervated by the parasympathetic nervous system, S2, 3, 4 nerve roots\textsuperscript{52}, with the neurons located in the sacral parasympathetic nucleus, and postganglionic neurons terminate in both the detrusor wall and the pelvic plexus\textsuperscript{52}. The end organ receptors are muscarinic, with M2 being the most common subtype, but M3 playing a prominent role in mediating detrusor contraction\textsuperscript{55-57}. The detrusor muscle is also innervated by the sympathetic system, whereby $\beta$ receptor activation results in muscular relaxation and increased tissue compliance\textsuperscript{57-59}.

The urinary sphincter is quite complex, and complete knowledge is rapidly evolving. It is well accepted that two separate, and independent, systems exist. The intrinsic sphincter (bladder neck / involuntary) and the extrinsic (striated / voluntary) are innervated by the sympathetic and somatic nervous systems, respectively. The sympathetic nerve roots are found in L2, L3 and the axons pass through the sympathetic ganglia, to the inferior mesenteric ganglia, and then travels with the hypogastric nerves to the pelvis plexus\textsuperscript{60}. The bladder outlet receptor types are primarily $\alpha$-adrenergic\textsuperscript{61-63}, with both $\alpha1$ and $\alpha2$ subtypes represented\textsuperscript{57}. The extrinsic sphincter is mediated via the pudendal nerve (S2, 3, 4), with motor neurons residing in the lateral border of the ventral horn, referred to as Onuf’s nucleus\textsuperscript{64}.

Therefore, congenital and acquired abnormalities of the spinal cord play result in fundamental changes to the function of the lower urinary tract. The most common etiology in children is spina bifida, which results in a very unpredictable outcome, depending upon the degree of parasympathetic, sympathetic, and somatic nerve dysfunction.

1.1.3.2. Physiology

Safe storage of urine, as well as urinary continence, mandates low intravesical pressures\textsuperscript{51, 65}. A fundamental property is tissue compliance, defined as the change in volume / change in pressure. The histology of the bladder is uniquely designed for maximal compliance, and the ability to hold large amounts of urine with a minimal rise in intravesical pressure. The tissue consists of smooth muscle and a unique extracellular matrix, which must work in concert for proper function. Complete relaxation of the detrusor requires healthy smooth muscle cells and active inhibition, mediated via
sympathetically mediated \(\beta\)-adrenergic receptors\(^{57-59}\). Intrinsic factors include the unique viscoelastic properties of the detrusor, which results in remarkable tissue compliance\(^{66}\).

The bladder wall stroma is composed of fibroblasts, collagen (types I, III, IV most commonly found) and elastin in a proteoglycan matrix\(^{66, 67}\). The complex interaction of these elements allows for the bladder to fill with urine and remain at low pressures until maximum capacity has been reached. Recent work has demonstrated that this stroma is not passive, but pays a fundamental role in response to stress; both physiologic compensation and pathophysiologic decompensation\(^{68}\).

### 1.1.3.3. Pathophysiology

With pBOO, the detrusor is exposed to elevated intravesical pressures. The initial response, as with many organ systems, is compensatory, but prolonged stress results in decompensation. Animal models have been very informative in eliciting the natural history of pBOO. The initial compensation is marked by an increase in the mass of the bladder wall\(^{69}\), increased bladder capacity\(^{70}\), blood flow, and smooth muscle hypertrophy\(^{69-71}\). However, more severe or prolonged obstruction will lead to a decrease in contractility, loss of normal tissue architecture, and collagen deposition\(^{70, 72, 73}\). The net result is a loss of the vital functions of the bladder, as it is no longer able to store urine safely.

The smooth muscle hypertrophy results in hypercontractility, where the continual uninhibited contractions, in combination with the increased outlet resistance, result in elevated bladder pressure. This is compounded by the collagen deposition, and overall change in extracellular matrix, as this decreases tissue compliance. Therefore, normal urine volumes are not tolerated, and result in a further increase in bladder pressures. These factors are especially dangerous in combination, as the contractility is less tolerated in a stiff, non-compliant bladder.

### 1.1.4. Clinical Implication

Elevated bladder pressures place the patient at risk for urinary incontinence and kidney damage. Even in the presence of pBOO, bladder pressure can involuntarily rise above urethral resistance, and thus result in an inappropriate loss of urine. This has significant implications with respect to the patient’s quality of life, especially in the pediatric population\(^{74-76}\). However, the threat to renal function is the most feared consequence of pBOO. Acutely, the increased pressure within the urinary tract results in a decrease in renal blood flow, an increase in renal vascular resistance\(^{77}\) and a decrease in glomerular filtration rate\(^{78, 79}\). Chronic exposure to elevated pressure results in VUR,
hydronephrosis, and renal compromise. The landmark clinical paper was published by McGuire in 1981, where he demonstrated that maintenance of bladder pressures below 40 cm H₂O pressure was associated with significantly improved renal outcomes. This had a dramatic effect on patients’ health and quality of life, and deaths from renal failure have decreased dramatically. However, morbidity and mortality from renal compromise remains significant, still accounting for up to 1/3 of deaths in the spina bifida population. The persistent pathology and lack of progress is likely secondary to our incomplete understanding of the molecular biology, resulting in contemporary treatments that remain relatively crude and fraught with complications.

1.2. Contemporary Management

Contemporary management is directed at: 1) identifying high-risk patients; 2) eliminating the obstruction; 2) medical treatment; and 3) surgical reconstruction. Unfortunately, all three modalities remain relatively crude, aimed at end-organ function, and have not yet been able to capitalize on recent advances in molecular biology.

1.2.1. Diagnosis

1.2.1.1. Urodynamics

Because we are currently unable to reverse the underlying cause of pBOO and the end-organ damage that has occurred, we rely on diagnostic testing to determine the need for intervention. Urodynamic monitoring is the urologist’s primary tool to measure lower urinary tract function. It is used to determine bladder capacity, compliance, and contractility, all of which are affected by pBOO. The pioneering work by McGuire gave us the threshold of 40 cm H₂O, so we could justify instituting or escalating treatment once this threshold had been exceeded. Although today many clinicians use lower thresholds, the fundamental problem remains: that contemporary treatment is based on instituting therapy after damage has occurred, and we are unable to definitively prevent organ damage secondary to increased urethral resistance and bladder decompensation.

1.2.2. Surgical Relief of pBOO

1.2.2.1. Endoscopic

If the cause of the obstruction is anatomic, as with BPH and PUV, this can be surgically corrected. Both can be treated endoscopically, with a trans-urethral resection of the prostate (TURP) being one of the most common procedures in adult urology. Children with PUV can be treated with a trans-urethral resection of valves, with a very high technical success rate. Surgical intervention can even be performed before birth,
whereby a catheter is placed into the fetal bladder to allow drainage into the amniotic fluid. However, these seldom result in a cure, as end-organ damage has already occurred, and the decrease in resistance is unable to reverse the end-stage, fibrotic, non-compliant and hypercontractile bladder.

1.2.2.2. Vescicostomy

Neonates with PUV, however, are often too small to undergo definitive endoscopic surgery, and a vescicostomy offers an excellent means of reducing intravesical pressure until definitive surgery is possible. Infants with a neuropathic bladder and unsafe storage pressures may require vescicostomy if the patient’s caregivers are unwilling or, despite maximal medical treatment, unable to maintain safe bladder pressures. Several studies have confirmed the effectiveness of a vescicostomy, with regards to either a reduction in upper tract dilation or improvement in renal function\textsuperscript{85-90}. Complications of cutaneous vescicostomy include stomal stenosis, prolapse, peristomal dermatitis and bladder calculi, but it is generally very well tolerated. The primary negatives include that a vescicostomy is incontinent (requiring a diaper to collect urine) and that it is limited to young children. At best it represents a safe and simple means of lowering intravesical pressures, at worst, it represents a crude, temporary, and incontinent treatment that is unable to address the primary pathology.

1.2.3. Medical Treatment of pBOO

1.2.3.1. Clean Intermittent Catheterization

Most high-pressure bladders are adequately treated with more conservative measures, and these included catheterization and medical treatment. Clean intermittent catheterization (CIC), introduced by Lapides, revolutionized urology and the treatment of high-pressure bladders; as it replaced the need for a continuous, indwelling catheter\textsuperscript{91}. Initiated either in all patients at risk, or those identified with urodynamics, it is extremely common and an integral part of conservative or medical management. CIC allows for effective, intermittent drainage of the bladder, which is mostly continent and avoids the complications of an indwelling catheter\textsuperscript{92-94}. Complications of CIC include an increased incidence of UTI and ascending epididymo-orchitis, with bacteriuria being nearly universal\textsuperscript{92, 95, 96}. The potential for urethral trauma is also omnipresent, but the incidence is of complicating stricture is relatively low\textsuperscript{97, 98}. In patients with intact penile sensation, however, it is nearly impossible to institute catheterization in the young child and teenager, due to the sensation and emotional components.
Overall, CIC is generally well tolerated and accepted by patients and caregivers\textsuperscript{99,100}, but is subject to patient compliance and appropriate social supports. Therefore, many patients and physicians will not initiate this invasive treatment unless there is evidence of renal compromise\textsuperscript{49} and many more receive less than ideal treatment, secondary to non-compliance, and their kidneys remain at risk.

