Myoblast and Fibroblast Therapy for Post-Prostatectomy Urinary Incontinence: 1-Year Followup of 63 Patients

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Purpose: We assessed the efficacy and safety of the application of autologous fibroblasts and myoblasts for treatment in post-prostatectomy urinary incontinence after a minimal followup of 1 year.

Materials and Methods: Sixty-three patients with stress urinary incontinence after radical prostatectomy were treated with transurethral ultrasound guided injections of autologous fibroblasts and myoblasts obtained from skeletal muscle biopsies. All subjects were evaluated preoperatively and 12 months postoperatively in terms of incontinence and Quality of Life Instrument scores, urodynamic parameters, and morphology and function of the urethra and rhabdosphincter.

Results: Of the 63 patients 41 were continent 12 months after implantation of cells, 17 showed improvement and 5 did not show any improvement. Incontinence and Quality of Life Instrument scores as well as thickness and contractility of the rhabdosphincter were significantly improved postoperatively.

Conclusions: The use of myoblast and fibroblast therapy represents a minimally invasive, safe and effective treatment for post-prostatectomy incontinence after a followup of 1 year.

Key Words: myoblasts; fibroblasts; endoscopy; urinary incontinence, stress

Prostate cancer is the most common cancer in men.[1] For curative therapy of localized prostatic cancer, radical prostatectomy is considered the treatment of choice. Estimates of post-prostatectomy continence may vary widely depending on how soon after surgery the results are reported, the experience of the operating surgeons and centers reporting the results, the method of interview and record retrieval as well as the consideration of subjective vs objective results.[2] Data from several reports from centers of excellence have indicated that continence rates after radical prostatectomy vary from 88% to 95%.[2]

Factors that contribute to urethral closure include urethral smooth as well as striated muscle tone and the supportive properties of the urethral mucosa. The striated urinary sphincter, the so-called rhabdosphincter, has a pivotal role in urinary continence after radical prostatectomy.[2] The rhabdosphincter is a tubular structure that surrounds the urethra at its ventral and lateral aspects like a coat.[3,4] Several recent studies have further extended earlier findings that sphincteric damage is the primary cause of post-prostatectomy incontinence, and even when associated with detrusor overactivity, sphincteric injury remains the primary cause.[2] In addition, spontaneous apoptosis leads to a continuous decrease in the density of striated muscle cells in the rhabdosphincter. This loss of rhabdosphincter cells eventually reaches a critical state which is accompanied by reduced function of the rhabdosphincter, which may finally result in stress urinary incontinence after prostate surgery.[4]

When significant and bothersome urinary incontinence persists despite active conservative treatment measurements, surgical therapy of incontinence is inevitable. Standard therapeutic modalities in treatment of urinary incontinence do not treat the pathophysiological causes of urinary incontinence. They are ineffective in some cases, repeated operations are therefore necessary and can further lead to severe side effects.[5] Thus, there is a need for effective and minimally invasive therapeutic approaches with low postoperative morbidity for therapy of post-prostatectomy stress urinary incontinence.

Recent animal experiments have indicated a role for the use of muscle derived stem cells in treatment of stress urinary incontinence.[6–8] In preclinical studies fibroblasts have been injected to treat atrophy of the urethral mucosa and myoblasts have been used to regenerate the rhabdosphincter. A combined application of these cells may restore normal morphology and function of urethral submucosa and the rhabdosphincter in incontinent patients. We present the 1-year postoperative results of transurethral ultrasound guided injections of autologous myoblasts and fibroblasts to regenerate the lower urinary tract in male patients with post-prostatectomy stress urinary incontinence.

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MATERIALS AND METHODS

Patients and Investigational Design

The Ministry of Health of the Federal Government of Austria approved the therapy. The study included 63 male patients (age range 52 to 82 years) treated between January 2004 and December 2005. The 1-year postoperative results are presented. Informed consent was obtained from all patients.

Radical prostatectomy was performed in all patients for localized prostate cancer. The time between prostate surgery and myoblast and fibroblast therapy of incontinence was at least 1 year, and all patients had performed pelvic floor exercise without improvement of symptoms. Urodynamic investigation objectified stress urinary incontinence in all patients. A total of 10 patients underwent additional surgery including internal urethrotomy for urethral strictures and radiation therapy for positive surgical margins after radical prostatectomy. Of the 63 patients 55 were referred to our department from other hospitals.

Urodynamic and clinical tests (including cystoscopy) were performed to investigate the lower urinary tract before therapy and exclude patients with urge incontinence. Preoperatively, the defined success parameters were investigated in all patients.

After myoblast and fibroblast therapy the patients were instructed to perform regular training of the rhabdosphincter and transrectal electrical stimulation for 4 weeks to support integration and regeneration. One year postoperatively the defined success parameters were investigated in all patients.

