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Myoblast therapy for stress urinary incontinence and bladder dysfunction

Abstract The field of tissue engineering and gene therapy has an exciting and promising future. During the past few years we have begun a comprehensive effort to investigate the use of myoblasts to improve and expand the treatment of stress urinary incontinence and bladder dysfunction. Moreover, we can expect the application of myoblast-mediated ex vivo gene transfer in the field of urology. In this paper we discuss the compositions of and methods involving the use of myogenic or muscle-derived cells for tissue engineering and cell-mediated gene therapy.

Key words Myoblast · Bladder · Urethra · Urodynamics · Incontinence · Gene therapy · Tissue engineering

Now that impotence has been ushered "out of the closet" by sildenafil citrate (Viagra), urologists worldwide are hoping to break the silence surrounding another, equally embarrassing, health condition. What problem could be as embarrassing and hidden as erectile dysfunction? The last medical taboo of the twentieth century in our field is urinary incontinence and bladder-control problems. Perhaps the most important meeting ever held in the field of urinary incontinence was convened in the summer of 1998 as the first International Consultation on Incontinence and was sponsored by the World Health Organization [19]. Between June 28 and July 1, 1998, 200 of the world's most highly respected experts in the field of incontinence gathered in Monaco. The congress identified urinary incontinence as a devastating medical problem not only in developed countries such as the United States but also in Third World countries. In the United States alone an estimated 17 million men and women are afflicted with urinary incontinence. Annually, the management and treatment of urinary incontinence costs over US $1000 per patient. The total cost of urinary incontinence, just to Americans, exceeds US $26 billion per year. It has been estimated that only 10–20% of patients with urinary incontinence and voiding dysfunction have overcome their embarrassment and discussed these problems with their doctor. Taken together, the field of neurourology and the problem of urinary incontinence currently represent the greatest growth area in urology practice and will continue to do so for the next decade.

To conquer this major medical problem we are exploring a novel and potentially promising therapy involving tissue engineering and gene therapy. This report explores in depth the role of cellular tissue engineering using myoblasts and muscle-derived cells, which are injected into the urethra for the treatment of stress urinary incontinence when injected into the urethra. In addition, the injection of myoblasts into the bladder wall may improve detrusor contractility. This article also describes cell-mediated gene therapy for the lower urinary tract using autologous muscle-derived cells. We hypothesize that: (1) myoblast injection can improve bladder and urethral smooth-muscle function, (2) autologous myoblasts can be harvested and injected to achieve long-term success, and (3) cell-mediated gene

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therapy using myoblasts transduced with trophic factors can further repair urinary-tract muscle damage.

Our experiment will have direct clinical utility in the near future. On the basis of the anticipated results we propose that autologous muscle-derived stem cells be harvested by aspiration of a very small amount of skeletal muscle from a patient's arm, that the muscle-derived cells be grown in culture, and that these myoblasts be engineered with viruses carrying the gene responsible for the expression of growth and trophic factors or nitric oxide synthase. The engineered myoblasts would then be injected into the impaired urethra or bladder of the same patient during a simple outpatient cystoscopy procedure (Fig. 1).

Myoblasts

Myoblasts, the mononucleate precursor cells of skeletal muscle, are different from other cells in many ways. Myoblasts naturally fuse to form postmitotic multinucleate myotubes, which results in the long-term expression and delivery of bioactive proteins [18]. Myoblasts have been extensively used as a vehicle for gene delivery to muscles for the treatment of muscle-related diseases such as Duchenne-type muscular dystrophy [9]. In addition, ex vivo gene transfer, which involves the injection of engineered myoblasts transduced with different viral vectors, has successfully delivered proteins such as dystrophin to muscle tissues [2].

Myoblasts have also been extensively used to deliver genes in many non-muscle-related gene-therapy applications, such as the transfer and expression of factor IX for hemophilia B [7], the systemic delivery of human growth hormone for growth retardation, delivery of the human adenosine deaminase gene for the adenosine deaminase deficiency syndrome [13], gene transfer of human proinsulin for diabetes mellitus, gene transfer for the expression of thyrosine hydroxylase for Parkinson's disease [11], and gene transfer for the expression of fasL to prevent immunorejection of pancreatic islet-cell transplants [12]. Myoblasts have been used for these non-muscle-related diseases because of their ability to become postmitotic and create a gene reservoir of secreted molecules that play a therapeutic role. Indeed, transplanted myotubes have been shown to persist and express therapeutic proteins in the brain for 6 months and in the subcutaneous tissue for at least 3 months posttransplantation [18].