1.2.3.2. Anticholinergic Therapy

The second integral component of conservative treatment involves anticholinergic therapy. This class of drugs act at the detrusor level, by inhibiting the binding of acetylcholine to its muscarinic receptors, and decreasing involuntary bladder contractions\textsuperscript{101-103}. Oxybutinin chloride was the first oral agent to gain widespread use, due to its effectiveness and tolerability\textsuperscript{103-105}. However, bothersome side-effects, including dry mouth, heat intolerance, and constipation, have been attributed to patient non-compliance\textsuperscript{106-108}. This has lead to the design of controlled release formulas and novel drugs with improved side effect profiles, both of which have modestly improved patient care\textsuperscript{107-110}.

Together, CIC and anticholinergic therapy can be very effective at reducing bladder pressures, increasing tissue compliance, and bladder capacity in up to 93\% of patients with spina bifida\textsuperscript{103-105,111}. This has resulted in both improved renal outcomes and improvements in urinary continence and quality of life\textsuperscript{104,105}. However, concerns remain over patient compliance\textsuperscript{106-108,111} and the lack of molecular specificity. This treatment modality however, despite its crude nature, has demonstrated that pharmacologic intervention can effectively prevent end organ damage secondary to congenital defects that otherwise place the essential function of the bladder at risk of irreversible damage\textsuperscript{112}.

1.2.4. Surgical Reconstruction

Despite even the best efforts and aggressive medical therapy, approximately 20\% of patients will require surgical reconstruction for renal health or urinary continence. The goals of reconstructive surgery mirror all bladder management: increased bladder capacity, increased tissue compliance, and an abolition of involuntary contractions. Strictly from a bladder point of view, this is currently best achieved by performing a laparotomy and augmenting the bladder with a section of the patients’ own intestinal tract.

Complications from such major interventions are numerous, and are amplified with incorporating the gastrointestinal tract into the urinary tract. The complications that occur
can be categorized into those that are: 1) structural complications, 2) secondary to the loss of an intestinal segment, 3) those secondary to intestinal secretions, and 4) complications that occur secondary to the intestinal reabsorption of urinary solutes.

1.2.4.1. Structural Complications

Laparotomy and the removal of an intestinal segment places the patient at risk for a bowel obstruction, with an occurrence rate of approximately 3% in these cases\textsuperscript{32,113-116}. Roughly 9.4% of patients required a secondary augmentation as reported in the Indiana University series\textsuperscript{116}. These patients were diagnosed secondary to persistent or de-novo incontinence or hydronephrosis. Spontaneous perforation of the augmented bladder can be a fatal complication\textsuperscript{117,118}, and has been reported in 6-13% of augmented patients\textsuperscript{117,119-121}.

The risk of malignancy in the augmented bladder is an increasing concern, since first described in 1971\textsuperscript{122}. The etiology is multi-factorial, involving chronic inflammation and carcinogens resulting in metaplastic changes and carcinoma\textsuperscript{123}. Tumors have been seen in all of the intestinal segments, arising from the intestinal segment, urothelium, or the ureterointestinal anastomosis. Transitional cell carcinoma, adenocarcinoma, anaplastic carcinoma, and benign polyps have all been reported\textsuperscript{124}. Hussman’s review reports a 33% mortality rate, with 29% of the 94 patients having benign tumors\textsuperscript{124}.

1.2.4.2. Loss of intestinal segment

The removal of a gastrointestinal segment for bladder augmentation is usually well tolerated, however, bowel dysfunction has been reported in 10-54% of patients\textsuperscript{125,126}. Vitamin B\textsubscript{12} deficiency can develop in up to 35% of patients following an 80 cm small bowel resection for a Kock pouch\textsuperscript{127}, but it has not been demonstrated following ileocystoplasty\textsuperscript{128,129}, perhaps because it is limited to a 25 cm resection that is easily kept proximal to the ileocecal valve.

1.2.4.3. Complications due to Secretions

Mucus production is an inherent property of gastrointestinal segments, and can result in incomplete bladder emptying, predispose the patient to UTIs, and become a nidus for bladder stone formation\textsuperscript{32}. Bladder stones occur in approximately 10-15%\textsuperscript{116,130-132} of bladder augmentations.
Bacteriuria is nearly universal in any patient performing CIC, especially when combined with an enterocystoplasty. A UTI most frequently presents with malodorous urine, but symptoms may include hematuria, incontinence exacerbation, suprapubic pain, or increased mucus production. Rink et al. reported that symptomatic urinary tract infections occur in 22.7% of patients with an ileal augmentation, while overall occurrence of a febrile UTI was reported to be 14%. Treatment of asymptomatic bacteriuria is not indicated unless culture indicates a urease producing organism, or a very virulent organism.

1.2.4.4. Complications due to absorption

The use of bowel for a urinary reservoir can be associated with profound metabolic changes due to its absorptive nature. Colon and ileum readily absorb ammonium, hydrogen ion, and chloride and this will result in a hyperchloremic metabolic acidosis. This is tolerated in many patients with normal renal function, but may require medical therapy in others. The acidosis prompts mobilization of phosphate buffers from bone, and can result in demineralization and Ca2+ excretion in urine. The acidosis will also result in hypocitraturia, which further compounds the risk of both renal and bladder stone formation. Medications such as dilantin and methotrexate are readily absorbed across the bowel and levels must be closely monitored to avoid toxicity. Glucose is also absorbed, making urinary monitoring of hyperglycemia less reliable.

Therefore, although the treatment of pBOO and the resultant effects on the bladder have improved greatly, there are many areas that merit significant improvement. Both medical and surgical treatments have tremendous ability to maintain renal health and urinary continence. CIC remains a fundamental component of contemporary management, but is obviously invasive and can be poorly tolerated. Unfortunately, our pharmacology is limited to the cellular / organ level and is susceptible to untoward side effects. Ideally, medical intervention should be specifically aimed at intra-cellular second-messenger pathways. However, currently we are unable to effectively prevent tissue damage, and far too many patients end up with end-organ failure, requiring a major surgical intervention with multiple, life-long potential for complications.

1.3. Current Research

1.3.1. Molecular Pathways

1.3.1.1. Cellular
A great deal of work is being performed to further understand the molecular events that occur secondary to pBOO. Significant progress has been made recently with respect to understanding the molecular pathways in normal bladder physiology and pathophysiology. These pathways underlie the changes in detrusor function, such as smooth muscle hypertrophy, detrusor hyperactivity, and end-stage fibrosis. Normal and abnormal bladder function exposes the bladder to signals arising from neural input, local mechanical stimulation, and the extracellular matrix. Several signal transduction pathways have been identified, including: receptor tyrosine kinase (RTK), G-protein coupled receptors (GPCR), calmodulin, Rho-associated kinase (ROCK), mitogen-activated protein kinase (MAPK), Jun N-terminal kinase (JNK), and numerous others. Intracellular nitric oxide synthase (NOS), however, has received significant attention with respect to its up-regulation with inflammation and role in detrusor remodelling. Accordingly, the extracellular molecular pathway generating prominent interest involves matrix metalloproteinases, and also involves MAPK. These are up-regulated in response to mechanical stretch and result in increased expression of collagen types I and III.

1.3.1.1.1. Nitrous Oxide

The discovery of nitric oxide (NO) as a major signaling transmitter in 1980 resulted in the 1998 Nobel Prize, and its role and importance appears to be growing. Like many other organ systems, NO in the lower urinary tract has been found to play a prominent role in the relaxation of smooth muscle. It has also gained recognition for its role as a neurotransmitter, in the nonadrenergic, noncholinergic nerves (NANC). It also has been discovered to be a cell mediator, limiting cell proliferation and organ hypertrophy. Therefore, its role in the urinary tract has begun to be explored, and there is considerable excitement regarding its potential therapeutic role in all manners of lower urinary tract dysfunction, and especially pBOO.

NO is synthesized from L-arginine, by the family of enzymes known as nitric oxide synthase (NOS). NOS is currently known to exist in 3 separate isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Both eNOS and nNOS are normally present in cells, and are referred to as constitutive, while iNOS is only present after induction by inflammatory mediators.

After production, NO is able to freely diffuse to its target tissues, where it binds to the heme moiety of guanylyl cyclase. This stimulates the production of cGMP from GTP, which results in a further cascade to induce smooth muscle relaxation. Protein kinase $A_2$ and $G$ are responsible for the phosphorylation of actin-myosin complexes and the
activation of Ca2+ channels, resulting in a reduction of cytosolic Ca 2+, and smooth muscle relaxation\textsuperscript{144}.