Outcome Criteria

To quantify the treatment success rates, patients were evaluated before and after therapy based on 4 criteria derived from an extensive literature search. The incontinence score, validated in previous clinical studies, was used as the primary parameter to evaluate the postoperative success of the treatment.[9] It comprises 3 different criteria of 24-hour voiding diary, 24-hour pad test and patient questionnaire. The total score ranges from 0 (continent) to 6 (completely incontinent). The Incontinence Quality of Life Instrument, which assesses quality of life before and after treatment of urinary incontinence, was also used for outcome measurement.[10] The total score for all answered questions ranges between 22 (no quality of life at all) and 110 (no restrictions on quality of life). Urodynamic tests, including pressure flow studies and urethral pressure profiles, were defined as outcome measures for determining whether obstruction of the lower urinary tract occurred after therapy and to demonstrate the therapeutic effect on urethral closure pressures.

Transurethral ultrasonography using high frequency ultrasound transducers (8Fr, 15 to 20 MHz) was used to evaluate the lower urinary tract before and after therapy. Previous studies have shown that this is the only currently available imaging technique that enables investigation of morphology and function of the urethra and the rhabdosphincter.[4] The distance between the ultrasound transducer and the inner aspect of the rhabdosphincter was measured at rest and during voluntary contraction of the muscle. The difference between these 2 readings served as a parameter for contractility of the rhabdosphincter.[4] The thickness of the urethra and rhabdosphincter as well the contractility of the rhabdosphincter were also defined as outcome measures.

Muscle Biopsy and Cell Application

Muscle biopsy and transurethral ultrasound guided injection of adult autologous fibroblasts and myoblasts into the urethral submucosa and the rhabdosphincter were performed similarly as described by Strasser.[11] From the biceps muscle a small biopsy (approximately 0.5 cm^3) was taken and transported to a Good Manufacturing Practice facility with official authorization for the production of fibroblasts and myoblasts for therapy of urinary incontinence (IGOR, Innovacell Biotechnologie GmbH, Austria).

Two different types of muscle derived autologous cells, fibroblasts and myoblasts, were isolated from these muscle biopsies, grown separately for 6 to 8 weeks and then harvested (fig. 1). Thereafter, the fibroblasts were suspended in 1 ml DMEM/F12 with 20% autologous serum mixed with 2.5 ml collagen (Contigen®, Bard) to prevent the cells from migrating away from the site of injection after application of cells. The myoblasts that are not mobile cells were suspended in 1.4 ml DMEM/F12 with 20% autologous serum. The cells were then filled into separate sterile syringes and transported to the operating room.

For application of the cells, the transurethral ultrasonography transducer was inserted into the urethra to visualize the urethral wall and rhabdosphincter. With a specially designed injection device (fig. 2) 15 to 18 portions (50 to 100 μl per depot) of the myoblast suspension were injected directly into the omega-shaped rhabdosphincter at 2 different levels to promote regeneration of the muscle (fig. 3). Then 25 to 30 depots (50 to 100 μl per depot) of the fibroblast/collagen suspension were injected into the submucosa circumferentially

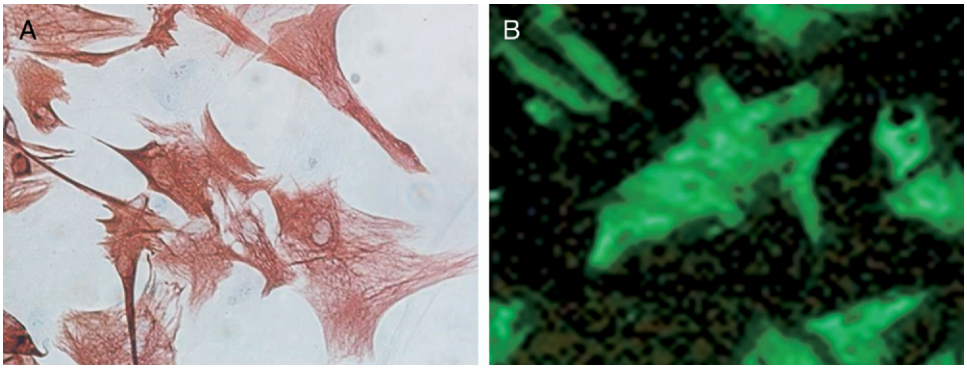

FIG. 1. *A*, immunohistochemical image of human myoblast culture with anti-desmin labeling (reduced from ×40). *B*, immunofluorescence image of human fibroblast culture with anti-vimentin labeling (reduced from ×40).

