Regenerative medicine based applications to combat stress urinary incontinence

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Abstract

Stress urinary incontinence (SUI), as an isolated symptom, is not a life threatening condition. However, the fear of unexpected urine leakage contributes to a significant decline in quality of life parameters for afflicted patients. Compared to other forms of incontinence, SUI cannot be easily treated with pharmacotherapy since it is inherently an anatomic problem. Treatment options include the use of bio-injectable materials to enhance closing pressures, and the placement of slings to bolster fascial support to the urethra. However, histologic findings of degeneration in the incontinent urethral sphincter invite the use of tissues engineering strategies to regenerate structures that aid in promoting continence. In this review, we will assess the role of stem cells in restoring multiple anatomic and physiological aspects of the sphincter. In particular, mesenchymal stem cells and CD34+ cells have shown great promise to differentiate into muscular and vascular components, respectively. Evidence supporting the use of cytokines and growth factors such as hypoxia-inducible factor 1-alpha, vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor and insulin-like growth factor further enhance the viability and direction of differentiation. Bridging the benefits of stem cells and growth factors involves the use of synthetic scaffolds like poly (1,8-octanediol-co-citrate) (POC) thin films. POC scaffolds are synthetic, elastomeric polymers that serve as substrates for cell growth, and upon degradation, release growth factors to the microenvironment in a controlled, predictable fashion. The combination of cellular, cytokine and scaffold elements aims to address the pathologic deficits to urinary incontinence, with a goal to improve patient symptoms and overall quality of life.

Core tip: Stress urinary incontinence is a condition which affects millions of women on a world-wide basis. Current surgical strategies to alleviate the symptoms involved with this condition are temporary stop-gap measures. With the advent of tissue engineering strategies in combination with stem cells, the reality of creating a functional replacement for anatomic structures involved in stress urinary incontinence can be a reality.

INTRODUCTION

Symptoms of the lower urinary tract in women, such as urinary incontinence, account for a significant number of

outpatient consultations to urogynecologists[1]. Several risk factors have been identified to explain the onset and persistence of this condition, particularly since the prevalence of urinary incontinence among adult women is approximately 16%-30%[2,3]. The occurrence of urinary incontinence is closely correlated with rising age[4], along with obesity through increased pressure on the pelvic floor, diabetes mellitus causing microvascular and neuropathic changes, and prior pelvic surgeries for the fascial, muscular and nerve stress it imparts. Among younger women, pregnancy and subsequent vaginal delivery leads to postpartum incontinence in up to 28% of the population. Bladder outlet compression, pelvic floor strain that elongates the pudendal nerve, and a prolonged second stage of labor all contribute towards urinary leakage[5-7]. Less often, patients with a chronic cough or those with fascial weakness secondary to a defect in collagen metabolism may have incontinence as well[8]. In adult men, direct injury to the neurovascular bundle and fascial planes during radical prostatectomy is a leading cause of incontinence[9].

A Scandinavian study reports that as many as 64% of women with urinary incontinence issues do not consult their primary care physician for a diagnostic work up of their symptoms[10]. A similar study in the United States (US) observed a rate of about 45%[11]. The prevalence of urinary incontinence, therefore, could be much higher than documented. Though not life-threatening, patients endure a dramatic decline to their quality of life. A survey across Europe and the US documented this subjective aspect, showing that confidence, self-perception, levels of physical activity and social engagement were negatively impacted by incontinence[12-14]. Not surprisingly, reports show a higher prevalence of anxiety and depression among these patients as well[15,16]. For the elderly in nursing homes, uncontrollable passage of urine irritates the perineal skin, causing dermatitis and discomfort that is difficult to alleviate[17].

Urinary incontinence is a condition in which there is involuntary leakage of urine that can be attributed to a number of differing factors. Depending on the presentation and history, incontinence is divided into several groups. Stress urinary incontinence (SUI) is an involuntary loss of urine that occurs with increased abdominal exertion, exemplified during coughing, sneezing, laughing or lifting. If the bladder is retaining urine in excessive amounts, small increases to abdominal pressure will produce an unwanted leak. Yet this symptom can still occur within the normal range of bladder fullness, such as in defects to the urethra or the intrinsic sphincter in retaining urine. A laxity of supportive structures to the pelvic floor and bladder means that increased pressures cannot be counteracted. Urge urinary incontinence presents with frequency causing low volume output, urgency and nocturia. The leakage of urine is typically accompanied by or preceded by a sense of urgency. A combination of these two types is described as mixed urinary incontinence, where increased abdominal pressures cause urinary leak-

age and concomitant urgency. Overactive bladder is characterized by urgency, with or without incontinence. Less common voiding abnormalities include conditions such as nocturnal enuresis and continuous urinary incontinence. An acute onset of urinary incontinence may suggest an underlying neurologic degenerative disease or malignancy, and should therefore be considered in the differential diagnosis[18-20].