The half-life of NO is extremely short, measured in seconds, as it is inactivated by oxygen to form NO2, and in solution forms the inactive nitrite (NO2-) and nitrate (NO3-)\textsuperscript{145}. However, the physiologic effects occurring downstream of NO must also be inactivated, and this occurs by an ever expanding list of phosphodiesterase inhibitors (PDE). The PDEs are responsible for de-phosphorylating cAMP and cGMP, thus removing the stimulus for relaxation. To date, over 11 families and 50 isoenzymes have been described and several have achieved clinical application\textsuperscript{138}. PDE inhibitors are currently used in erectile dysfunction and pulmonary hypertension, and further work is being done examining cardiac remodeling and bladder overactivity\textsuperscript{146, 147}.

Nitric oxide is currently felt to be a prominent inhibitory factor in the lower urinary tract\textsuperscript{148, 149}. Both animal and human LUTs have demonstrated both nNOS and iNOS in smooth muscle, but conclusive evidence regarding its local production is lacking\textsuperscript{150}. Its role in normal bladder function is not completely understood, but may play a role in the active relaxation of the detrusor during filling\textsuperscript{151}, an essential property for reducing bladder pressure and maintaining urinary continence and renal health. The intracellular increase in cGMP may potentiate the effects of other mediators, which has lead some authors to postulate that the role of NO may be more modulatory in nature\textsuperscript{150}.

Inflammatory stimuli, such as lipopolysaccharides or cytokines, result in a definite increase in iNOS activity\textsuperscript{140, 152}. However, even more pertinent is the finding that iNOS is up-regulated after pure pBOO\textsuperscript{153}. This has been characterized with an increase in cDNA and protein levels observed as soon as 1 week after obstruction, implying a fundamental role in the early response to pBOO\textsuperscript{153}. NO has also demonstrated a role in the inhibition of smooth muscle hypertrophy\textsuperscript{154-156}. Felsen et al. have also shown that iNOS expression was up-regulated in obstruction induced ischemia, and that its inhibition reduced both contractility and fibrosis\textsuperscript{154}. This makes it an ideal target for investigation and potential intervention, although early efforts have not been successful, likely due to an incomplete understanding of its role\textsuperscript{150}. Further work implies that increasing tissue levels of NO may decrease the pro-inflammatory and pro-fibrotic effects of TGF-\textgreek{p}\textsuperscript{157}. PDE5 inhibition has been shown prevented smooth muscle hypertrophy, a result often attributed to TGF-\textgreek{p}\textsuperscript{147}. The mechanisms of this effect are still under investigation.

1.3.1.1.2. TGF-\textgreek{p}
TGF-β has been long been recognized as having a prominent role in the pathophysiology of pBOO\(^{158-160}\). Its biology is complex and is subject to significant molecular regulation; the increased mRNA synthesis certainly implies an up-regulation of its inflammatory cascade.

Transforming growth factor beta (TGF-β) is clearly a master regulator of the immune system, but its mechanism of action is far from understood. A highly pleiotropic cytokine, it exists in three isoforms in mammals (TGF-β1, TGF-β2, TGF-β3)\(^{161}\) and is a key member of a genetic superfamily involved with diverse developmental and physiologic roles\(^{162}\). TGF-β is stored in an inactive form, covalently bound to a latency-activated peptide (LAP). Once activated through a variety of potential mechanisms, it binds to a heterodimeric receptor complex, consisting of a type I (5 different type I receptors identified) and a type II (7 different type II receptors identified) complex\(^{162}\).

Intracellular signal transduction occurs via Smad proteins, where phosphorylation allows nuclear translocation and binds to the Smad binding element (SBE)\(^{163, 164}\). TGF-β also results in stimulation of the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (Erk), and the Jun-N-terminal kinase (JNK) pathways\(^{165}\).

TGF-β has been shown to effect many cellular functions, including differentiation, proliferation, migration, and survival\(^{166}\). It has been shown to have a prominent role in wound healing, angiogenesis, and cancer\(^{167}\). However, its actions are clearly context dependent, and it has been shown to have opposing effects in several scenarios.

Fibrosis is often perceived as the end-stage or final result of severe inflammation, and TGF-β has been shown to play a prominent role. The primary cell in pathological fibrosis is the myofibroblast, a cell derived from epithelial cells by a recently discovered process known as epithelial mesenchymal differentiation (EMT)\(^{168}\). It has been shown that TGF-β is one of the primary driving forces behind EMT\(^{169}\), and therefore a fundamental aspect of the fibrotic, end-stage result of the inflammatory process.

Connective tissue growth factor (CTGF) is often seen as mirroring the effects of TGF-β, as over-expression of TGF-β results in increased secretion in fibrotic conditions\(^ {170, 171}\). It is an ECM-associated protein secreted by fibroblasts. It has been shown to regulate elements of extra-cellular matrix (ECM) production, cell migration, proliferation, adhesion, and differentiation\(^ {172}\). CTGF has also been shown to be an early event in response to stretch\(^ {173}\) and hypoxia\(^ {174}\), as both have been shown to result in bladder fibrosis\(^ {175, 176}\).
with increased collagen and extracellular matrix deposition\textsuperscript{177}. Its persistent expression in skin has been implicated in chronic fibrosis\textsuperscript{178}. Furthermore, in chronic fibrotic conditions, increased CTGF secretion is seen in response to stimulation with TGF-β\textsuperscript{179}. CTGF inhibition will not prevent fibrosis, but it is ameliorated\textsuperscript{180}.

### 1.3.1.1.3. HIF-1α

Bladder outlet obstruction has been shown to induce hypoxia\textsuperscript{181}, and in turn hypoxia has been shown to increase HIF-1α and PDGF\textsuperscript{174, 182}. HIF-1α is a heterodimer ubiquitous among oxygen dependent species and deletion results in perinatal death. The α unit is rapidly degraded in normoxic conditions, as its specific proteosome requires oxygen as a co-substrate. Hypoxia results in the inactivation of the proteosome and decreased degradation of the alpha subunit allowing it to dimerize with the constitutively expressed β unit. The heterodimer is then an active transcription factor with several target genes\textsuperscript{183}. HIF-1α results in the up-regulation of many genes, including vascular endothelial growth factor (VEGF) and PDGF\textsuperscript{184}. Adam et al. further demonstrated that mechanical stretch in-vitro will selectively up-regulate PDGF\textsuperscript{185}, and this may relate to hypertrophy\textsuperscript{186}. Akbal used hydrostatic pressures of 20-40 cm H\textsubscript{2}O on bladder smooth muscle and fibroblast cell cultures to demonstrate an increase in PDGF\textsuperscript{187}. Huang et al. proved a relationship between HIF-1α and CTGF, as HIF-1α deficient mice were not able to produce CTGF after hypoxia\textsuperscript{174}.

A great deal of contemporary knowledge of HIF-1α stems from a hereditary form of renal cell carcinoma, von Hippel Lindau (VHL) syndrome. This autosomal recessive trait led to the identification of a VHL tumor suppressor gene, which stabilizes HIF-1α. The loss of this inhibition results in tumorigenesis and metastasis. Furthermore, it has resulted in the discovery and development of novel therapeutics that have shown promise in disrupting the pathway\textsuperscript{188}.

### 1.3.1.2. Extracellular Matrix

Although our comprehension of smooth muscle physiology and pathophysiology has expanded extremely rapidly, it is unable to completely explain detrusor decompensation. Because the unique viscoelastic property of the bladder wall relies on both the smooth muscle and the extracellular matrix (ECM), the latter has also come under extensive investigation. The ECM is a remarkably active and vital component in nearly all organ systems. It has been found to play a fundamental role in cell
proliferation, migration, and differentiation\(^{189}\). The initiation and propagation of signals resulting in these changes is under intense study in several organ systems, including cardiac, vascular, bone, cartilage, lung, and bladder. Specifically, its role in remodeling secondary to stretch and pressure is referred to as “mechanobiology” and the signaling “mechanotransduction”\(^{190}\).

The ECM is composed of structural and functional proteins, including collagen glycoproteins, and elastin. The ECM confers much of the integral properties of the tissue: mechanical strength, structural support, and attachment for cellular receptors. Specifically for the bladder, and pBOO, it is responsible for the viscoelastic properties and compliance and is host to multiple signaling molecules. The major component of the bladder’s ECM is collagen, with types I and III predominating in normal bladders. With pBOO and the resulting fibrosis, there is a general accumulation of ECM proteins, a decrease in the ration of collagen I to III ratio, increased growth factor activity, an increase in growth factor and matrix receptors, and an increase in proteolytic enzymes\(^{142, 191-194}\).