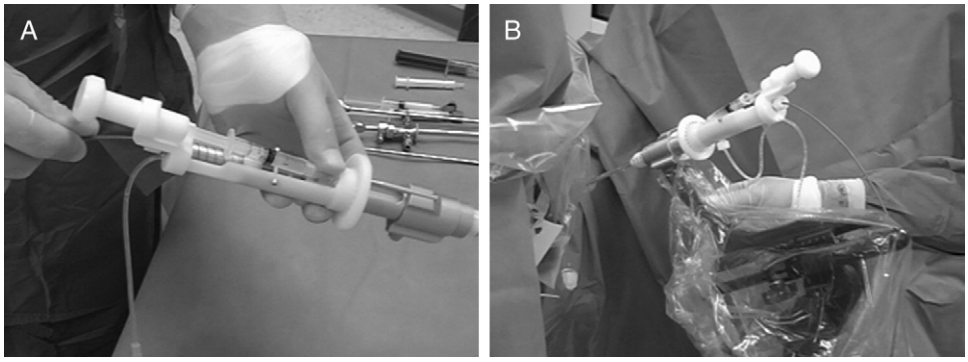

FIG. 2. Ultrasound guided injection of autologous myoblasts and fibroblasts. *A*, myoblasts and transurethral ultrasound transducer have been attached to injection device. *B*, similar to brachytherapy transurethral application device for cells is mounted on tripod to avoid any unintentional displacement of applicator. Therefore, cells can be injected step by step in many small depots all along the submucosal urethra and rhabdosphincter.

at 3 levels (slightly cranial to, slightly caudal to and between the 2 levels of the injected myoblasts) to treat atrophy of the urethral submucosa and improve its sealing effect (fig. 3).

Statistical Analysis

Data were saved in an Excel 11.0 database. For data processing SPSS® 11.5.1 for Windows was used. Mean values and standard deviations were calculated for numeric variables, and median values and range for ordinal variables for statistical data description. The Wilcoxon test was used for preoperative vs postoperative comparisons. A p value of 0.05 or less was considered statistically significant.

RESULTS

Ultrasound guided transurethral injection of adult autologous fibroblasts and myoblasts could be performed in all 63 patients without intraoperative or postoperative complications. All patients received on average injections of 3.8×10^7 fibroblasts (range 5.4×10^6 to 6×10^7) and 2.8×10^7 myoblasts (range 5.1×10^6 to 3.6×10^7, figs. 1 to 3).

After a followup of 1 year 41 of the 63 patients were continent and did not need to wear pads during daily life. An additional 17 patients showed improvement. In 5 patients no improvement was noted. No patient had deterioration of