Though the development of urinary incontinence is multifactorial in nature[21], all variations of disease in SUI ultimately manifest as either urethral hypermobility or urethral sphincter dysfunction. The effects of aging are particularly well described to demonstrate this. Increasing age leads to urethral musculature degeneration and neurologic injury[22]. The number of striated muscle fibers diminishes with histologic thinning noted at the proximal vesicle neck (supported by the U-shaped detrusor) and at the dorsal wall of the urethra (encircled by striated sphincter muscles). In addition, an age-related weakening of the endopelvic fascia reduces the support to the urethra, causing an inability to maintain the physiologic 90-120 degree vesicourethral angle required to maintain continence[23]. What results is termed urethral hypermobility. The endopelvic fascial layer is a dense, fibrous connective tissue layer surrounding the vagina, with attachments to the arcus tendinous fascia, the pubic bone ventrally, and the ischial spine dorsally[23]. Degenerative changes diminish the ability of the urethra to generate pressure against stress. As an example, a staccato cough creates a 150 cmH$_2$O increase in abdominal pressure, which the urethra must counteract during non-micturation times. A more compliant fascial layer threatens to lose continence during such transient pressures changes.

Initiating therapy for SUI requires the identification and understanding of the functional components comprising the urinary sphincter. The nerve supply to the lower urinary tract consists of three important innervations (Figure 1). First, pelvic nerves (S2-S4) provide parasympathetic innervations to the detrusor muscle and urethral smooth muscle sphincter. As an excitatory motor efferent nerve, the pelvic nerves initiate and coordinate micturition by contracting the detrusor and relaxing the sphincter. Second, hypogastric nerves (T2-L3) are sympathetic innervations to the trigone, bladder neck internal sphincter and detrusor muscle. These nerves allow for urinary retention and inhibit detrusor activity. Lastly, the pudendal nerves (S2-S4) of the sacral plexus innervate the external urethral sphincter (EUS) and striated muscles of the pelvic floor. The EUS consists of circular striated "slow twitch" fibers that sustain long periods of contraction, and pelvic basin muscles like the pubococcygeus contains slow and fast twitch fibers for reflex contraction during a cough or sneeze. The pudendal nerve arises from Onuf's nucleus, traverses Alcock's canal and enters the ischiorectal fossa to innervate the EUS[6]. This trajectory places the nerve between the sacrospinous and sacrotuberous ligaments, which makes it vulnerable to compression and injury.
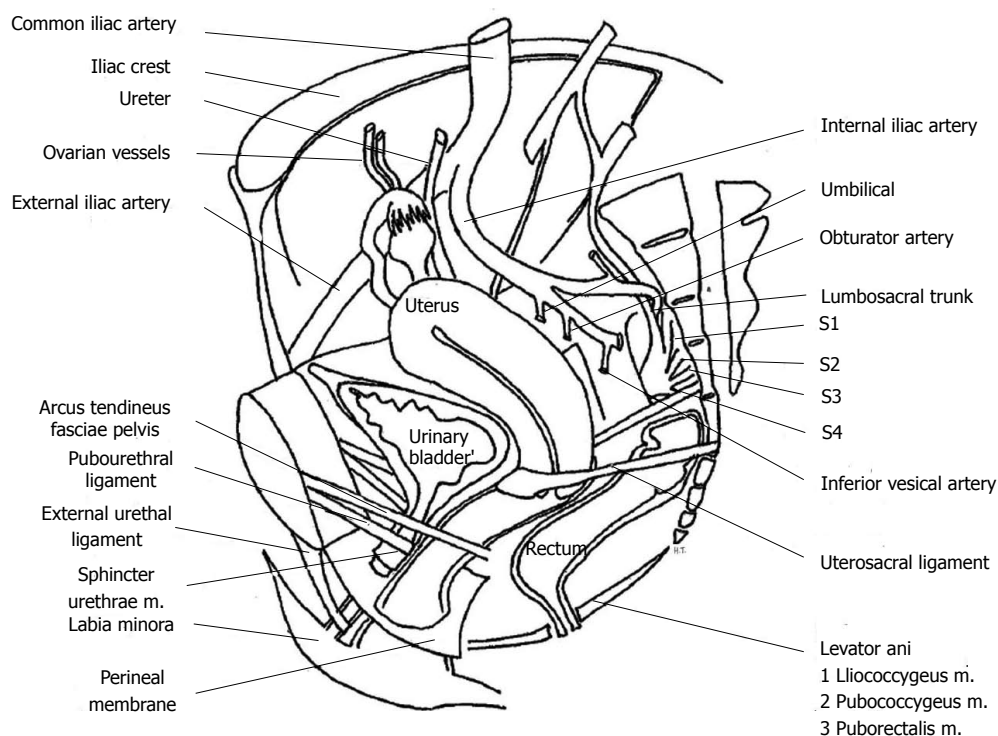
Figure 1 Sagittal section of female pelvis illustrating the position of the sphincter urethrae muscle in relation to adjacent structures and associated neurovascular components. Adapted from reference [21].