A second vital component of the bladder ECM is the vast reservoir of growth factors and cytokines\(^{195}\). TGF-β, CTGF, numerous interleukins, insulin-like growth factor, platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and many others are all present and linked to the pathophysiology of pBOO\(^{196}\). Degradation of collagen and other individual components of the ECM is the responsibility of the family of matrix metalloproteinases (MMP). MMPs are fundamental to physiologic and pathophysiologic tissue remodeling, with multiple isoforms categorized into 5 families based on substrate specificity\(^{197}\). Growth factors and mediators interact, as MMPs have been reported to activate growth factors and cytokines\(^{197}\), and therefore are felt to play a fundamental and regulatory role in ECM remodeling\(^{198}\). Tissue homeostasis is also maintained with tissue inhibitors of metalloproteinases (TIMP), which are endogenous inhibitors of proteolysis.

1.3.2. Animal Models

One of the primary difficulties in studying and translating this knowledge is its reliance upon in-vitro methodology. Potentially confounding variables include; the fact that the extracellular response is very dependant on the type of stretch the tissue culture is subjected to. Most of the aforementioned experiments use bladder smooth muscle cells that are cultured on a deformable membrane. This is then subjected one of many possible stretch / stress scenarios, including a cyclic stretch, which may be sinusoidal or wave, or a static stretch\(^{72, 199}\). The type of stretch may result in different cellular and
extracellular responses, so it is difficult to compare results\textsuperscript{72, 199}. However, there is no consensus as to which most accurately replicates in-vivo hydrodynamics, which highlights the need for a whole animal model of pBOO. Furthermore this is even more important when considering assessment of therapeutic interventions before human application.

1.3.3. Regenerative Medicine / Tissue Engineering

Due to our incomplete understanding of the pathophysiology and the current inability to inhibit progression, our best preventative efforts still occasionally result in an end-stage bladder. The gold standard, to achieve either continence or preserve renal function, is bladder augmentation. The intestinal tract provides the best means of increasing compliance and preventing uninhibited contractions. However, an intestinocystoplasty is a major surgical intervention with potential significant morbidity. Therefore, alternatives are sought, and at the forefront of these is augmentation with bio-engineered materials. The ideal bladder construct is able to reproduce the fundamental characteristics of the bladder: an impermeable barrier with the ability to store increasing amounts of urine at a low pressure and empty completely when desired. Despite the enthusiasm in the lay-press from Atala's report on human application\textsuperscript{200}, most bladder augmentation in humans using tissue engineering methods is being carried out at a preliminary level with significant hurdles left to overcome.

1.3.3.1. Unseeded Scaffolds

Current techniques are based upon dissolvable scaffolds, whereby normal urothelium is directed to infiltrate and the scaffold is completely replaced with a natural process of regeneration. Many different scaffolds have been studied, the more popular of which include: small intestinal submucosa (SIS), polyglycolic acid (PGA), and bladder acellular matrices (ACM). These may be either produced in the local laboratory, or attained via several commercial sources. However, successful incorporation requires rapid revascularization to support cell survival, healthy ECM, and the regeneration of a healthy bladder wall. Vascular and cellular in-growth is facilitated by a porous membrane, but, the graft must be capable of providing a watertight barrier to urine. This dichotomy, combined with the poor preliminary results, has prompted the use of seeded scaffolds.

1.3.3.2. Seeded Scaffolds

The porous nature of the scaffolds, lends itself well to the potential to be “seeded” with cellular components, growth factors, or living cells. This potential has
heralded the development of tissue engineering, and is fundamental to its progress as a bladder replacement. Hyaluronic acid (HA) was incorporated into an ACM because of its potential to aid in wound healing. It has been shown to moderate inflammation, and promote fibroblast and endothelial cell migration\textsuperscript{201}, and in-vitro, its addition to the ACM decreased its permeability\textsuperscript{202}. HA also offers potential to be a carrier for other molecules, which has enormous potential for additional therapies. For example, Cartwright et al. fortified their HA with VEGF, and this was successful in improving vascularity in-vitro\textsuperscript{203}.

Another approach has been to seed the scaffold with living cells, because despite successful development of a urothelial layer, the muscular and ECM layers have not been as effective\textsuperscript{204, 205}. Various cell types have been examined with advantages and disadvantages to each. The patients own bladder cells are readily available and can be incorporated into a matrix, but these are inherently abnormal resulting in physical characteristics which are less than ideal\textsuperscript{204}. Smooth muscle cells can be harvested from normal bladders, and their application have had some degree of success\textsuperscript{205, 206}. Co-cultures with urothelial cells have been reported to improve tissue characteristics, presumably secondary to cell to cell interactions\textsuperscript{207}. In an attempt to use a pleuripotential cell, capable of differentiation along several cell lines, bone marrow stromal cells have been used, and they have differentiated into normal smooth muscle cells, biologically similar to those in the bladder\textsuperscript{208, 209}. These cells have advantages over embryonic stem cells, as well as adult stem cells due to their availability, and concerns over ensuring proper direction of differentiation\textsuperscript{208, 210}. Although progress is being made, all of these studies acknowledge the need to further understand the impediments to successful regeneration and the promotion of healthy tissue development.

1.4 Formulating the Research Problem

Due to both the quantity and quality of research being performed in this field, the clinical potential is very exciting. We need to ensure that the work continues to progress and clinical application is achieved for the benefit of our patients. The translation of bench research to clinical application is a fundamental component, and there are several avenues readily available to exploit. The fundamental aspect required, however, is an appropriate model, which allows us to accurately elicit pathways and assess interventions. Despite their theoretical and in-vitro potential, it is inappropriate to consider human application without a carefully designed and documented mammalian model. This need is further magnified by the rapid development of tissue engineering, as
its efficacy and safety needs to be fully assured in a diseased animal model before it can be accepted for human use.

At present translational studies are hampered by the absence of a reproducible, inexpensive, yet effective animal model that has been well characterized for its response to pBOO. Although many investigators have used models to examine molecular pathways and clinical feasibility, few have progressed to assess therapeutic interventions. We submit that a comprehensive model needs to be developed in a readily accessible animal model, so that comprehensive study can occur. Furthermore, quantification of damage needs to be performed in a manner that replicates the human clinical situation, with physiologic parameters such as urodynamics being equated to bladder and renal pathology. This will then allow us to assess the effectiveness of translatable therapeutic interventions. Data will be generated regarding the efficacy of TGF-β and HIF-1α inhibition, which is interpretable and applicable to clinical scenario.

Progression of regenerative medicine techniques requires significant safety and efficacy evaluations in stringent and clinically applicable models. Most current models have only examined results in-vitro or in healthy bladders. Limited data in diseased models, demonstrates the increased difficulty in regenerating healthy tissue from a pathologic milieu\(^{206,211}\). Because these studies have been performed in large animals, their numbers are limited. This prevents the detailed assessment of experimental variation and therefore, the needed intricate details required to optimize results. This can be overcome with a small animal model, which will facilitate the advancement of both medical prevention and the application of regenerative medicine, as both of these fields need intense scrutiny to ensure that we are able to deliver the most effective care possible to our patients.

Inhibition of the pathophysiologic decompensation has to be a priority, and requires an effective animal model to determine its true benefit. Contemporary management is targeted at minimizing end-organ effects by rather crude means. Ensuring the bladder is not overfilled via CIC and minimizing gross bladder contractions has been effective, but carries significant morbidity. By exploiting cell messengers, such as NO, we may be better able to prevent muscle hypertrophy and instability. Recently, very effective NO agonists have flooded the pharmacologic marketplace in the form of phosphodiesterase inhibitors (PDE). Originally conceived for pulmonary hypertension, their efficacy in relaxing vascular smooth muscle has propelled them to notoriety in the treatment of erectile dysfunction. Recent developments have progressed in other organ systems, and
we are encouraged that these well characterized, safe, and effective oral medications can be used on the bladder to decrease smooth muscle hypertrophy and decompensation.

Likewise the inhibition of the hypoxia-induced damage could be a significant advancement in treating pBOO. Rapamycin is a commonly used in whole organ transplantation\textsuperscript{212} and is being explored for the treatment of metastatic renal cell carcinoma\textsuperscript{213}. There is also some evidence that it may prevent renal fibrosis after ureteric obstruction\textsuperscript{214}. This effect has been attributed to its anti-inflammatory and immune suppressive nature. Therefore, the exploration and characterization of this pathway in a clinically relevant animal model readily translates into pharmacologic inhibition. Furthermore, a well-characterized animal model will easily allow for its assessment in prevention of bladder fibrosis secondary to pBOO\textsuperscript{214}.

Ideally, tissue engineering requires improved scaffolds that are degradable, porous enough to allow development of normal tissue architecture, impermeable to urine, and able to deliver molecules and medications to further develop the aforementioned pathways. The successful incorporation of HA and VEGF herald the potential to deliver other molecules directly to the scaffold and infiltrating cells. Further work with cell lines, perhaps with genetic modifications, will result in an effective and controllable cell population that readily differentiates into bladder tissue with physiologically indistinguishable characteristics. If the PDE inhibitors are effective at reducing hypertrophy and inflammation, their addition as an oral agent could further potentiate results.