Treatment of stress incontinence

There are myriad treatment modalities for stress urinary incontinence. The most common treatments currently used for stress include absorbent products, indwelling catheters, pessaries (vaginal rings placed for support of the bladder neck), medication, exercise, urethral plugs, biofeedback and electrical stimulation, surgery, and injection therapy.

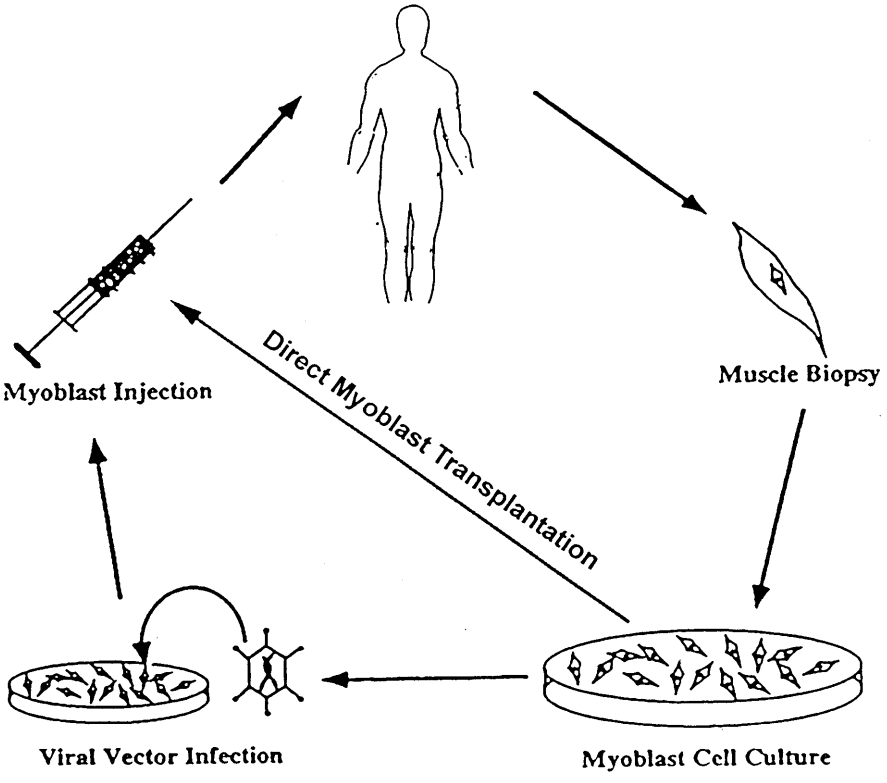

Fig. 1 Our experimental concept: myoblast mediated tissue engineering and gene transfer

Medication

Several drugs have been approved for the treatment of urge incontinence. However, no drug has been approved for or found effective in the treatment of stress urinary incontinence.

Exercise

Kegel exercise is a common and popular method used for the treatment of stress incontinence. Although 50% of the patients who practice the exercise four times daily for 3–6 months achieve some improvement, only 5% are cured. Moreover, most patients skip exercises and eventually drop out of the protocol because it is very time-consuming and requires considerable daily discipline.

Urethral plug

This device is a new disposable "cork"-like plug for women with stress incontinence that costs US $2. The woman is supposed to use a new plug after each micturition episode. The estimated daily cost for its use is US $15–20, and the estimated annual cost exceeds US $5000. Use of the plug is associated with urinary tract infection in over 20% of cases. The plug does not cure incontinence. For these reasons the sale of this device has been disappointing since its approval by the Food and Drug Administration (FDA) in 1996.

Biofeedback and functional electrical stimulation

Biofeedback and functional electrical stimulation involve the use of a vaginal probe for the treatment of urge and stress urinary incontinence. These methods are time-consuming and expensive. The results are somewhat better than those obtained with Kegel exercise.

Surgery

The surgical techniques applied in patients with urinary incontinence include laparoscopic or open abdominal bladder-neck suspensions, carried out using the transvaginal approach, and the creation of an artificial urinary sphincter. The latter is an expensive, complex surgical procedure that requires revision in 40% of cases.