MEDICAL AND SURGICAL MANAGEMENT OF SUI

Conservative management of SUI involves lifestyle and behavioral changes, weight loss[24], bladder training[25], Kegel exercises to strengthen the pelvic floor and pharmacotherapy to improve urethral muscle tone[26-28]. Unlike other forms of urinary incontinence, there is a dearth of pharmacologic options that are effective for SUI. Alpha-adrenergic receptors at the bladder neck and urethra can be targeted with pseudoepherine and ephedrine to stimulate smooth muscle contraction[27,29]. Though this use may theoretically be suited to improve symptoms, the cardiovascular side effect profile precludes many patients from this therapy[30]. Imipramine, a tricyclic antidepressant with strong anti-cholinergic effects, also promotes urethral sphincter closure and urinary retention. This drug, however, poses a significant risk of orthostatic hypotension, which is often contraindicated in the elderly population. Lately, the use of duloxetine outside of the US has shown some promise, though the mechanism of action hardly addresses the underlying cause[31,32]. Patients with SUI and concomitant urge symptoms have more therapeutic options, including anti-muscarinic agents and oxybutynin[33].

Despite these initial efforts to gain continence, most of these patients are refractory to treatment and are invariably referred for surgical intervention. The goals of surgery are to reduce the number of episodes of incontinence each day or to reduce the volume of leaked urine, with the ultimate aim of achieving complete continence.

Surgery can either support the compression of the urethra, or augment coaptation to create a better seal. Pre-operative assessment of surgical candidates begins with a detailed a history with bladder diary recordings, and a physical, including a bimanual exam. A urinalysis to rule out infectious causes is indicated, followed by a cough stress test. In this test, the clinician visualizes the leakage of urine from a full bladder while the patient coughs. The volume of urine leak, collected on chux padding, may signify the degree of stress incontinence. Once the diagnosis of SUI is confirmed, most patients move on to urodynamic testing.

Urodynamic testing is expensive and subject to operator variability[34,35], but it provides two important measurements for the management of SUI. The first is leak point pressure (LPP). LPP measures the intravesical pressure at which urine leakage occurs when the patient is asked to strain or subjected to the Valsalva manuever. The patient must be careful to not induce a detrusor contraction. This is a measurement of intrinsic sphincter deficiency, and a LPP less than 60 cmH$_2$O is suggestive of SUI. The second measurement is the maximum urethral pressure generated, which is part of the urethral pressure profile. Profilometry also documents maximum urethral closure pressure, functional urethral length and the pressure transmission ratio. Maximum urethral pressures less than 20-30 mmHg are suggestive of SUI, and warrants the use of sling placement or periurethral bulking agent injections[36].

The surgical approach to SUI involves either placement of slings to support of the urethra, or injection of

biomaterials to increase urethral coaptation. Minimally invasive sling placement is currently the most common intervention for SUI[37]. Through a vaginal incision, a thin mesh of polypropylene thread material is positioned at the midurethral or at the bladder neck[38]. The sling behaves like an immobile floor, onto which the urethra can contract[39]. This strategy assumes the incontinent sphincter is indeed caused by urethral hypermobility, so supporting the plane of contraction would limit the angulation. Midurethral slings may be placed with either a retropubic or transobturator approach. A multicenter, randomized trial shows equivalent outcomes regardless of approach[40], though some studies still report better outcomes with a tension-free vaginal tape retropubic sling[41]. Newer interventions using a single incision sling or an adjustable sling have also shown promise[42].

Midurethral sling procedures have largely replaced the older Marshall-Marchetti-Krantz and Burch techniques of colposuspension[38,43]. Though these procedures have success rates of up to 88%[44], complications of urinary retention, de novo urgency, posterior vaginal wall prolapse and osteitis pubis have been documented[45]. In contrast, a Cochrane review of sling surgeries showed that the minimally invasive approach lessens operative times and decreases post-operative voiding dysfunction[38].

