available at http://ajws.elsevier.com/tcmj

Tzu Chi Medical Journal

Review Article

Regenerative Therapy for Stress Urinary Incontinence

*Ron Jankowski[1], Ryan Pruchnic[1], David Wagner[1], Michael B. Chancellor[2]**

[1]*Cook MyoSite, Inc., Pittsburgh, PA, USA*
[2]*Department of Urology, William Beaumont Hospital, Royal Oak, MI, USA*

Article info

Article history:
Received: January 2, 2008
Revised: January 29, 2008
Accepted: February 1, 2008

Keywords:
Adipose
Muscle
Stem cells
Urethral sphincter
Urinary incontinence

Abstract

In anatomical and functional studies of the human and animal urethra, the middle urethral contained rhabdosphincter is critical for maintaining continence. Transplanted muscle and/or stem cells may have the ability to undergo self-renewal and multipotent differentiation, leading to sphincter regeneration. In addition, such cells may release, or be engineered to release, neurotrophins with subsequent paracrine recruitment of endogenous host cells to concomitantly promote a regenerative response of nerve-integrated muscle. Cell-based therapies include the use of autologous multipotent stem cells, such as the bone marrow stromal cells. However, harvesting bone marrow stromal stem cells is difficult, painful, and may yield low numbers of stem cells upon processing. In contrast, alternative autologous adult stem cells such as muscle-derived stem cells and adipose-derived stem cells can be easily obtained in large quantities and with minimal discomfort. We will review the neurophysiology of stress urinary incontinence (highlighting the importance of the middle urethra); current injectable cell sources for cystoscopic treatment; and the potential of muscle-derived cells. (*Tzu Chi Med J* 2008;20(3):169–176)

*Corresponding author. Department of Urology, William Beaumont Hospital, 438 Medical Office Building, 3535 West 13 Mile Road, Royal Oak, MI 48073, USA.
E-mail address: chancellormb@gmail.com

1. Introduction

There are over 200 million people worldwide with incontinence, a condition that is associated with social impact and a reduced quality of life (1,2). Stress urinary incontinence (SUI) has been reported as the most common type of urinary incontinence (3). Risk factors for developing SUI include increasing parity, age, and obesity (4). Injury during childbirth to pelvic floor musculature, connective tissue, and nervous structures appears to be the most important risk factor for urinary incontinence in later life (5–8).

SUI can be grouped into two major categories: urethral hypermobility and intrinsic sphincter deficiency (ISD) (9). Urethral hypermobility, or loss of bladder neck support, results in a lack of intra-abdominal pressure transmission to the proximal urethra. In contrast, ISD is characterized by malfunction of the urethral closure mechanism. However, the grouping of patients into dichotomous categories has not translated into diagnostic and therapeutic improvements (10). Most researchers now believe that SUI varies between the extremes of urethral hypermobility and ISD, with most patients having elements of both disorders (11).

The role of pharmacotherapy for SUI, including α-adrenoceptor agonists, has been disappointing (12). Duloxetine, a selective serotonin and norepinephrine reuptake inhibitor, is available in Europe but it was

not approved by the FDA in the United States for the indication of SUI [13]. The use of injectable bulking agents including polytetrafluoroethylene [14], bovine collagen [15], silicone particles [16], carbon beads [17], and autologous ear chondrocytes [18] has yielded short-term success in the treatment of SUI. However, use of bulking agents has resulted in chronic inflammatory reactions, foreign body giant cell responses, periurethral abscess, particle migration, erosion of the urinary bladder or the urethra, obstruction of the lower urinary tract with urinary retention, severe voiding dysfunction, and pulmonary embolism [19–22].

The potential of stem cell therapy for the regenerative repair of the deficient rhabdosphincter is currently at the forefront of incontinence research [23]. Overall, the aim of stem cell therapy is to replace, repair, or enhance the biological function of damaged tissue or organs. There are two general types of stem cells potentially useful for therapeutic treatment, embryonic stem cells (ESCs) and adult stem cells. Although theoretically appealing, the practical use of ESCs is limited due to problems of cell regulation and ethical considerations [24]. In contrast, adult stem cells have no significant ethical issues related to their use.

We envision that in the near future, treatment of SUI will involve a routine urologist or urogynecologist's office visit in which a muscle biopsy using a small caliber needle biopsy device is performed. The biopsy is preserved and shipped to a central approved stem cell facility for processing, where a number of muscle-derived cells (MDCs) are isolated, prepared, and stored. Within a period of weeks, the MDCs are shipped back to the doctor's office and, using a cystoscopy or spinal needle, the cells are injected into the patient's urethral sphincter under local anesthesia (Fig. 1).