The potential for the prevention and improved treatment of pBOO is remarkable, and a great deal of work has been done. However, progress requires a comprehensive and reproducible animal model of pBOO to confirm safety and efficacy. From our proposed model, molecular pathways, medical interventions, and the application of tissue engineering can be accurately assessed and their potential fully elicited.


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Chapter 2: Experiments and Results

2.1 Introduction

Partial bladder outlet obstruction (pBOO) is a ubiquitous problem in the aging male\textsuperscript{1,2} and results in significant morbidity in the pediatric population\textsuperscript{3,4}. Prostatic hypertrophy can progress to overt urinary retention and renal insufficiency\textsuperscript{5}. Pediatric patients, that develop pBOO secondary to spina bifida and posterior urethral valves (PUV), are at risk for the development of significant renal injury, including vesicoureteral reflux, hydronephrosis and renal insufficiency\textsuperscript{3,6-8}. It has been established that the renal injury that occurs secondary to prolonged exposure to elevated bladder pressures\textsuperscript{3}; however, pathophysiologic mechanisms which result in these high pressure bladders are not fully understood. It has been well demonstrated that the progression to an end-stage bladder is the result of multiple complex, well-regulated biochemical pathways\textsuperscript{9-11}; however, these studies have often been performed in isolation, examining a single time point or an isolated mediator, which does not resemble the clinical scenario of a progressive deterioration from a healthy bladder. Therefore, a representative, comprehensive and clinically representative model would be invaluable to the advancement of knowledge and patient care.

It is postulated that the bladder passes through several distinct physiological phases en-route to decompensation. An initial phase of inflammation, with adaptive muscle hypertrophy and the ability to generate sufficient pressure to compensate for the increased pressures needed to void occurs across the increased urethral resistance, and finally a high pressure, poorly compliant bladder that has decompensated into a fibrotic organ develops. Therefore, any assessment of the pathophysiologic decompensation must analyze changes over several time points, as the bladder passes through distinct phases en route to this decompensated state. Delineating these phases and their progression over time may help improve clinical care and outcomes, as medical prevention can be more tailored to the particular pathophysiology of the patient’s bladder. Further elucidation of biochemical mechanisms could lead to novel therapies and decrease progression to renal damage.

Therefore, we have used a well described an animal model\textsuperscript{12-14} and compared the urodynamic, histologic and biochemical changes over several time periods after pBOO. It is important for our model to effectively replicate the clinical scenario, whereby
these changes occur insidiously and without significant acute symptoms or morbidity. Our observations, and those of others, demonstrate the presence of distinct phases along the path to bladder decompensation including an initial inflammatory response whose mediators initiate the subsequent effects, a compensatory response with muscle hypertrophy and the final phase which results in a fibrotic, end-stage bladder responsible for the significantly elevated bladder pressures. These features mimic the clinical situation, which places the patient at significant risk for an end-stage bladder and subsequent renal failure.

2.2 Methods

2.2.1 Animal Model of pBOO

The University of Alberta’s Animal Care and Use Committee approval was obtained for the research. Adult female rats underwent pBOO at a weight of 200 gms as described by Sutherland et al. After induction of isoflurane anesthesia the bladder was exposed via a midline incision and an 18-gauge catheter was advanced through to the urethra. The bladder neck region was dissected, ureters were identified and a right angle clamp was placed around the urethra. A 2-0 silk tie was then gently tied down to the tissue to ensure sufficient resistance without completely occluding the urethra. The catheter was then removed and the suprapubic incision closed with absorbable suture. Four separate experimental groups (2, 4, 8, and 13 weeks of pBOO) were compared to shams. Sham animals underwent the same procedure including, urodynamics, advancement of the angiocatheter, and dissection of the urethra, except that no suture was placed around the urethra.

2.2.2 Animal Health

To ensure that the animals remained healthy during the period of pBOO, the rats were food and water intake were monitored and the animals weighed prior to pBOO and at the end of the experiment as a general measure of overall health. At animal sacrifice, blood was drawn and sent for serum creatinine levels. Necropsy was performed, with the bladders and kidneys weighed and grossly inspected for hydronephrosis or bladder stones.

2.2.3 Urodynamic Measurements

Urodynamics were performed under anesthetic, both prior to the creation of pBOO and at animal sacrifice at 2, 4, 8 or 13 weeks. These were performed under anesthetic with the bladder exposed through a midline incision. An 18-gauge angiocatheter was placed into the dome of the bladder and secured. The bladder was
completely drained, and the catheter was connected to a Y tubing connector, with one port attached to a syringe pump, which infused a normal saline solution at 0.1 ml per minute. The second arm was attached to a pressure transducer. Pressures were zeroed to atmospheric pressure and then recorded at 15-second intervals once the infusion started.

The primary parameters recorded were maximal bladder capacity and detrusor pressure. Bladders were infused with normal saline solution at 0.1 ml per min and recording proceeded until bladder incontinence was observed when urine was expelled per urethra. Pressures were recorded throughout the urodynamics and the maximum detrusor pressure was defined as the maximum pressure recorded before urination. The animals were determined to have phasic contractility if they demonstrated a sustained amplitude variation of at least 10 cm H₂O.

2.2.4 Organ and Tissue Analysis

At the time of animal sacrifice, bladders were surgically removed above the level of the suture, opened and weighed after the removal of all urine, stones and adherent adipose tissue. The bladders were sectioned and either snap frozen in liquid nitrogen or submersed in 4% paraformaldehyde. Kidneys were grossly inspected for the presence of hydronephrosis, weighed, and then preserved as for the bladders.

Paraffin blocks created from the paraformaldehyde sample were 5µm sections were mounted on slides and stained with hematoxylin and eosin (H+E) or Masson's trichrome stain. Photomicrographs were taken using a digital camera (Nikon Coolpix 4500) mounted on a microscope (Nikon Optishot 2) using 20x optical magnification. All photographs were taken with identical lighting and with a micrometer to ensure comparability.

Mean bladder thickness was measured by sectioning a 0.5 x 0.5 cm sample of the posterior bladder wall. To minimize contraction, the bladder wall was placed in a microscopic cassette and held firmly in place with foam padding during fixation in paraformaldehyde. This effectively prevented tissue stretch or contraction that would influence thickness of the bladder wall. Histologic sections were viewed under 20x magnification with a micrometer. The bladder wall thickness was calculated by measuring the distance between the urothelium and the detrusor-fat junction in five randomly selected fields from each animal in every group and calculating the mean.

Immunohistochemistry
Immunohistochemistry was performed on frozen sections by embedding them in Shandon Cryomatrix™ (Thermo Electron Corp., Pittsburgh, PA) and 8 µm sections were cut, fixed in acetone, treated with hydrogen peroxide to quench endogenous peroxidase and blocked with corresponding serum from a secondary antibody for 1 hour. Slides were then incubated for 2 hours at room temperature with rat anti-mouse F4/80 monoclonal antibody (mAb) (5 µg/ml; eBioscience Inc., San Diego, CA), for identification of connective tissue growth factor (CTGF) and alpha-smooth muscle actin (α-SMA)(eBioscience Inc., San Diego, CA). Sections were then incubated with anti-F4/80 mAb and conjugated with horseradish peroxidase (Sigma, Saint Louis, MS) for 1 hour at room temperature. Signals on tissue were revealed using diaminobenzidine and hydrogen peroxide. Thereafter, counterstaining was performed with hemotoxylin.

2.2.5 Real-time reverse transcription–polymerase chain reaction

Total RNA was extracted from bladder samples using RNeasy™ spin columns (Qiagen, Mississauga, ON, Ca) according to the manufacturers’ recommendations. To eliminate contamination with genomic DNA, DNase digestion was performed for 60 minutes. First-strand cDNA was synthesized using an enhanced avian first strand synthesis kit (Sigma, Oakville, ON, Ca) at 42°C using 500 ng total RNA extract. Real-time reverse transcription–polymerase chain reaction (RT-PCR) was conducted using Power SYBR® Green PCR Master Mix (ABI, Foster, CA) in a 25 µL tube with a total reaction volume of 25 µL containing 1 µL of a 1:2 dilution of first-strand reaction product, 0.2 µM gene specific upstream and downstream primers. Amplification and analysis of cDNA fragments was carried out using a 7300 real time PCR system (ABI, Foster, CA). Cycling conditions were initial denaturation at 95°C for 3 minutes, followed by 40 cycles consisting of a 15-second denaturation interval at 95°C and a 30 second interval for annealing and primer extension at 60°C. Amplification of the housekeeping gene β-actin mRNA was used as to normalize the results. mRNA levels were measured as CT threshold levels and normalized with the individual β-actin control CT values. Altered mRNA levels in pBOO bladders are expressed as fold changes.