Sling materials have traditionally been sourced from autologous rectus fascia, fascia lata or small intestinal submucosa[46-48]. Cadaveric allografts from the dura, dermis or fascia lata have been shown to be inferior to autologous grafts in the long term based on the frequency of recurrent incontinence[49]. However, harvesting autologous fascial layers requires longer operative times, longer recovery times and more pain for the patient[50], despite evidence suggesting they are equivalent to synthetic slings[51]. For this reason, synthetic materials have become the mainstay in slings.

Sling placement, albeit a minimally invasive procedure, is not without risks and complications. Establishing an appropriate tension across the urethra is challenging, and can result in urinary retention or failure to alleviate symptoms. Both outcomes require release and adjustment of the sling, which is another surgical procedure. Other risks include bladder or urethral laceration, perforation and urinary tract infections (UTI)[52]. Occasionally, these issues are secondary to erosion of the mesh through soft tissue. These patients suffer from vaginal discharge, post-coital spotting, dyspareunia, and frequent UTIs. Despite these potential complications, the majority of patients do achieve freedom from incontinence, and self-reported improvements to sexual activity[53,54], and reductions in coital incontinence[55].

Injecting biomaterials to augment the urethral mucosa has become an alternative to urethral slings. Bulking agents have the greatest benefit for intrinsic sphincter defects with a LPP of less than 60 cmH2O. Regardless of where the injection is placed, studies have shown equivalent success with biomaterials in the periurethral, transurethral, midurethral and proximal neck areas[56,57]. The differences then lie in the biomaterial composition.

APPLICATIONS OF TISSUE ENGINEERING TO SUI

Tissue engineering the urethral sling

Current surgical standards use non-antigenic synthetic materials for slings. Over the past few years, studies have investigated the potential role of stem cells in SUI treatment. In a study by Zou *et al*[58], acellular silk slings were tested against slings seeded with autologous bone marrow derived mesenchymal stem cells (MSCs) on rats with bilateral sciatic nerve transection. MSCs were isolated from bone marrow aspirates using flow cytometry against CD34, CD44 and CD105 cell surface epitopes. Sciatic nerve transection, among other methods, is a means to produce genuine SUI pathology[59]. At 12 wk post-implantation, MSC/silk slings had double the collagen fiber formation of silk slings alone, evidenced by a higher Young's modulus (4.468 ± 0.510 MPa) and higher failure force (2.436 ± 0.192 N) as compared to silk slings alone. The mean Young's modulus of silk slings alone was 3.045 ± 0.388 MPa, with a failure force of 1.521 ± 0.087 N. The collagen formation improved sling integration with the native urethral tissue. However, both MSCs/silk and silk alone constructs performed equally in increasing the LPP (MSCs/silk at 36.3 ± 3.1 cmH2O *vs* silk alone at 38.0 ± 3.3 cmH2O). Nonetheless, this study demonstrates that the introduction of MSCs into the urethral environment does not cause any significant inflammation, scarring or adverse effects. Other scaffolds may be better suited in lieu of silk. It is important to note that even though a stem cell seeded construct could improve integration of slings into the urethra, the operative risks remain the same as that for current sling placements. Additionally, there is no evidence demonstrating that cellular slings have a decreased risk of mesh erosion over commercially available slings.

Tissue engineering the urethral sphincter

Attribution of SUI to intrinsic sphincter degeneration poses a challenging problem from a therapeutic standpoint. Sphincter degeneration involves the loss of multiple functional tissue types. Efforts to recreate the function of urethral sphincters are best demonstrated through artificial fluid-filled cuffs encircling the urethra. Artificial urinary sphincters have three components: a cuff of 4.5 cm in size, a reservoir with 61-70 cmH2O to mimic urethral pressures, and a pump to permit inflation and deflation controlled by the patient. Sphincters are most commonly placed at the bulbar urethra in men who suffer from post-prostatectomy SUI[9]. Though theoretically purposeful, artificial sphincters are associated with a multitude of complications. Acutely, urethral edema produces pain and discomfort for the patient. Chronically, patients experience atrophy and erosion of the sphincter resulting in irritative voiding symptoms, perineal pain and hematuria[60]. There are presently no controlled trials showing an improvement to symptoms using an artificial device over conventional therapy[61]. As such, we consider the employment of stem cells and tissue engineering

Table 1 Periurethral stem/progenitor cell injections improving leak point and urethral closure pressures in various studies