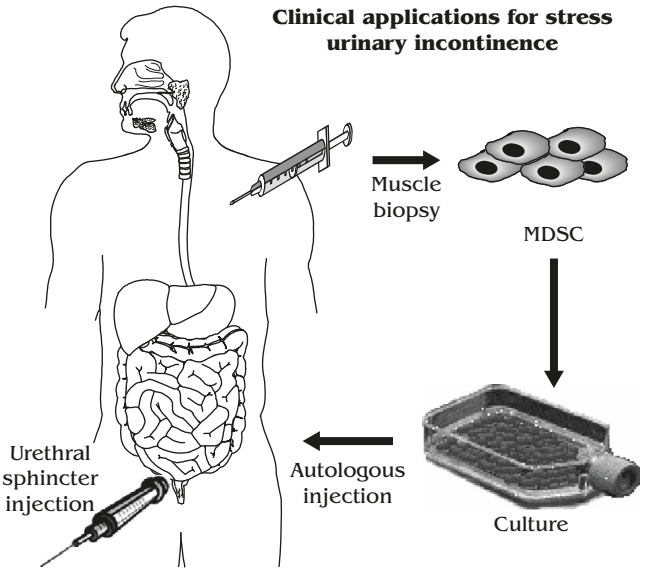

Fig. 1 — Diagram showing autologous stem cell injection therapy for stress urinary incontinence. Autologous stem cells are obtained with a biopsy of tissue, the cells are dissociated and expanded in culture, and the expanded cells are implanted into the same host. MDSCs=muscle-derived stem cells.

2. New concepts in SUI physiology

All of the urethral muscles, pelvic floor muscles, and surrounding connective tissues contribute to urethral resting tone. When intra-abdominal pressures are increased by coughing, sneezing, or exercise, the urethra is passively or actively closed to prevent urinary leakage. This passive urethral closure mechanism is well understood. Contraction of the pelvic floor muscles (levator ani muscles) pulls the vagina forward toward the pubic symphysis, creating a backstop for the urinary tract. This stable backstop compresses the two walls of the urethra, preventing urinary leakage during elevation of intra-abdominal pressures [25]. In addition, the position of the bladder neck is important for pressure transmission to the properly positioned structures during increased intra-abdominal pressure to remain equal. Descent of the structures causes a pressure gradient that can result in urinary leakage.

The active urethral closure mechanism that maintains urinary continence during elevation of intra-abdominal pressures is currently still under investigation. The external urethral sphincter (EUS), commonly referred to as the rhabdosphincter, is composed of both type I and type II striated muscle fibers located in the middle urethra [26]. In addition, urethral smooth muscles are deposited in longitudinal and circular layers. The urethral muscles are controlled by three sets of peripheral nerves: sacral parasympathetic nerves (pelvic nerves), thoracolumbar sympathetic nerves (hypogastric nerves), and sacral somatic nerves (pudendal nerves) (Fig. 2).

Sympathetic preganglionic pathways emerge from the thoracolumbar spinal cord, pass through the sympathetic chain ganglia (SCG), the inferior splanchnic nerves (ISN), and to the inferior mesenteric ganglia (IMG). Preganglionic and postganglionic sympathetic axons then travel through the hypogastric nerves to the pelvic plexus and the urogenital organs. Parasympathetic preganglionic axons that originate in the sacral spinal cord pass through the pelvic nerves to ganglion cells located in the pelvic plexus and then to distal ganglia in the target urogenital organs. Sacral somatic pathways are contained in the pudendal nerves, which provide innervation to the EUS. The pudendal and pelvic nerves also receive postganglionic axons from the caudal SCG. These three sets of nerves contain afferent axons from the lumbosacral dorsal root ganglia (DRG) [27].

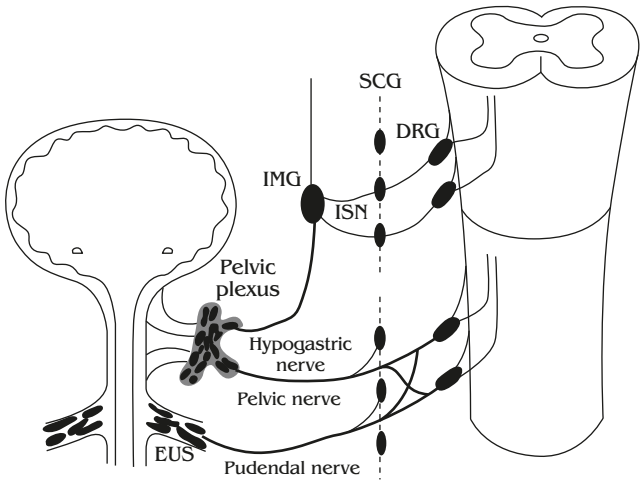

Fig. 2 — Diagram showing the sympathetic, parasympathetic, and somatic innervation of the urogenital tract. IMG=inferior mesenteric ganglia; SCG=sympathetic chain ganglia; DRG=dorsal root ganglia; ISN=inferior splanchnic nerves; EUS=external urethral sphincter.