Urethral injection therapy for stress incontinence

A major advance has been made over the past 10 years in the treatment of stress urinary incontinence through the use of injectable bulking agents to build up the deficient urethral sphincter valve. This minimally invasive outpatient approach can be applied to all types of stress urinary incontinence, and excellent results have been achieved. Three substances have been used for injection therapy: polytetrafluoroethylene (Teflon), glutaraldehyde cross-linked collagen, and autologous fat. The injection of a bulking agent has a significant advantage over conventional surgical repair of incontinence. Injection therapy can be done in a 10-min outpatient procedure using minimal sedation, after which patients can return home and immediately resume their full activity and work loads. Moreover, patients do not experience the pain or risks associated with surgery and hospitalization. We demonstrate below that injection of the patients' own myoblasts is superior to and represents a significant advance over treatment with conventional injectable substances.

Teflon

Despite the encouraging results obtained using Teflon for the treatment of vesicoureteral reflux and stress urinary incontinence, within the medical community, significant reservations about its use remain because of complications and particle migration. Some of the serious complications observed following Teflon injection include granuloma, diverticulum, cyst, and urethral polyp formation [8]. Of greatest concern, however, has been the documentation of distant Teflon-particle migration, resulting in fever and pneumonitis. Animal studies have demonstrated that the periurethral administration of Teflon may result in migration via the lymphatics and blood vessels. Particles with diameters of up to 80 μm have been found to cause granuloma formation at distant locations, including the brain and lungs [14].

Collagen

Glutaraldehyde cross-linked bovine collagen, manufactured in the form of an injectable paste, is the material most commonly and most successfully used for the correction of stress incontinence. Collagen gained FDA approval in 1993 for the indication of intrinsic sphincteric deficiency. Approximately 80% of patients show improvement following collagen injection; up to half of the 80% whose condition improves are cured or almost cured, and the other half show only a partial improvement. The remaining 20% of patients receive no benefit from collagen injection. There are three major disadvantages to collagen injection. First, collagen is often resorbed. This has an adverse effect on the outcome and necessitates repeated injections in all patients; a mean of 3 sessions of collagen injection are required for partial or complete improvement. The second disadvantage is that the processed bovine collagen is expensive, costing US $120/ml. The mean volume required per injection is

7.5 ml, and each patient receives a mean of three injections. The total cost of the collagen alone would be US $2700 per patient. The third drawback of bovine collagen is that 5% of patients are allergic to it. Most collagen-injected patients eventually develop antibodies to the bovine antigen.

Autologous fat

The concept of periureteral injection has proved to be efficacious, but the ideal material for injection has yet to be identified. This led to the investigation of the use of autologous fat as an injectable bulking agent. Histology studies reveal that although some injected fat survives in the area of injection as an autogenous graft, most of the injected fat is resorbed [16]. The extent and duration of fat-graft survival, however, remains a matter of controversy. An inflammatory reaction generally occurs at the site of implantation. Studies examining this problem indicate that between 10% and 30% of the fat survives after injection [16]. A fibrous reaction, which may be responsible for the persistence of bulk in the face of fat resorption, peaks at 40 days postimplantation. The occurrence of neovascularization along the graft periphery to any clinically relevant extent is unknown [16]. A report by the Ad Hoc Committee of the American Society of Plastic and Reconstructive Surgery noted that autologous fat injections can cause complications such as fat resorption, nodules, and tissue asymmetry [1]. Olson and associates [17] recently reported the disappointing long-term outcome of autologous fat injection in a controlled rat model.

Myoblast injection for the treatment of stress incontinence

Our initial experience with myoblast research involved urethral myoblast injection therapy for the treatment of stress urinary incontinence. For this study we used a myoblast cell line [6]. The myoblasts were first transduced with an adenovirus carrying the lacZ reporter gene. The cells were incubated with fluorescent latex microspheres (FLM) such that the fate of the injected cells could be followed. Cells were injected into adult female Sprague-Dawley rats ($n = 20$). A midline incision was made and myoblasts were injected into the proximal urethral wall at two sites using a 10-μl Hamilton microsyringe. The injection dose ranged from 1.33×10^5 to 1×10^6 myoblasts. The tissue was harvested at 2–4 days postinjection and was flash-frozen in liquid nitrogen. The tissue was sectioned, stained with x-gal substrate, and then counterstained with hematoxylin and eosin (H&E). We observed in the urethral wall a large number of cells expressing β-galactosidase (Fig. 2). This study demonstrates the feasibility of myoblast injection into the urethral wall and the survival of injected myoblasts. We hypothesize that myoblasts can serve as an injectable agent for bulking up of the urethral wall, enhancement of coaptation, and improvement of the urinary sphincter muscle.