Study	Stem/progenitor cell source	SUI model	LPP findings in cmH2O at 4 wk status post injection	Absolute difference in LPP in cmH2O and percent improvement
Lee *et al*[117]	Allogenic gastrocnemius MDSC	Rat, sciatic nerve transection	MDSC = 44.1 ± 6.6 Saline = 18.6 ± 5.2 Control (-) = 25.8 ± 2.5	MDSC = 18.3 ± 9.1, 70.93%
Kwon *et al*[67]	Muscle derived cells and fibroblasts	Rats, sciatic nerve transection	MDSC = 38.2 ± 4.3 Fibroblast = 38.8 ± 1.2 MDSC/fibroblast = 34.5 ± 3.3 Control (-) = 25.8 ± 1.4 Control (+) = 43.3 ± 2.5	MDSC = 12.4 ± 5.7, 48.06% Fibroblast = 13.0 ± 2.6, 50.38% MDSC/Fibroblast = 8.7 ± 4.7, 33.72%
Fu *et al*[118]	Adipose derived stem cells induced into myoblasts with 5-azacytidine	Rats, vaginal balloon dilation	Myoblast = 32.43 ± 2.05 ADSCs = 30.75 ± 3.17 Control (-) = 36.19 ± 2.25 Control (+) = 45.42 ± 1.71	Myoblast = -3.76 ± 4.3, -10.38% ADSC = -5.44 ± 5.42, -15.03%
Kinebuchi *et al*[69]	Bone marrow derived mesenchymal stem cells	Rats, urethrolysis and cardiotoxic injection	BMSC = 25.66 ± 4.38 Control (-) = 18.19 ± 1.55 Control (+) = 35.98 ± 5.14	BMSC = 7.47 ± 5.93, 41.06%
Lim *et al*[119]	Human umbilical cord blood mononuclear cells	Rats, electrocauterization of periurethral soft tissue	HUCS = 91.75 ± 18.99 Control (-) = 65.02 ± 22.09	HUCS = 26.76 ± 41.08, 41.16%
Kim *et al*[120]	Allogenic mesenchymal stem cells	Rats, pudendal nerve transection	MSC = 43.1 ± 3.2 Control (-) = 22.0 ± 2.2 Control (+) = 29.1 ± 2.1	MSC: 21.1 ± 5.4, 95.90%
Zhao *et al*[93]	Autologous adipose derived stem cells + NGF	Rats, pudendal nerve transection	ADSC/PLGA = 17.8 ± 3.1 ADSC/NGF = 18.3 ± 2.4 ADSC/PLGA/NGF = 22.5 ± 6.1 Control (-) = 11.6 ± 2.7	ADSC/PLGA = 6.2 ± 5.8, 53.44% ADSC/NGF = 6.7 ± 5.1, 57.75% ADSC/PLGA/NGF = 10.9 ± 8.8, 93.96%
Corcos *et al*[72]	Bone marrow derived mesenchymal stem cells	Rats, pudendal nerve transection	MSC = 24.28 ± 1.47 Control (-) = 16.21 ± 1.26	MSC = 8.07 ± 2.73, 49.78%
Watanabe *et al*[121]	Adipose derived mesenchymal stem cells	Rats, pelvic nerve transection	Subtracted LPPs: Collagen = 9.39 ± 2.08 ASC = 8.86 ± 3.13 Control (-) = 10.94 ± 3.55	
Kim *et al*[122]	Human amniotic fluid stem cells	Rats, pudendal nerve transection	hAFCs = 20.2 ± 3.3 Control (-) = 15.2 ± 3.1 Control (+) = 27.6 ± 3.6	hAFCs = 5.0 ± 6.4, 32.89%
Li *et al*[123]	Adipose derived stem cells	Rats, vaginal balloon dilation	ADSC = 46.34 ± 2.63 Control (-) = 36.82 ± 1.68 Control (+) = 48.92 ± 2.71	ADSC = 9.52 ± 4.31, 25.85%
Chun *et al*[66]	Human amniotic fluid stem cells	Rats, pudendal nerve transection	hAFCs = 23.9 ± 1.85 Muscle progenitors = 38.43 ± 0.51 Control (-) = 15.24 ± 1.87 Control (+) = 36.54 ± 1.67	hAFCs = 8.66 ± 3.72, 56.82% Muscle progenitors = 23.19 ± 2.38, 152.16%
Lecoeur *et al*[124]	Myofiber core of satellite cells	Pigs, endoscopic destruction of striated urethral sphincter	Myofiber = 71.5 ± 17.8 Curarization sphincter = 33.5 ± 14.8	
Mitterberger *et al*[125]	Autologous myoblasts and fibroblasts	Humans (*n* = 123)	UCP = 28.8 ± 12.3 Post-operative UCP = 40.5 ± 15.8 after 1 year	11.7 ± 28.1, 40.62%
Mitterberger *et al*[126]	Autologous myoblasts and fibroblasts	Humans (*n* = 20)	Pre-operative UCP = 27.0 ± 13.3 Post-operative UCP (1 yr) = 39.4 ± 14.8 Post-operative UCP (2 yr) = 42.2 ± 12.1	66.4 ± 28.1, 45.92% (1 yr) 15.2 ± 25.4, 56.29%

SUI: Stress urinary incontinence; LPP: Leak point pressure; MDSC: Muscle derived stem cells; NGF: Nerve growth factor; ADSCs: Adipose derived stem cells; BMSC: Bone marrow stem cells; HUCS: Human umbilical cord serum; PLGA: Poly (lactic-co-glycolic acid); hAFCs: Human amniotic fluid cells; UCP: Pre-operative urethral closure pressures; MSC: Mesenchymal stem cell; ASC: Adipose stem cells.

techniques to reconstruct the urethral sphincter.