Recent studies have reported that the EUS can be activated voluntarily or by reflex mechanism elicited by bladder distension [28]. Nerve tracts from the central nervous system terminate at Onuf's nucleus in the sacral spinal cord and synapse with the pudendal nerves. Serotonin and norepinephrine are two key neurotransmitters that stimulate the proximal end of the pudendal nerves to control EUS contraction [28–30].

Nerve-mediated active urethral closure mechanism that maintains urinary continence during elevation of abdominal pressures may be divided into two groups: central nervous control passing through Onuf's nucleus during sneezing or coughing [31,32], and the bladder-to-urethral spinal reflex during laughing, exercise, or lifting heavy objects [33,34].

In an elegant series of experiments, Kamo et al [31–34] demonstrated that an increase in middle urethral pressure during sneezing was caused not only by passive transmission of increased abdominal pressure but also by active reflex contractions of the EUS and pelvic floor muscles. In addition, they were able to show that this sneeze-induced continence reflex in the middle urethra is impaired in a rat SUI model induced by vaginal distension. In contrast, the increase in proximal and distal urethral pressure during sneezing was dependent upon increases in intravesicular and/or intra-abdominal pressure. Another key observation was that passive elevation of intravesicular pressure in spinal cord transected rats elicited both pelvic afferent nerve-mediated contractile reflexes and bladder-to-urethral spinal reflexes in the middle-to-proximal urethra mediated by the activation of pelvic nerves.

In anatomical reports, Strasser et al [35] showed that hypogastric and pelvic nerves predominantly innervated and regulated the proximal urethra, whereas stimulation of pudendal nerves led to contraction of the middle-to-distal urethra. In addition, a decrease in the number of striated muscle cells was reported in conjunction with an age-dependent increase of apoptosis of the striated muscle fibers of EUS [36]. These results suggest that the middle urethra and EUS are critical for maintaining continence and represent a primary focus in the management of SUI.

3. Stress incontinence cellular therapy

Cell-based therapies and tissue engineering are most often associated with the use of autologous multipotent stem cells. One commonly described source of such cells is the bone marrow stroma. The bone marrow compartment contains several cell populations including mesenchymal stem cells (MSCs) that are capable of differentiating into adipogenic [37], osteogenic [37], chondrogenic [38], and myogenic cells [39,40]. However, autologous bone marrow procurement has significant inherent limitations including painful procurement procedures that frequently require general or spinal anesthesia, and often yield a low number of MSCs upon processing [37]. As an alternative source of autologous adult stem cells, MDCs and adipose-derived stem cells (ADSCs) are advantageous because they can be easily obtained in large quantities under local anesthesia.

MDC therapy, often referred to as myoblast transfer therapy, has in the past been hindered by numerous limitations including poor survival of the injected cells. Selection of specific stem cells from the pool of remaining MDC populations, through the use of techniques such as pre-plating, has led to improved cell survival rates following transplantation [41]. Such observations have since led to extensive investigation into the developmental origins of skeletal muscle progenitor cells and the functional heterogeneity displayed by various skeletal MDC populations [42,43]. While difficult to identify *in vitro* through expression of specific "marker" proteins, which are often in flux or may be downregulated quickly following placement in culture, MDCs display a remarkable regenerative capacity when compared to the commonly recognized and abundant striated muscle precursor "myoblast" cells. The total regenerative response elicited through the transplantation of such cells, including their survival, engraftment, induction of host tissue repair, and ability to restore functionality is elegantly displayed in a recent report comparing the fate of MDCs and myoblasts in a myocardial infarct model [44]. Therefore, not all MDCs are created equal and care should be taken when considering and comparing reports originating from different laboratories.

In our ongoing research, MDCs display an improved transplantation capacity with the ability to undergo long-term proliferation, self-renewal, and multipotent differentiation, including differentiation toward endothelial and neuronal lineages [45,46]. MDC injection therapy offers several advantages over conventional treatments for SUI. The use of cells that are derived from the incontinent patient (autologous cell transplantation) will not cause an immunogenic or allergic reaction and therefore may persist longer than injected foreign substances such as collagen [47,48]. MDCs are uniquely different from fibroblasts and smooth muscle cells since MDCs will fuse to form post-mitotic multinucleated myotubes. This limits persistent expansion and risk of obstruction that may occur with other cell sources such as fibroblasts [49]. Finally, MDCs form myotubes and myofibers that become innervated into the host muscle. Therefore, not only can they serve as a bulking agent, but they are also physiologically capable of improving urethral sphincter function [50–52].