2.2.6 Hydroxyproline Analysis

Bladder collagen content was determined by liquid chromatography / mass spectrometric analysis of 4-hydroxyproline from the snap frozen samples. An internal standard (N-methyl-L-proline) and 6N HCl solution were added to pre-weighed, freeze dried bladder tissue and each sample was then hydrolyzed overnight at 115°C.
The O-butyl ester derivatives were prepared with 10% BF$_2$-butanol for 30 minutes at 120°C after drying the hydrolysate. Liquid chromatography (column: Eclipse XDB-C18)/mass spectrometry analysis was performed on a Hewlett-Packard (series 1100, Atlanta, GA) mass selective detector monitoring the ions at m/z 186 and 188. Results were expressed as µg hydroxyproline per mg of dry sample weight.

**Data presentation and calculation**

Graphs were constructed using mean values and error bars representing the standard error of the means. Parametric statistical analysis was performed with a 2 tailed, non-paired student t-test, when comparing pre to post pBOO results. ANOVA, with the SNK test, was used to compare results between groups of animals. Differences were considered significant if p<0.05.

2.3 Results

2.3.1 Animal Health

Rats included in the analysis remained healthy for the duration of the experiment. Animals were weighed prior to pBOO and at the end of the experiment. There were no significant differences between pre pBOO and post pBOO weights when comparing the experimental and control sham animals at the 4 and 12-week time points. The experimental animals gained weight at the same rate as controls (Figure 2-1). Serum creatinine was drawn at the end of the experiment and there was no significant increase in serum creatinine when compared to control animals (Figure 2-2). At necropsy, there was no gross evidence of hydronephrosis in any of the animals and kidney weights did not change over the course of the experiment and histology remained normal as compared to controls.

2.3.2 Urodynamic Outcomes

Urodynamic data were used to confirm the physiologic response to the pBOO as this directly correlated to outcomes seen in the clinical setting and confirms its relevance. Animals were tested before surgical induction of pBOO and after the allotted time periods. Therefore, comparisons were made to the animal's own baseline and between different durations of obstruction. The findings are as follows:

a) Total Bladder Capacity

Total bladder capacity initially increased with pBOO then returned to baseline values by 13 weeks of pBOO (figure 2). Bladder capacity did not significantly change from baseline in neither sham nor experimental group and was not different compared to
any of the pre-obstruction values. In the experimental group with pBOO, there was a significant increase over pre-obstruction volumes after 2, 4 and 8 weeks of pBOO (p<0.05), and at 8 weeks, capacity was significantly higher than the 4-week group (p<0.05). Thereafter, capacity subsequently decreased, so by 13-weeks of pBOO, the bladder capacity of the experimental animals were not significantly different from either the shams or their pre-pBOO volumes (Figure 3).

b) Maximal Bladder Pressure

Maximal bladder pressure also increased with pBOO, but this occurred later than the peak increase in bladder capacity and then remained significantly increased throughout the remaining experimental period (Figure 4). Although the bladder pressure was not increased after 2 weeks of pBOO, it was significantly greater than sham controls and pre-pBOO after 4, 8 and 13 weeks of obstruction (p<0.05). After 8 weeks of pBOO, peak bladder pressures were observed which were significantly higher than 2, 4 and 13 weeks. Although the bladder pressures declined after 8 weeks, they remained also higher than sham controls as well as the earlier time points of 2 and 4 weeks (p<0.05).

c) Compliance

Compliance of the bladder was calculated by dividing the maximum capacity by the maximal pressure. The compliance increased initially as compared to the shams (0.02 mls / cm H2O, range 0.0075-0.075 ml/cm H2O) to 2 weeks (0.04 mls / cm H2O, range 0.005-0.10 ml/cm H2O) before it gradually decreased to a minimum at 13 weeks (0.008 mls / cm H2O, range 0.002-0.014). However, these values did not reach statistical significance (Figure 5).

d) Volume / Pressure Curves

Representative curves were selected from sham, 4 week, and 13-week animals, which demonstrated increased capacity at 4 weeks and pressure at 13 weeks (Figure 6).

Phasic contractions were seen in 3/5 of the animals after 2 weeks of pBOO and in 3/6 after 4 weeks; however, in only 1/4 at 8 weeks and none after 13 weeks of pBOO.

2.3.3 Organ and Tissue Analysis

a) Gross Appearance

On gross examination, the bladders of the animals with pBOO demonstrated obvious visible morphological changes secondary to prolonged obstruction. Initially, after
two weeks of pBOO the bladders appeared very inflamed, and by four weeks the bladders were very distended and thin walled which progressed to become very small and contracted by 13-week of pBOO (Figure 7).

b) Bladder Mass and Thickness

The weight of the bladders in the experimental group increased after 4 weeks of pBOO and remained elevated for the course of the experiment. Whole bladder weights were significantly increased when compared to sham operated controls in the 2, 4 and 8-week groups (p<0.05). The 4-week pBOO bladders were significantly heavier than the 2-week bladders (p<0.05) (Figure 8).

The thickness of the bladder walls both grossly and as measured histologically using the micrometer increased to its maximum by 4 weeks of pBOO and gradually decreased thereafter. The 4-week specimens were significantly thicker than both sham groups, as well as the 8-week and 13 week pBOO groups (p<0.05) (Figure 8).

c) Tissue Histology

Microscopic sections were consistent with our hypothesis that the bladder progresses through several phases, including inflammation, muscle hypertrophy and fibrosis en route to a high pressure, end-stage bladder. After 2 weeks there was an inflammatory infiltrate obvious on H+E staining. By 4 weeks, there was an appreciable increase in muscle mass, while maintaining basic tissue architecture. At 8 weeks, the muscle was less apparent and there was an increase in connective tissue with a general loss of tissue architecture, which was pronounced by 13 weeks (Figure 9). Masson’s trichrome staining demonstrates mature collagen fibers, and several samples showed a near complete replacement of the bladder wall by 13 weeks (Figure 10).

d) Immunohistochemistry

The detection of inflammatory mediators was consistent with our hypothesis that the presence of CTGF is detected throughout the urothelium at 2 weeks, and seems to decrease thereafter (Figure 2-11). The detection of myofibroblasts is also time dependent, with a minimal detection of α-SMA in the sham groups, a significant presence seen at 8 weeks and a gradual increase to a dense collection in the sub-urothelial layer by 13 weeks (Figure 12).

2.3.4: Real Time RT-PCR
HIF-1α, PDGF, CTGF and TGF-β1 are cytokines which have been previously shown to play a prominent role in pBOO and fibrosis\textsuperscript{18-21}. In our pBOO model, an initial up-regulation of mRNA for each of the cytokines measured was seen in the inflammatory phase. A statistically significant (p<0.05) increase in the mRNA for collagen type I, CTGF and TGF-β occurred at two weeks, before a return to baseline levels (Figure 13).

HIF-1α mRNA also increased by 2 weeks, but did not reach statistical significance, compared to shams, until it peaked at 4 weeks, and remained significantly higher than shams through to 13 weeks (p<0.05) (Figure 14). PDGF-A mRNA was the only cytokine to decrease, where all values were statistically significantly lower than shams (p<0.05).

2.3.5 Hydroxyproline analysis

With the initiation of the pBOO, an initial decrease in the amount of collagen in the bladder wall occurred where significantly less hydroxyproline was measured in the 2, 4 and 8-week samples as compared to shams (p<0.01). By 8 weeks, collagen levels began to increase, until at 13 weeks, the amount of collagen in the bladder wall decreased and was no different from sham controls and 2-week samples, but remained significantly higher than the 4 and 8-week samples (p<0.05) (Figure 15).
2.4 Discussion

Partial bladder obstruction (pBOO) is a pervasive problem throughout urology, from the child with a neuropathic bladder or PUV to the adult male with prostatic hyperplasia or urethral stricture. Although a great deal of knowledge has been accrued, our current treatment regimes remain crude and can contribute to significant morbidity. This has forced a re-examination of the pathophysiology of pBOO, whereby recognizing that while the outlet resistance may have initiated the process, ultimately pBOO leads to permanent pathologic changes in the bladder wall. As of yet, current treatment is unable to reverse the molecular and biochemical processes, and thereby prevent the very high-pressure end-stage bladder.

Thus, a representative animal model would be invaluable to the further development of medical and surgical treatment and to advance the field of regenerative medicine. This current model demonstrates that it is very applicable to the clinical scenario urologists face. Despite the fact that the animals remained apparently healthy, clinically relevant measurements including urodynamics demonstrated marked pathologic changes associated with high-pressure bladders. Importantly, this model demonstrates progression of pathologic changes over time with a pBOO, a feature not described by previous models, which lead to a more thorough understanding of the pathology and potential corrective therapies.