Several studies have established a foundation of infusing stem cells directly into the urethral sphincter. Preparations of MSCs, autologous progenitor muscle cells[62], adipose cells[63], processed lipoaspirate[64,65], human amniotic stem cells[66] and fibroblasts[67] have all been used with variable results[62,68,69] to bolster smooth muscle regeneration and to improve LPPs and urethral closure pressures (Table 1). Few studies have assessed the role of stem cells for the subset of male patients with SUI from prostate-related surgery. In one study, transurethral injections of autologous muscle derived fibroblasts and myoblasts produced complete continence in 65% of the 63 participants, quantified by a pre-operative LPP of 46.3 ± 17.1 cmH2O, and a post-operative LPP of 68.2 ± 24.3 cmH2O[70]. Another study using a similar approach

reported improvements to merely 12% of 222 male patients, with no improvements in 46%[71]. While both studies showed that stem cell implantation is a safe procedure in eligible patients, the results do not show a clear benefit as seen in trials with women and SUI.

Using MSCs seems to show the greatest promise, as MSCs have displayed the potential to regenerate both muscle and ganglion components in the sphincter. Corcos *et al*[72] demonstrated in an animal model that injecting BMSCs into denervated urethral sphincters improved LPPs to almost normal, non-SUI levels. This result is argued to be due to the differentiation of MSCs into striated muscle within the urethral microenvironment[72]. Though this evidence is merely histologic, and not in an improvement to symptoms for patients, the concept of creating a functional contractile tissue in the sphincter is worthy of further development.

MSC use in tissue engineering has become a prominent strategy in a multitude of fields, including urogynecology[73-79]. MSCs express cell surface markers CD29, CD44, CD105, CD166, and are negative for hematopoietic markers such as CD14, CD34, CD40 and CD45[80]. MSCs are also negative for leukocyte common antigen CD45, suggesting that these stem cells escape lymphocyte detection, and thus avoid immune rejection[81,82]. Sourced from the bone marrow, MSCs can be easily isolated from other hematopoietic cells through flow cytometry. MSCs have the capacity to divide 24-40 times *in vitro*, allowing for multiple passages of expansion without losing their multipotent properties or differentiating spontaneously[83]. This advantage, described by Pittenger *et al*[84], permits the differentiation of MSCs by external forces, such as the microenvironment of target tissue itself. Coupled to this environment, MSCs display an immunomodulatory effect[85] that includes the secretion of cytokines to initiate and support tissue regeneration[86]. For these reasons, MSCs must, by minimal criteria, differentiate into osteoblasts, chondrocytes and adipocytes. Yet, the plasticity inherent in MSCs has pushed researchers to generate neural, cardiac, muscular and other soft tissue lineages. In the realm of urinary tract healing, MSCs are considered a prime candidate since their presence has great therapeutic potential with minimal complications[87,88].

Some centers are already offering stem cell injections into the urethra for patients[89-91]. However, it is unclear whether these cells serve a functional purpose in regenerating damaged sphincters, or whether the cells are merely a bulking agent not unlike injectable biomaterials. In addition, it is recognized that inflammation at the implant site diminishes the ability of injected cells to survive long enough to participate in regeneration[92]. If growth were not sustained over a 7-10 d period, the applications of MSCs would be significantly stunted. Providing a means to enhance cell viability *in vivo* could be achieved by introducing synthetic scaffolds and growth factors.

The harmony of using scaffolds, stem cells and growth factors together has shown promise in a number of tissue engineering projects. Zhao *et al*[93] harvested adipose derived stem cells (ADSCs) and seeded them onto poly (lactic-co-glycolic acid) (PLGA) microparticles containing nerve growth factor (NGF). As a synthetic scaffold, PLGA has been shown to be safe in the urinary tract[94,95]. With periurethral injection into mice, this combination improved the LPP to 22.5 ± 6.1 cmH2O over treatments lacking either the PLGA or NGF. This result was explained by NGF prolonging the survival of ADSCs, enhancing the urethral muscle area on histology, and increased the density of neurofilaments supporting the sphincter lamina propria. This is the first iconic study where tissue engineering directly addresses the pathology underlying intrinsic sphincter defects. We propose a similar approach, where scaffolds and MSCs are injected not into the periurethral space, but directly into the urethral sphincter.