The feasibility of this concept was first demonstrated in rat models of SUI [23,53]. Chermansky et al [54] showed that MDCs had integrated within the striated muscle layer of the cauterized middle urethra 4 weeks after injection. In addition, the striated muscle layer of the MDC-injected urethra was contiguous with an increase in nervous tissue when compared to those of the cauterized urethra injected with only saline solution. These results suggest that MDCs may have the capacity for multipotent differentiation in the host urethral tissue or have the capacity to elicit a paracrine effect resulting in a more complete regenerative muscle-nerve healing response. In addition, the increase in leak point pressure (LPP) seen in the groups injected with MDCs was significant when compared with the saline-injected cauterized rats. Importantly, the difference in LPP both 4 and 6 weeks after MDSC injection was not significant when compared with the uncauterized control rats.

In a recent report, Kwon et al [49] compared MDCs and fibroblasts with regard to their potential for restoration of urethral function following injection. Using LPP for functional comparison, the short-term experiment revealed no significant difference between MDCs and fibroblasts, or a combination of both, when the cell dosage was equal across the groups. However, when the dosage was varied by two 10-fold increases, only a high dose of fibroblast injection led to urinary retention. Importantly, even high doses of MDCs did not result in such adverse events. These results suggest that fibroblasts may be producing a bulking effect, as evidenced by the increase in LPP, but may also make the tissue less compliant. It has been well documented that the volume and location of synthetic substance injections are extremely critical to achieve a low incidence of adverse events such as retention [55].

Studies supporting the potential of ADSCs are also emerging. Zuk et al [56] described the differentiation of ADSCs *in vitro* into adipogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors. In addition, ADSCs exhibited the functional ability to contract and relax in direct response to pharmacologic agents [57]. ADSCs may also represent an alternative stem cell source for the treatment of SUI [58]. Feasibility of ADSC use has been suggested through reports of improvements in LPP and urethral function in a rat model of SUI when animals were injected with ADSCs in conjunction with biodegradable microbeads as a carrier [59]. By providing a potential cost-effective source for genitourinary reconstruction, cell therapies using MDCs and ADSCs are emerging as a promising technology for the treatment of SUI.

4. *Ex vivo* delivery of trophic factors

The use of growth factor proteins to promote healing is severely hindered by the difficulty of ensuring their delivery to the injured site, their short biologic half-lives, and the rapid clearance of these molecules from the bloodstream [60]. Various growth factors appear to regulate skeletal myoblast proliferation and differentiation, play a role in different stages of muscle regeneration, and enhance the healing process [61]. It is intriguing that adult knockout mice expressing a neutralizing antibody against nerve growth factor (NGF) display a severely reduced muscle mass [62]. In addition to acting as a target derived factor for developing neurons, NGF has an autocrine effect on myoblast proliferation and fusion [63]. In fact, myoblasts express low-affinity p75 NGF receptors [64] and high-affinity tyrosine kinase A (trkA) NGF receptors [65]. Presence of receptor and exposure of myoblasts to NGF resulted in upregulation of antiapoptosis/prosurvival proteins.

This suggests that NGF mediates survival of myoblasts prior to differentiation and is important for muscle fiber development. Since Schwann cells can survive inside the intramuscular nerve trunks of denervated skeletal muscles for a 25-month period without axonal contact in rats [66], NGF release by transplanted MDCs may also promote axonal regeneration and functional recovery after nerve injury. It stands to reason that transplanted MDCs that have the capacity to undergo self-renewal and multipotent differentiation, as well as release growth factors such as NGF, may promote a more complete response due to both autocrine and paracrine effects, leading to both a muscle and integrated nerve regenerative response of donor transplanted and host cells.

In a number of tissues, the development and survival of sympathetic neurons are dependent on the presence of target-derived neurotrophins, of which

Table 1 — Early results of initial clinical studies examining the efficacy of adult stem cell therapy for stress urinary incontinence

Clinical study	Patients (*n*)	Method of delivery	Stem cell source	Symptomatic improvement
Strasser et al [70]	63	TUUS	Autologous myoblasts and fibroblasts*	85%
Strasser et al [72]	42	TUUS	Autologous myoblasts and fibroblasts*	91%
Mitterbarger et al [73]	20	TUUS	Autologous myoblasts and fibroblasts*	90% at 1 yr 89% at 2 yr
Carr et al [74]	8	TUUS, EI, DPI	Pure MDSCs	63%

*Mixed cellular plus collagen injections. TUUS = transurethral ultrasound; EI = endoscopic injection; DPI = direct periurethral injection; MDSCs = muscle-derived stem cells.

the best characterized is NGF [67]. Sympathetic activation of adipocytic β-adrenoceptors induces lypolysis and a decrease in the number of adipocytes. This process is responsible for a loss of body weight during hibernation [68]. A recent study has demonstrated that NGF is synthesized and released by white adipose tissue with the expression of p75 and trkA NGF receptors in adipocytes [69]. This suggests that ADSCs may also have the potential of releasing NGF and regenerating urethral malfunction in patients with SUI.