These temporal relations can provide great insight into advancing medical treatment, as we can now further examine the effects of anticholinergics, or other novel therapies. This will allow us to manipulate multiple variables, such as timing of initiation and dosing schedules and accurately assess their effects. With respect to advancing the surgical treatments, a great deal of progress has been made in the field of tissue engineering, with respect to bladder substitution, but these grafts are usually placed into healthy bladders, with normal tissue architecture and blood supply. This is fundamentally different from what we would propose with our model, which much more closely resembles the clinical scenario. It is likely that end-stage bladder has lost its normal architecture and vasculature, which would be much less likely to integrate an engineered substrate. Therefore, progress in this field will likely require the implantation of grafts into a diseased animal model, such as described herein, that more closely resembles the human clinical scenario.

Our hypothesis is that the pathophysiology of pBOO involves several predictable phases. The bladder initially is able to compensate to the increased stress and pressure,
but the sustained pressure results in a decompensation phase. Our results are consistent with this, as the initial increase in bladder capacity seen at 2 weeks after pBOO apparently related to the inflammatory response, with an increase in cellularity histologically and the up-regulation of the inflammatory cytokine mRNA. It is likely that if earlier time points had been chosen such as 1, 2, or 7 days, the greater increase in inflammatory cytokines may have been observed. TGF-β and CTGF were selected for this study, as these have been well documented to play fundamental roles in fibrosis in many organ systems, and specifically the bladder.

TGF-β1 has been long been recognized as having a prominent role in the pathophysiology of pBOO. Although its biology is complex and its regulation occurs at many levels, the increased mRNA levels implies an up-regulation of this fibrogenic cytokine. CTGF has also been shown to be an early event in response to stretch and has been shown to result in bladder fibrosis, with increased collagen and extracellular matrix deposition. Although the effects of the increased collagen and extracellular matrix may not yet be seen until after prolonged obstruction, but it is likely up-regulated very early in the pathophysiology.

Previously, others have implicated HIF-1α and PDGF in fibrosis due to hypoxia, and it is likely that our model of bladder outlet obstruction induces hypoxia, as described by others in the past. PDGF comprises a family of growth factors, related to both fibrosis, with TGF-β, and hypoxia via the HIF-1α pathway. Adam et al. demonstrated that mechanical stretch in-vitro will selectively up-regulate PDGF-BB, leading to bladder hypertrophy. Akbal et al. used hydrostatic pressures of 20-40 cm H₂O on bladder smooth muscle and fibroblast cell cultures to demonstrate an increase in PDGF-BB. Huang et al established a relationship between HIF-1α and CTGF, as HIF-1α deficient mice were not able to produce CTGF after hypoxia. However, our animals did not show a significant increase in HIF-1α until 4 weeks after pBOO, which likely corresponds to the time required for bladder pressures to become sufficiently elevated before hypoxia develops. TGF-β1 has been shown to have no discernible effect on PDGF expression in cultured hair follicles, and only a minimal stimulatory effect in retinal cells, while TGF-β1 was shown to down-regulate PDGF-A in lung fibroblasts. PDGF-A expression may be biphasic in that initially a decrease occurs during the inflammatory period of pBOO, before the subsequent increase in HIF-1α results in its up-regulation. Since PDGF is secreted by myofibroblasts, the up-regulation may be to their increased proliferation.
The inflammatory cascade initially results in the bladder being able to increase its capacity while maintaining normal bladder pressures. However, the cytokines initiate smooth muscle hypertrophy, seen histologically associated with the proliferation of myofibroblasts. TGF-β also promotes the development of myofibroblasts\textsuperscript{40}, and its increased expression in this model likely accounts for the subsequent elevation in pressures at 4 and 8 weeks, as the bladder is becoming fibrotic as shown by the loss of phasic contractions seen in many of the animals at 2 weeks and 4 weeks, which was lost 13 weeks when no contractile activity was seen. The histologic features observed correspond to significant of muscle hypertrophy, with the preservation of the general bladder tissue architecture. These functional and histologic changes seen correspond with the increased thickness of the bladder wall and increased weight of the bladder itself, which are common markers for hypertrophy and loss of tissue compliance throughout the entire bladder\textsuperscript{34, 41, 42}.

The role of the myofibroblast in neuropathic bladders was first described by Wiseman \textit{et al}\textsuperscript{43} and its role in neuropathic bladders is actively being explored\textsuperscript{44, 45}. Increased sub-urothelial myofibroblasts, compared to controls, were found in patients with neuropathic bladders which failed conservative treatment\textsuperscript{44}. Our study confirms not only their presence in a high-pressure bladder, but also their recruitment with the progressive pBOO. It is widely believed that the pro-fibrotic cytokines TGF-β and CTGF have a prominent role in the differentiation and activation of myofibroblasts\textsuperscript{40, 46, 47}. Therefore, the initial inflammatory cascade and upregulation of TGF-β and CTGF are likely associated with the increase in sub-urothelial myofibroblasts seen at 8 and 13 weeks.

By 8 weeks of pBOO in this model, the bladders have begun to decompensate, with very high pressures and a significant decrease in capacity by 13 weeks. This likely occurs from progressive fibrosis leading to less contractility and more fibrotic, poorly compliant bladder. The increased in collagen synthesis in the bladder wall is consistent the marked increases in bladder pressures seen at 13 weeks. The initial decrease in collagen content is likely due to development muscle hypertrophy early after pBOO, which decreases the relative proportion of collagen in the tissue. As the bladder decompenses, the collagen content increases to its maximum at 13 weeks as seen histologically where pathologic deposition of collagen leads to the loss of normal bladder architecture. This likely also accounts for the increase in thickness, as the hypertrophied muscle is replaced by fibrosis.
This model appears to be the first to demonstrate the progression through discreet phases of inflammation, followed by a compensated, muscular hypertrophy phase, before a final decompensated, fibrotic phase develops. Different therapeutic strategies may be required for the different phases of pBOO. Early treatment must be aggressive and tailored to preventing the inflammatory cascade, and focused on preventing muscle hypertrophy and contractility. Once pressures become elevated, then treatment is tailored at reducing contractility and preventing deterioration to fibrosis. Treatment of the final phase, therefore, needs to focus on fibrosis, as there is minimal normal detrusor tissue left. However, the proliferation of myofibroblasts may provide another biological target. Our model also further encourages the early and aggressive use of current regimes such as clean intermittent catheterization and anticholinergics in the hopes of minimizing the inciting inflammatory cascade and preventing the pathologic progression from increased intravesical pressure to fibrosis.
2.5 Conclusions

Based on our results we feel that this model effectively demonstrates the fundamental changes to the bladder that occur over time with a pBOO in a clinically relevant manner. Furthermore, it correlates changes to findings at a tissue and organ level and highlights discreet phases. This programmed and predictable progression certainly is translatable into an increased number of therapeutic options. For example, medical prevention may be more effective if specifically targeted at inflammation and muscle hypertrophy initially, versus prevention or reversal of fibrosis during later stages of the disease process. Furthermore, this effective and reproducible model will allow for precise testing of novel medical therapies, including tissue replacement strategies.

Finally, this model can be used to examine changes that occur on a molecular and cellular level. We hypothesize that the inflammatory and fibrotic pathways will vary according to the duration of pBOO and this will be invaluable information for further development of prevention and therapeutic strategies.
2.6 Figures

**Figure 1:** The animals maintained healthy weights throughout the experiment, gaining weight at the same rate as control animals. Animals obstructed for 2 weeks trended towards weight-loss, implying significant physiologic stress, but this was not statistically significant.
Figure 2: Serum creatinine levels were drawn at the end of the experimental period. There were no statistical differences between the groups.
Figure 3. Urodynamic bladder capacity, comparing the pre to post pBOO values and between groups of animals. Animals had significantly higher capacity than both sham groups and their pre-pBOO capacity after 2, 4 and 8 weeks of pBOO. Capacity at 13 weeks was not different from shams or pre-operative levels. Maximal capacity was seen at 8 weeks, which was also significantly higher than the capacity seen at 4 weeks (p<0.05). All values represent the means with the standard error.
Figure 4. Maximal bladder pressures, comparing pre to post-pBOO values and between groups. Bladder pressures were significantly higher than both shams and pre pBOO levels at 4, 8 and 13 weeks. Mean pressure at 13 weeks exceeded levels recorded at 4 weeks, while the 8 week animals had pressures significantly higher than those seen at 2, 4 and 13 weeks. All values represent the means with the standard error.
Figure 5: Compliance was calculated by dividing bladder volume by the pressure. Although there were no significant differences calculated, an initial improvement (compensation) and subsequent deterioration (decompensation) is apparent.
Figure 6: Plotting the change in pressure vs. bladder volume gives an excellent representation of the changes seen in the obstructed bladders and the difference with prolonged observation.
Figure 7: Digital photography demonstrates the obvious gross changes occurring in the bladder. The inflamed appearance progressed to larger volumes at 4 weeks, and finally demonstrated a small capacity bladder at 13 weeks. “Ruler” is a 1 ml tuberculin syringe with increments of 0.1 ml corresponding to 5 mm.