Poly(1,8 octanediol-co-citrate) scaffolds to support urethral sphincter regeneration

The number and variety of polymers synthesized for tissue engineering is rapidly expanding. A popular selection for research is PLGA[96], possessing elastic properties that adapt well to dynamic soft tissue structures. A similar material, poly (1,8-octanediol-co-citrate) (POC), is used by our group for urologic tissue engineering efforts. First established by the Ameer Research Lab[97], POC thin film (POCf) scaffolds are a highly reproducible elastomeric material[98]. The POCf allows for cell growth, cell infiltration, and for unimpaired exchange of oxygen and nutrient delivery. During polymerization of the scaffold, several aspects of construction can be customized to mimic the compliance, elasticity, and tensile strength measured through Young's modulus. Equimolar amounts of citric acid and 1,8-octanediol are combined, melted and cooled to make a pre-polymer, and parameters such as temperature and time can be adjusted. Higher temperatures with short polymerization times produce dense films, while low temperatures and long polymerization times yield scaffolds that are less cross-linked. With these modifiable ester-bonding schemes, highly adaptable, labile and reproducible scaffolds can be created specifically for urinary tract tissue targets. Tailoring these parameters also reflects the degradation scheme of POCf, which degrades to nontoxic byproducts of CO_2 and H_2O *via* nonenzymatic hydrolysis.

Another feature of POC scaffolds, besides providing a highly conducive substrate for cell growth and proliferation, is the ability to deliver growth factors through a controlled release upon scaffold degradation[99]. During the polymerization of POC, small peptides including growth factors and cytokines may be chemically coupled to the scaffold and released upon surface erosion. POCf scaffolds modified with heparan sulfate to hold vascular endothelial growth factor (VEGF), fibroblast growth factor 2 and insulin-like growth factor were studied by Sharma *et al*[99] in a rat model. Heparan sulfate, a highly sulfated glycosaminoglycan, protects bound growth factors to prevent enzymatic degradation. Delivery of the

pro-angiogenic growth factors upon break down of the scaffold led to increased vascular growth *in vivo* as compared to controls. The difference in results demonstrates that using POCf for a protracted but focused delivery of growth factors improves tissue healing.

The versatility of POCfs used in consonance with MSCs and growth factors offers the basis to potentially correct for sphincter deficiencies. For pregnancy related SUI, one pathway has been identified to be upregulated in response to vaginal distention and subsequent tissue damage[100]. The pathway involves hypoxia inducible factor-1α, a transcription factor stabilized in hypoxic conditions to induce expression of VEGF. This marker of tissue injury, and the resulting drive for angiogenesis, could potentially home stem cells to the site of injury. Studies by Cruz *et al*[101] have shown that pelvic injury *via* vaginal distention is a sufficient nidus for MSC homing to the urethra and levator ani. This phenomenon is speculated to be through chemokine ligand-7. Adding these chemokines to the POC delivery system could therefore boost the response of MSCs in tissue regeneration, and recruit circulating progenitors as well[102,103]. Progenitors stationed in the tissue, such as intrinsic satellite cells, may also be recruited to striated muscle reconstruction[104]. Direct and strong evidence exists that VEGF promotes the growth of myoblasts and increases capillary growth to the regenerating tissue. Interestingly, VEGF was capable of advancing the growth of myoblasts sourced even from older mice, where cells have less capacity to proliferate into functional tissue[105]. Growth factors like basic fibroblast growth factor (bFGF), hepatocyte growth factor and insulin-like growth factor have also contributed to muscle regeneration[106]. Characterization studies have also outlined a multitude of paracrine factors secreted by MSCs that are anti-apoptotic, immunomodulatory, anti-fibrotic and pro-angiogenic[107]. As previously confirmed, VEGF from MSCs, along with IL-6, MCP-1 and extra-cellular matrix components, assist in supporting angiogenesis, laying down extracellular matrix, and preventing apoptosis secondary to hypoxia[108].

The significance of bioactive compounds in regeneration was further strengthened by Choi *et al*[109]. In this study, plasmid DNA encoding bFGF was injected into rat periurethral submucosa *via* a PLGA synthetic delivery system. Results indicated that the levels of SM α-actin were elevated due to the bFGF, corresponding to a proliferation of tightly packed smooth muscle. Furthermore, contraction studies, conducted through electrical stimulation, showed a marked elevation in contractile properties for pDNA transfected urethras. Normal, continent urethras generated a contraction force of 36.4 ± 2.5 tension/mg of tissue, which is not dramatically different from the measured 32.3 ± 1.5 tension/mg tissue generated in pDNA/PLGA treated incontinent mice.