5. Clinical results of cellular therapy

Results of the first clinical studies have recently become available (Table 1). Strasser et al [70] reported their comparison of 63 patients undergoing autologous myoblast and fibroblast injection versus 28 patients undergoing collagen injection for SUI. Under ultrasound guidance, a transurethral probe was used to inject fibroblasts into the urethral submucosa to treat mucosal atrophy, and myoblasts into the rhabdosphincter for muscle reconstruction [71]. The myoblasts and fibroblasts were obtained from an upper arm biopsy. Despite significant increases in *Incontinence Quality of Life (I-QOL) Instrument Scores* and decreases in *Incontinence Scores* in the collagen treated group, these results did not translate into clinical improvement, and only two (10%) female patients were cured of incontinence. In comparison, 85% of the stem cell-treated group was cured of incontinence, and thickness of urethra and rhabdosphincter were increased significantly at the 12-month follow-up on transurethral ultrasonography. Comparing stem cell versus collagen injections in female clinical trial participants revealed a 91% incontinence cure rate in the stem cell-treated group compared to 10% in the collagen-treated group [72]. Efficacy at 2 years (89% cure, 11% improvement) was demonstrated in 20 female patients from the same study [73]. However, in these studies, fibroblasts were mixed with 2.5 mL of collagen as carrier material to prevent site migration. The fractional benefit of myoblasts versus fibroblasts versus collagen used in the mixed cellular plus collagen injection approach is unclear. Randomized, controlled clinical trials are necessary to clarify the benefit of the mixed cellular plus collagen injection therapy.

Pure cellular clinical therapy with MDCs obtained from biopsies of the lateral thigh were reported by Carr et al [74] at the 2006 AUA annual meeting, and represented the first trial of North American patients. Eight patients received treatment using either a transurethral or periurethral injection into the middle urethra and EUS. The two transurethral injections using a 10-mm needle and the two periurethral injections resulted in measurable improvement, but the two initial injections using the shorter 8-mm needle were not effective. Five of the eight patients with follow-up for over 1 year reported significant improvement occurring between 3 and 8 months after injection (mean follow-up of 16.5 months). In addition, cystoscopy at study exit and surgical exploration in two patients at the time of midurethral tape surgery did not demonstrate any appreciable tissue change. Subsequent midurethral tape placement and outcome was not negatively impacted upon by previous MDC injection. These results suggest the ability of pure cellular therapy to treat SUI and emphasize the importance of proper cell placement in resulting effectiveness. Onset of improvement was delayed following injection, suggesting that restoring muscle function may be the mechanism of action in comparison to standard bulking agents. Deeper delivery of MDCs into the external sphincter appears to be important for successful outcome.

6. Conclusions

The transvaginal tape (TVT) procedure has gained popularity for the treatment of SUI. Several authors have reported on the surgical outcome of TVT procedures, demonstrating 85–89% objective cure rates at 3 or 5 years [75–77]. In contrast, Ward and Hilton [78] published the largest randomized, controlled trial comparing subjective and objective outcomes after abdominal colposuspension or insertion of TVT. At 2 years, 63% of the TVT group and 51% of the colposuspension group were objectively cured. The subjective cure rates at 2 years, however, were only 43% and 37%, respectively.

Suburethral sling procedures are regarded as a hammock to reinforce the weakness of pelvic floor muscles and supportive ligaments or fascia, whereas stem cell injection therapy into the middle urethra may restore the contractile response of the striated muscle and rhabdosphincter. The hope is that stem cell treatment of SUI will result in improved cure rates with minimal risks. Not all cellular therapies are the same, as demonstrated by the differences in safety and efficacy among MDCs, myoblasts, and fibroblasts. Autologous MDC and ADSC pure injection therapy may be a promising treatment to restore urethral sphincter function.

Acknowledgments

Michael B. Chancellor is consultant to Cook and has received research grants from Cook. David Wagner, Ryan Pruchnic and Ron Jankowski are employees of Cook. Our SUI research has been supported by National Institutes of Health grants (DK67226, AR49398, DK55387) and Cook MyoSite funding.