Long-term survival of primary myoblasts

Primary myoblasts obtained from normal mouse skeletal muscle have been studied [20]. The myoblasts, transduced with an adenovirus carrying the lacZ reporter gene, were injected into the proximal urethra and bladder wall of severe combined immunodeficient (SCID) mice ($n = 30$). The number of myoblasts injected ranged from 0.5×10^6 to 1×10^6 cells. The tissue was harvested at 5, 15, 35, and 70 days as well as 6 months postinjection. The tissue was sectioned and assayed for β-galactosidase activity. We observed a large number of cells expressing β-galactosidase at each time point investigated. Many myofibers expressing β-galactosidase were also seen in the bladder wall and urethra (Fig. 3). Primary myoblasts injected into the bladders of SCID mice survived for over 6 months, and 60% of the transgene expression remained at 70 days postinjection. This study demonstrates the feasibility and survival of myoblast-mediated gene transfer into the urethral wall and bladder wall. We hypothesize that autologous myoblasts (myoblasts harvested from and cultured for a specific patient with stress incontinence) can serve as a nonallergenic agent for bulking up of the urethral wall, enhancement of coaptation, and improvement of the urinary sphincter muscle. Myoblast injection opens up new opportunities to improve detrusor and urethral sphinctor function.

Development of myoblast cellular myoplasty for smooth-muscle dysfunction

Although we are proposing the first investigation using skeletal muscle for the repair of urinary tract smooth-muscle dysfunction, we are basing our work on a strong foundation of success in skeletal myoblast therapy for myocardial regeneration and gene therapy to the myocardium. Recently, several important reports have been published on the use of myoblast transplantation for the repair of myocardial dysfunction [15]. In addition, the cryoprobe model, which we will use to induce bladder and urethral damage, has proved to be successful and reliable in inducing myocardial damage [15].

Myoblast-mediated ex vivo gene transfer involves the harvest of muscle, the cultivation of myoblasts followed by their transduction in vitro with viral vectors, and injection of the transduced cells into the animal-model joints, bone injury, or muscle contusion or laceration. Our preliminary experiment demonstrated the differentiation of myoblasts [10]. Primary myoblasts transduced with an adenovirus carrying the lacZ reporter gene were injected into the bladder of SCID mice. The tissue was harvested at 3, 35, and 70 days postinjection; stained for smooth-muscle α-actin (specific for smooth muscle) and fast heavy-chain myosin (specific for skeletal muscle); and