Three important concepts come out of this study: (1) that a sustained release of pDNA expressing bFGF through PLGA proved beneficial to regeneration; (2) the use of pDNA ensures that regeneration continues beyond the half-life and denaturation of biogenic compounds; and (3) since neither MSCs nor any other cell lines were not utilized in the injection, the improvement to symptoms can be specifically attributed to bFGF.

Integrating POCfs with MSCs and cytokines addresses the muscular aspect of sphincter regeneration. But a more comprehensive approach pays attention to the vascular and neural components as well. Seeding POC with progenitor cells from the bone marrow alongside MSCs could complete these components. Recent insights into CD34+ hematopoietic stem cells (HSCs), harvested from the same bone marrow origin as MSCs, points to a promising adjunct to MSCS. HSCs express von Willebrand Factor, vascular endothelial-cadherin and Flk-1[110-112]. These markers, in addition to CD133 and CD34[113], allow HSCs to be distinguished from MSCs and other primitive cells[114]. Placement of CD34+ HSCs onto compatible POCfs improved neovascularization and reduced fibrosis when injected into the site of injury[115].

Angiogenesis in the diseased urethral sphincter is beneficial for two reasons. First, it will nourish the proliferation and regeneration of MSCs into muscular components. Second, the blood supply will contribute to the vascular plexus that surrounds the urethral smooth muscle lumen. This plexus, when perfused, helps forms a tight seal of the mucosal surfaces, just as muscle contraction would.

Addressing the need for neural components in tissue engineering has been challenging. One study from our own group demonstrated the ability of MSCs combined with CD34+ cells to form muscular, vascular and even neural tissue in a rat bladder augmentation model[115]. Stem/progenitor cells were seeded onto POC scaffolds prior to implantation, which yielded well-organized fascicles of smooth muscle supported by collagen. CD34+ cells contributed greatly to the levels and distribution of blood vessels in MSCs/CD34+/POC constructs. Novel to this study was the detection of peripheral nerve regeneration from the surrounding healthy tissue. Stained with neuronal specific antibodies β Ⅲ tubulin and synaptophysin, rat nerve bundles innervated the regenerated tissue significantly more in MSC/CD34+/POC grafts than in controls. The authors suggest that renewal of a blood supply to the area improves delivery of growth factors and cytokines promoting neuronal growth.

CONCLUSION

In the evolving field of tissue engineering, there has been an overwhelming trend towards therapy against the exact mechanism of disease causing SUI. Individual studies have lent credence to the importance of MSCs, CD34+ HSCs, scaffolds and growth factors in efforts to regenerate the urethral sphincter. A combination of these four components would create a plausible scenario in which to restore function in a structure as complex as the sphincter. Even with the advances in surgical slings, there still remains an inherent need to establish normal physiologi-

cal function. Paired with POC scaffolds, we exploit the vast potential MSCs to differentiate into muscle, and hematopoietic precursors to proliferate into blood vessels in the presence of cytokines and growth factors. The indices of LPP readings from pre-operative urodynamic studies can be correlated to different levels of POCf elasticities, suited for a specific patient. Immunohistochemical and calcium release assays would support the MSC contractile properties as muscle regenerates, and nicotinic receptors targeted by α-bungarotoxin would illustrate the presence of neuronal fibers[116].

At least one study has observed the restoration of skeletal muscle and ganglionic elements from MSC injection into the rat urethral sphincter. Conducted by Kinebuchi *et al*[69], this study is a step in the right direction. However, follow up results did not confirm any improvement to LPP when compared to a control of cell free medium injection. The authors attribute the finding to inflammatory changes and to an insufficient bone marrow stem cells volume. In spite of this, the fallbacks can perhaps be accounted for by the absence of scaffolds and growth factors. Applications of this system expand beyond the treatment of SUI in adult women. Foremost, children born with neurogenic bladders secondary to myelomeningocele often have coexistent sphincter dysfunction. Likewise, post-prostatectomy men occasionally complain of incontinence as well. Patients with multiple sclerosis may have S2-S4 damage, leading to neuromuscular degeneration from the loss of incoming sensory nerve impulses and outgoing motor signals. The concept of urinary incontinence is similar to that of vesicoureteral reflux, so tissue engineering strategies provide an additional avenue to explore alongside ureteral reimplantation. To improve the symptoms of lower urinary tract symptoms in these patients, MSCs, HSCs, POC, and growth factors may one day supplement current surgical tactics.

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