References

1. Corcos J, Beaulieu S, Donovan J, et al. Quality of life assessment in men and women with urinary incontinence. *J Urol* 2002;168:896–905.
2. Norton P, Brubaker L. Urinary incontinence in women. *Lancet* 2006;367:57–67.
3. Hampel C, Wienhold D, Benken N, Eggersmann C, Thuroff JW. Prevalence and natural history of female incontinence. *Eur Urol* 1997;32(Suppl 2):3–12.
4. Bump RC, Norton PA. Epidemiology and natural history of pelvic floor dysfunction. *Obstet Gynecol Clin North Am* 1998;25:723–46.
5. Peschers U, Schaer G, Anthuber C, Delancey JO, Schuessler B. Changes in vesical neck mobility following vaginal delivery. *Obstet Gynecol* 1996;88:1001–6.
6. Thom DH, van den Eeden SK, Brown JS. Evaluation of parturition and other reproductive variables as risk factors for urinary incontinence in later life. *Obstet Gynecol* 1997; 90:983–9.
7. Meyer S, Schreyer A, De Grandi P, Hohlfeld P. The effects of birth on urinary continence mechanisms and other pelvic-floor characteristics. *Obstet Gynecol* 1998;92: 613–8.
8. Sampselle CM, Miller JM, Mims BL, Delancey JO, Ashton-Miller JA, Antonakos CL. Effect of pelvic muscle exercise on transient incontinence during pregnancy and after birth. *Obstet Gynecol* 1998;91:406–12.
9. McGuire EJ, Lytton B, Pepe V, Kohorn EI. Stress urinary incontinence. *Obstet Gynecol* 1976;47:255–64.
10. Blaivas JG. A modest proposal for the diagnosis and treatment of urinary incontinence in women. *J Urol* 1987; 138:597–8.
11. Kayigil O, Iftekhar Ahmed S, Metin A. The coexistence of intrinsic sphincter deficiency with type II stress incontinence. *J Urol* 1999;162:1365–6.
12. Radley SC, Chapple CR, Bryan NP, Clarke DE, Craig DA. Effect of methoxamine on maximum urethral pressure in women with genuine stress incontinence: a placebo-controlled, double-blind crossover study. *Neurourol Urodyn* 2001;20:43–52.
13. Chancellor MB. Duloxetine for treatment of stress urinary incontinence. *Evid Based OBGYN* 2004;6:196.
14. Herschorn S, Glazer AA. Early experience with small volume periurethral polytetrafluoroethylene for female stress urinary incontinence. *J Urol* 2000;163:1838–42.
15. Sakamoto K, Sharma S, Wheeler JS. Long-term subjective continence status and use of alternative treatments by women with stress urinary incontinence after collagen injection therapy. *World J Urol* 2007;25:431–3.
16. Maher CF, O'Reilly BA, Dwyer PL, Carey MP, Cornish A, Schluter P. Pubovaginal sling versus transurethral macroplastique for stress urinary incontinence and intrinsic sphincter deficiency: a prospective randomised controlled trial. *BJOG* 2005;112:797–801.
17. Chrouser KL, Fick F, Goel A, Itano NB, Sweat SD, Lightner DJ. Carbon coated zirconium beads in beta-glucan gel and bovine glutaraldehyde cross-linked collagen injections for intrinsic sphincter deficiency: continence and satisfaction after extended followup. *J Urol* 2004;171:1152–5.
18. Bent AE, Tutrone RT, McLennan MT, Lloyd LK, Kennelly MJ, Badlani G. Treatment of intrinsic sphincter deficiency using autologous ear chondrocytes as a bulking agent. *Neurourol Urodyn* 2001;20:157–65.
19. Sweat SD, Lightner DJ. Complications of sterile abscess formation and pulmonary embolism following periurethral bulking agents. *J Urol* 1999;161:93–6.
20. McKinney CD, Gaffey MJ, Gillenwater JY. Bladder outlet obstruction after multiple periurethral polytetrafluoroethylene injections. *J Urol* 1995;153:149–51.
21. Papa Petros PE. Tissue reaction to implanted foreign materials for cure of stress incontinence. *Am J Obstet Gynecol* 1994;171:1159.
22. Kiilholma PJ, Chancellor MB, Makinen J, Hirsch IH, Klemi PJ. Complications of Teflon injection for stress urinary incontinence. *Neurourol Urodyn* 1993;12:131–7.
23. Lee JY, Cannon TW, Pruchnic R, Fraser MO, Huard J, Chancellor MB. The effects of periurethral muscle-derived stem cell injection on leak point pressure in a rat model of stress urinary incontinence. *Int Urogynecol J Pelvic Floor Dysfunct* 2003;14:31–7.
24. Edwards RG. A burgeoning science of embryological genetics demands a modern ethics. *Reprod Biomed Online* 2007;15(Suppl 1):34–40.
25. DeLancey JO. Structural support of the urethra as it relates to stress urinary incontinence: the hammock hypothesis. *Am J Obstet Gynecol* 1994;170:1713–23.
26. Schroder HD, Reske-Nielsen E. Fiber types in the striated urethral and anal sphincters. *Acta Neuropathol* 1983;60: 278–82.
27. De Groat WC. Integrative control of the lower urinary tract: preclinical perspective. *Br J Pharmacol* 2006;147 (Suppl 2):S25–40.
28. Chancellor MB, Perkin H, Yoshimura N. Recent advances in the neurophysiology of stress urinary incontinence. *Scand J Urol Nephrol* 2005;39:21–4.
29. Thor KB, Katofiasc MA. Effects of duloxetine, a combined serotonin and norepinephrine reuptake inhibitor, on central neural control of lower urinary tract function in the chloralose-anesthetized female cat. *J Pharmacol Exp Ther* 1995;274:1014–24.