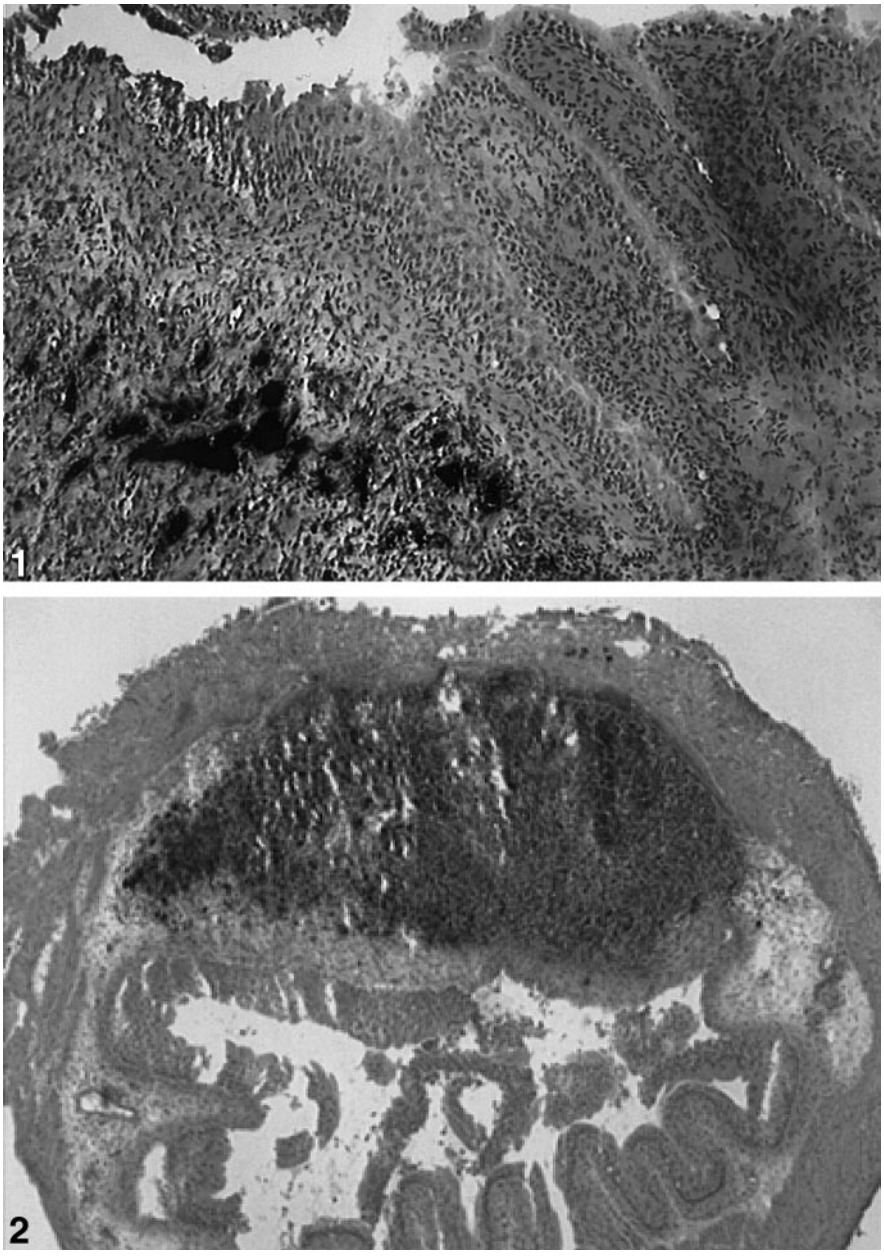

Fig. 2 LacZ staining: microscopic findings of myoblasts injected into the urethra. The *dark area* shows injected myoblasts

Fig. 3 LacZ staining: microscopic findings of myoblasts injected into the bladder wall. The *dark area* shows injected myoblasts

assayed for β-galactosidase expression. We observed long-term survival of the injected myoblasts in the bladder, and at 35 and 70 days postinjection we noted positive staining for smooth-muscle α-actin within the myofibers. These results suggest that after their injection into the bladder these skeletal myoblasts can differentiate into smooth muscle. These results support the existence of a population of muscle-derived stem cells within skeletal muscle.

Can myoblast injection into the bladder wall improve detrusor contractility?

Apart from the sphincter deficiency causing stress urinary incontinence, another common and serious cause of urinary incontinence (urge and overflow types) is impaired bladder contractility. This condition is becoming increasingly common in the geriatric population and in patients with neurological diseases, especially diabetes mellitus. Inadequate bladder contractility results in incomplete voiding, which can lead not only to incontinence but also to urinary tract infection and even renal insufficiency. Clinicians are currently very limited in their ability to treat impaired detrusor contractility. There is no effective medication for the improvement of detrusor contractility. Although urocholine can produce a slight increase in intravesical pressure, it has not been shown to aid effective bladder emptying in controlled studies. The most commonly approach is circumvention of the problem by intermittent or indwelling catheterization. The Brindley method [3] of intradural sacral

nerve stimulation is not effective in contracting the bladder in cases of impaired contractility.

Chancellor et al. [4] have attempted to augment the poorly contractile detrusor with dynamic bladder myoplasty, whereby a segment of neurovascularly intact rectus muscle is wrapped around the failed bladder. The rectus myoplasty is connected via intramuscular electrodes to a pulse generator via an external programmer that activates and stimulates the rectus muscle to contract and empty the patient's bladder. Although animal experiments [5] have been successful, the initial clinical results have been only partially favorable and the surgery is difficult. The tissue-engineering technique of cellular myoplasty has great potential as an improved and expanded form of treatment for impaired bladder contractility. This is an area of high priority in our research.

Conclusions and future directions

We hope that in the not-too-distant future, patients with stress urinary incontinence can come to their urologist's office for a simple needle aspiration of their muscle that takes fewer than 5 min. The muscle cells are shipped to a biotechnology center, where they are also cultivated in a step taking a few weeks. The myoblasts, vastly increased in number, are shipped back to the urologist in charge of treatment and are injected back into the patient in a brief 10-min outpatient endoscopy procedure. The injection is performed using a small cystoscope and a cystoscopic needle. Under the surgeon's direct vision the needle tip is inserted into the urethral sphincter mechanism and the myoblast suspension is injected into the urethral wall for the induction of urethral coaptation and closure. The cultured myoblasts can be frozen and stored indefinitely for additional future injections, if necessary. Muscle-derived cells can also be used for cell-mediated gene therapy with various tropic factors. A similar procedure of myoblast injection and gene therapy can also be carried out in the bladders of patients with impaired bladder contractility.

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