Legend: a) Sham b) 2 weeks pBOO at 20x; c) 4 weeks; d) 8 weeks; e) 13 weeks.
Figure 8. pBOO bladders were significantly heavier at 2, 4 and 8 weeks than shams, but not at 13 weeks (p<0.05). However, bladder wall thickness was not significantly different from shams until 4 weeks, and then the 8 and 13 weeks bladders reverted back to a thickness not significantly different from shams. This is consistent with significant muscle hypertrophy at 4 weeks resulting in an increase in mass and thickness, and the progression to fibrosis manifested by a thinner and lighter bladder.
**Figure 9.** H&E staining is consistent with an initial inflammatory response at 2 weeks, muscle hypertrophy at 4 weeks, then a progressive increase in fibrosis and loss of normal tissue architecture through 8 and 13 weeks. All photographs are at 20x magnification, except c, which was photographed at 100x to demonstrate the increased cellularity and inflammation. Legend: a) sham; b) 2 weeks; c) 2 weeks pBOO at high magnification; d) 4 weeks; e) 8 weeks; f) 13 weeks.
Masson’s trichrome staining more aptly demonstrates the progressive increase in collagen deposition. All photographs are at 20x magnification. Legend: a) Sham b) 2 weeks pBOO at 20x; c) 4 weeks; d) 8 weeks; e) 13 weeks. This is consistent with a gradual, but definite increase in collagen deposition that nearly replaces the entire bladder wall by 13 weeks.
**Figure 11.** IHC for CTGF demonstrate time-dependent changes in the pro-fibrotic mediator, with a maximum at 2 weeks and a gradual decrease to sham levels seen in the fibrotic tissue at 13 weeks. Legend: a) sham, b) 2 weeks, c) 4 weeks, d) 8 weeks, e) 13 weeks.
Figure 12. IHC for α-SMA demonstrates an increase in sub-urothelial myofibroblasts with duration of pBOO. With prolonged exposure to pBOO, we see progression from minimal detection in the shams and 2-week samples, to a significant presence in the 13-week samples, correlating to the increase in bladder pressures on urodynamics. Legend: a) Sham b) 2 weeks pBOO at 20x; c) 4 weeks; d) 8 weeks; e) 13 weeks.
Figure 13. Early up-regulation of mRNA, with significantly increased levels of CTGF, TGF-β1 and collagen seen in the first 2 weeks of pBOO and a decrease to baseline levels with prolonged pBOO. This is fundamental to our hypothesis of an initial inflammatory cascade resulting in the deleterious down-stream changes resulting in the end-stage bladder. All values represent the means with the standard error.
Figure 14: RT-PCR for HIF-1α and PDGF-A demonstrate reciprocal activity. HIF-1α increases significantly above shams whereby PDGF-A shows a significant decrease in mRNA production compared to shams. However, levels of PDGF-A do trend back to baseline, which may be a result of the increased HIF-1α.
Figure 15. Mass spectrometry for hydroxyproline shows a significant decrease in the amount of tissue collagen by 2 weeks, with the 2, 4, and 8-week samples significantly lower than shams. The 13-week samples were significantly higher than 4 and 8-week samples, and back to the level of the sham bladders. All values represent the means with the standard error.
Abbreviations

pBOO Partial bladder outlet obstruction
PUV Posterior urethral valves
RT-PCR Real-time reverse transcription-polymerase chain reaction
TGF-β Transforming growth factor Beta
CTGF Connective tissue growth factor
α-sma Alpha smooth muscle actin
HIF-1α Hypoxia inducible factor alpha
PDGF Platelet derived growth factor
mAb Monoclonal antibody
IHC Immunohistochemistry
H+E Hematoxylin and eosin
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Chapter 3: Future Direction

3.1 Future Direction:

The treatment of patients with neuropathic bladders is progressing rapidly, with novel medical applications and surgical treatments\(^1\). Although significant advances have been made with excellent in-vitro studies, progress to clinical application is limited by in-vivo testing. Therefore, our animal model represents a very clinically relevant and reproducible vehicle for assessing development. A controlled and reproducible model will allow us to determine the efficacy of varying doses and dosing schedules and assess the effects on the prevention of an end-stage bladders and the prevention of fibrosis. Furthermore, it provides an ideal means to test the development of synthetic grafts provided by the emerging field of regenerative medicine.

Recent work, including our own, has focused on multiple other molecular pathways to prevent detrusor overactivity and neuropathic bladders. These include MAPK\(^2\), NO\(^3, 4\), PDGF\(^5, 6\), HIF1\(\alpha\)^\(6, 7\), and TGF-\(\beta\)^\(8-11\). Although selective agents have been used to inhibit their activity in-vitro, a lack of a clinically relevant animal model has inhibited their translation into human practice. This model readily identifies pro-fibrotic pathways involving TGF-\(\beta\), CTGF, and HIF-1\(\alpha\) and provides an ideal means of observing the effect of new oral agents.

Despite the significant progress achieved in the lab, the translation to human practice has numerous significant obstacles to overcome\(^12\). The normal bladder tissue must be to store urine safely and empty completely upon command. Therefore, the bio-engineered tissue must be watertight, capable of significant expansion while maintaining a low-pressure environment, and capable of a coordinated and forceful contraction. This requires the development and integration of: the urothelium, a viscoelastic connective matrix, and smooth muscle cells.

Many investigators have been successful at developing a tissue scaffold and some of aforementioned elements, but have yet to replicate the normal conditions. Atala et al. published results from the human application of their scaffolds in 2006\(^13\), but the results have been controversial and have yet to be replicated\(^14, 15\). Most of the have shown promising in-vitro results, or have been implanted into healthy animals with normal bladders. However, this has significant limitations, as contemporary clinical indications for bladder augmentation result in avascular and high-pressure bladders.
Perhaps the most exciting direction involves the use of multi potential stem cells, which promise the ability to develop normal tissue. Much of the contemporary work involves manipulating the cytokine milieu to induce differentiation of the cells to normal detrusor. However, this also results in a significant clinical concern, as many of the signals capable of inducing differentiation are also many of the signals that become dysregulated with malignant differentiation.

Therefore, a diseased animal model is required to truly assess both novel medical preventative strategies using both established medications and novel therapeutics. We will expose groups of animals to standard dose oxybutinin and high dose oxybutinin to determine if we can prevent the muscle hypertrophy, elevated bladder pressures and subsequent fibrosis. Furthermore, as early intervention has also been reported to prevent bladder deterioration, we will also look at the effect of initiating therapy at the time of pBOO vs. after 4 weeks, when the pressures and contractility have been documented to increase.

Our model also offers an excellent opportunity to explore the use of novel therapeutics. Recently, there have been several publications regarding PDE-5 inhibitors, used for erectile dysfunction, who have reported improved lower urinary tract function\textsuperscript{16-18}. This is supported with scientific evidence that the increase in nitric oxide ameliorates the effects of TGF-β. Our previous work highlighted an inflammatory response to the bladder with induction of pBOO and upregulation of TGF-β, both of which contribute to eventual bladder decompensation and fibrosis. Therefore, we plan on testing the effect of a long-acting PDE-5 inhibitor (tadalafil) on outcomes after pBOO.

Another potential preventative medication is rapamycin, a powerful inhibitor of the mTOR pathway. Although originally used for its immunosuppressive effects in transplant medicine, it has recently gained popularity as an anti-cancer agent and anti-fibrotic agent due to its inhibition of the HIF-1α pathway. Therefore, we wish to explore a novel means of preventing fibrotic changes after pBOO, as our previous work suggests a prominent role for HIF-1α in the latter stages of pBOO. We will assess the effect of standard rodent doses of rapamycin after prolonged pBOO.

Our hypothesis is that a more aggressive approach to therapy (early and higher doses) will prevent further deterioration. Also, more selective inhibition will demonstrate effective prevention of fibrosis.
Our model also allows us to further explore the use of bio-engineered grafts in a diseased animal model system. We will be able to compare the efficacy of different grafts (e.g. seeded vs. unseeded) and assess for differences if grafts are implanted early in the disease process vs. later, into fibrotic and avascular environment. We hypothesize that the grafts seeded with urothelial tissue will have more “normal” properties. Furthermore, grafts implanted soon after pBOO will have more normal physiologic and histologic characteristics compared to implantation into end-stage bladders.

3.2 Summary

Our animal model has great potential to advance the medical treatment of neuropathic bladders as well as improve surgical outcomes by advancing the field of tissue engineering. The ability to test novel medications and strategies is required to complement and expand upon our rapidly expanding knowledge of pathways and mediators. Physiologic measurements with long-term outcomes are required for clinical relevance and will help promote clinical application and maximize results. It may also prevent further exploration of ineffective therapy.

Furthermore, the very young field of regenerative medicine has shown great promise with respect to bladder reconstruction, but a great deal more work need to occur to improve results and our understanding. Our model provides an ideal means to evaluate different grafts and allow for relevant short and long-term assessments.