30. Kaiho Y, Kamo I, Chancellor MB, Arai Y, de Groat WC, Yoshimura N. Role of noradrenergic pathways in sneeze-induced urethral continence reflex in rats. *Am J Physiol Renal Physiol* 2007;292:F639–46.
31. Kamo I, Torimoto K, Chancellor MB, de Groat WC, Yoshimura N. Urethral closure mechanisms under sneeze-induced stress condition in rats: a new animal model for evaluation of stress urinary incontinence. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R356–65.
32. Kamo I, Kaiho Y, Canon TW, et al. Functional analysis of active urethral closure mechanisms under sneeze induced stress condition in a rat model of birth trauma. *J Urol* 2006;176:2711–5.
33. De Groat WC. Anatomy of the central neural pathways controlling the lower urinary tract. *Eur Urol* 1998;34 (Suppl 1):2–5.
34. Kamo I, Cannon TW, Conway DA, et al. The role of bladder-to-urethral reflexes in urinary continence mechanisms in rats. *Am J Physiol Renal Physiol* 2004;287:F434–41.
35. Strasser H, Ninkovic M, Hess M, Bartsah G, Stenzl A. Anatomic and functional studies of the male and female urethral sphincter. *World J Urol* 2000;18:324–9.
36. Strasser H, Tiefenthaler M, Steinlechner M, Eder I, Bartsch G, Konwalinka G. Age dependent apoptosis and loss of rhabdosphincter cells. *J Urol* 2000;164:1781–5.
37. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284:143–7.
38. Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;279:1528–30.
39. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997;276:71–4.
40. Dezawa M, Ishikawa H, Itokazu Y, et al. Bone marrow stromal cells generate muscle cells and repair muscle degeneration. *Science* 2005;309:314–7.
41. Qu Z, Balkir L, van Deutekom JC, Robbins PD. Development of approaches to improve cell survival in myoblast transfer therapy. *J Cell Biol* 1998;142:1257–67.
42. Seale P, Asakura A, Rudnicki MA. The potential of muscle stem cells. *Dev Cell* 2001;1:333–42.
43. Jankowski RJ, Deasy BM, Huard J. Muscle-derived stem cells. *Gene Ther* 2002;9:642–7.
44. Oshima H, Payne TR, Urish KL, et al. Differential myocardial infarct repair with muscle stem cells compared to myoblasts. *Mol Ther* 2005;12:1130–41.
45. Lee JY, Qu-Petersen Z, Cao B, et al. Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. *J Cell Biol* 2000;150:1085–100.
46. Qu-Petersen Z, Deasy B, Jankowski R, et al. Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. *J Cell Biol* 2002;157:851–64.
47. Yokoyama T, Yoshimura N, Dhir R, et al. Persistence and survival of autologous muscle derived cells versus bovine collagen as potential treatment of stress urinary incontinence. *J Urol* 2001;165:271–6.
48. Lee JY, Paik SY, Yuk SH, Lee JH, Ghil SH, Lee SS. Long term effects of muscle-derived stem cells on leak point pressure and closing pressure in rats with transected pudendal nerves. *Mol Cells* 2004;18:309–13.
49. Kwon D, Kim Y, Pruchnic R, et al. Periurethral cellular injection: comparison of muscle-derived progenitor cells and fibroblasts with regard to efficacy and tissue contractility in an animal model of stress urinary incontinence. *Urology* 2006;68:449–54.
50. Chancellor MB, Yokoyama T, Tirney S, et al. Preliminary results of myoblast injection into the urethra and bladder wall: a possible method for the treatment of stress urinary incontinence and impaired detrusor contractility. *Neurourol Urodyn* 2000;19:279–87.
51. Yokoyama T, Pruchnic R, Lee JY, et al. Autologous primary muscle-derived cells transfer into the lower urinary tract. *Tissue Eng* 2001;7:395–404.
52. Huard J, Yokoyama T, Pruchnic R, et al. Muscle-derived cell-mediated *ex vivo* gene therapy for urological dysfunction. *Gene Ther* 2002;9:1617–26.
53. Cannon TW, Lee JY, Somogyi G, et al. Improved sphincter contractility after allogenic muscle-derived progenitor cell injection into the denervated rat urethra. *Urology* 2003; 62:958–63.
54. Chermansky CJ, Tarin T, Kwon DD, et al. Intraurethral muscle-derived cell injections increase leak point pressure in a rat model of intrinsic sphincter deficiency. *Urology* 2004;63:780–5.
55. Beckingham IJ, Wemyss-Holden G, Lawrence WT. Long-term follow-up of women treated with perurethral Teflon injections for stress incontinence. *Br J Urol* 1992;69:580–3.
56. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001;7:211–28.
57. Rodriguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ. Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. *Proc Natl Acad Sci USA* 2006;103:12167–72.
58. Jack GS, Almeida FG, Zhang R, Alfonso ZC, Zuk PA, Rodriguez LV. Processed lipoaspirate cells for tissue engineering of the lower urinary tract: implications for the treatment of stress urinary incontinence and bladder reconstruction. *J Urol* 2005;174:2041–5.
59. Zeng X, Jack GS, Zhang R, et al. Treatment of SUI using adipose derived stem cells: restoration of urethral function. *J Urol* 2006;175:291.
60. Robbins SG, Rajaratnam VS, Penn JS. Evidence for upregulation and redistribution of vascular endothelial growth factor (VEGF) receptors flt-1 and flk-1 in the oxygen-injured rat retina. *Growth Factors* 1998;16:1–9.
61. Florini JR, Ewton DZ, Magri KA. Hormones, growth factors, and myogenic differentiation. *Annu Rev Physiol* 1991;53: 201–16.
62. Ruberti F, Capsoni S, Comparini A, et al. Phenotypic knockout of nerve growth factor in adult transgenic mice reveals severe deficits in basal forebrain cholinergic neurons, cell death in the spleen, and skeletal muscle dystrophy. *J Neurosci* 2000;20:2589–601.
63. Rende M, Brizi E, Conner J, et al. Nerve growth factor (NGF) influences differentiation and proliferation of myogenic cells *in vitro* via TrKA. *Int J Dev Neurosci* 2000;18: 869–85.
64. Reddypalli S, Roll K, Lee HK, Lundell M, Barea-Rodriguez E, Wheeler EF. p75NTR-mediated signaling promotes the survival of myoblasts and influences muscle strength. *J Cell Physiol* 2005;204:819–29.
65. Wheeler EF, Gong H, Grimes R, Benoit D, Vazguez L. p75NTR and Trk receptors are expressed in reciprocal patterns in a wide variety of non-neural tissues during rat embryonic development, indicating independent receptor functions. *J Comp Neurol* 1998;391:407–28.
66. Dedkov EI, Kostrominova TY, Borisov AB, Carlson BM. Survival of Schwann cells in chronically denervated skeletal muscles. *Acta Neuropathol* 2002;103:565–74.

67. Shooter EM. Early days of the nerve growth factor proteins. *Annu Rev Neurosci* 2001;24:601–29.
68. Shi H, Song CK, Giordano A, Cinti S, Bartness TJ. Sensory or sympathetic white adipose tissue denervation differentially affects depot growth and cellularity. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R1028–37.
69. Peeraully MR, Jenkins JR, Trayhurn P. NGF gene expression and secretion in white adipose tissue: regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines. *Am J Physiol Endocrinol Metab* 2004;287: E331–9.
70. Strasser H, Marksteiner R, Margreiter E, et al. Transurethral ultrasonography-guided injection of adult autologous stem cells versus transurethral endoscopic injection of collagen in treatment of urinary incontinence. *World J Urol* 2007; 25:385–92.
71. Strasser H, Pinggera GM, Gozzi C, et al. Three-dimensional transrectal ultrasound of the male urethral rhabdosphincter. *World J Urol* 2004;22:335–8.
72. Strasser H, Marksteiner R, Margreiter E, et al. Autologous myoblasts and fibroblasts versus collagen for treatment of stress urinary incontinence in women: a randomised controlled trial. *Lancet* 2007;369:2179–86.
73. Mitterberger M, Pinggera GM, Marksteiner R, et al. Adult stem cell therapy of female stress urinary incontinence. *Eur Urol* 2007;53:169–75.
74. Carr LK, Steele D, Steele S, et al. 1-year follow up of autologous muscle-derived stem cell injection pilot study to treat stress urinary incontinence. *Int Urogynecol J Pelvic Floor Dysfunct* 2008;19:881–3.
75. Ulmsten U, Johnson P, Rezapour M. A three-year follow up of tension free vaginal tape for surgical treatment of female stress urinary incontinence. *Br J Obstet Gynaecol* 1999;106:345–50.
76. Nilsson CG, Kuuva N, Falconer C, Rezapour M, Ulmsten U. Long-term results of the tension-free vaginal tape (TVT) procedure for surgical treatment of female stress urinary incontinence. *Int Urogynecol J Pelvic Floor Dysfunct* 2001;12(Suppl 2):S5–8.
77. Jeffry L, Deval B, Birsan A, Soriano D, Darai E. Objective and subjective cure rates after tension-free vaginal tape for treatment of urinary incontinence. *Urology* 2001;58:702–6.
78. Ward KL, Hilton P. A prospective multicenter randomized trial of tension-free vaginal tape and colposuspension for primary urodynamic stress incontinence: two-year follow-up. *Am J Obstet Gynecol* 2004;190:324–31.