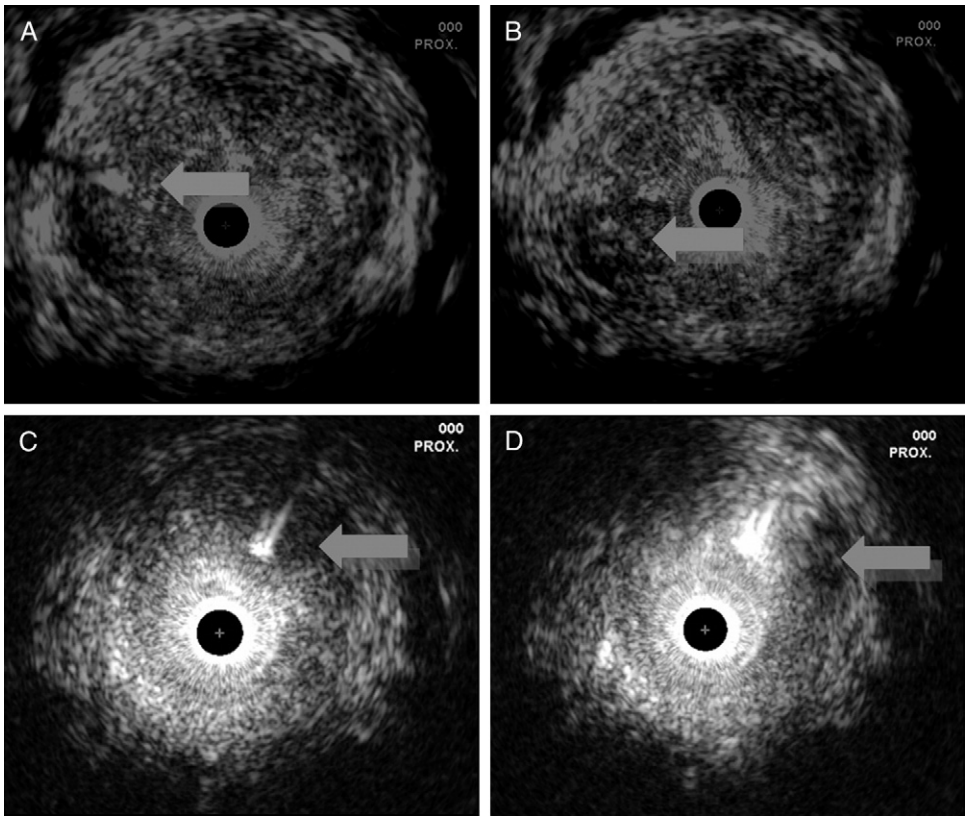

FIG. 3. Ultrasound guided injection of autologous myoblasts and fibroblasts in incontinent patient. *A*, tip of needle (arrow) is located at inner aspect of rhabdosphincter (9 o'clock position). *B*, subsequently small depot of myoblasts (50 μl) is injected (arrow). *C*, after injection of myoblasts, fibroblasts are injected into urethral submucosa (1 o'clock position). *D*, small depots of fibroblasts can be visualized.

incontinence after therapy. In addition, no severe postoperative side effects could be observed (such as pelvic pain, obstruction, inflammation or de novo urgency). Furthermore, no strictures or scars were detected on transurethral ultrasonography or cystoscopy. A urinary tract infection was found in 1 patient after implantation of cells, and in 5 patients a urinary catheter had to be placed postoperatively due to voiding problems. In all these cases the catheter could be removed on the first postoperative day.

A year after implantation of cells, incontinence score (median values 6 vs 1) was significantly improved compared to baseline values. The Quality of Life Instrument score (median values 52 vs 101) was also significantly improved. Furthermore, rhabdosphincter thickness (mean 2.2 ± 0.4 vs 3.3 ± 0.4) and contractility (mean 0.7 ± 0.3 vs 1.2 ± 0.3) were also significantly improved.

Measurement of urodynamic parameters revealed a statistically significant increase in maximum urinary flow and in maximum urethral closure pressure during voluntary contraction of the rhabdosphincter. A statistically significant decrease in residual urine and maximum detrusor pressure during voiding could be seen. There was no urodynamic sign of obstruction of the lower urinary tract postoperatively. Except maximum bladder capacity, all postoperative success parameters were statistically significantly improvement after therapy (see table).

DISCUSSION

Stress urinary incontinence represents the complication with the most significant impact on patients' quality of life after radical prostatectomy.[2] While conservative management is still recommended as the therapy of choice during the first 12 months (due to the possibility of spontaneous recovery of continence), persistent incontinence has to be treated by surgical therapy thereafter.

Surgical procedures to treat incontinence include bulking agents, various urethral sling procedures as well as implantation of inflatable balloons or artificial urinary sphincters. Bulking agents remain the most minimally invasive treatment for post-prostatectomy incontinence. All agents for which there is peer reviewed data available show only modest success rates with low cure rates.[12]

A variety of urethral sling techniques using different materials and different surgical approaches have been introduced as therapy for incontinence in men after radical prostatectomy. These male slings work by producing compression and obstruction of the urethra to alleviate the symptoms of incontinence. Sling procedures have been shown to result in short-term cure or improvement, but in many patients urinary incontinence invariably recurs, presumably as the pressure against the bulbous urethra decreases with time.[12,13]

Implantation of an artificial urinary sphincter has been the gold standard for the treatment of incontinence after radical prostatectomy, with success rates ranging from 59% to more than 87% in some series.[12] However, patients should be informed about the risk that surgical revisions may be necessary after implantation.

All the above mentioned injected or implanted materials and surgical procedures that have been employed to treat urinary incontinence can cause severe side effects. Injectables and implanted materials can lead to inflammation, erosions, urinary retention, obstruction and voiding dysfunction. All these surgical techniques lead to obstruction of the urethra. As they do not restore normal anatomy or function of the urethra and the rhabdosphincter, they do not treat the pathophysiological causes of urinary incontinence.[12–17]

In the present study 65% of patients were continent after ultrasound guided implantation of autologous myoblasts and fibroblasts. Continence was defined as no use of pads during normal daily life. In additional 27% of the treated patients incontinence was improved after a followup of 1 year. The injection of myoblasts led to a significant improvement of quality of life as well as an increase in thickness and improved contractility of the rhabdosphincter. The sonographic data show that additional muscle tissue is formed in the rhabdosphincter and that this new muscle tissue is functionally active.

It has been shown that myoblasts taken from skeletal muscle and those taken from the rhabdosphincter have the same physiological properties.[18] In animal experiments and clinical studies, autologous myoblasts and fibroblasts have been injected to treat various conditions, such as muscle defects, myocardial infarction, muscular dystrophies and facial rhytids.[19,20] Myoblasts have been shown to display adult tissue stem cell potential, as they can either proliferate or form new muscle tissue, and injected autologous myoblasts have already been used to aid the regeneration of skeletal muscle tissue.[11,19,20] No serious side effects, such as the development of hyperplasia, tumors or inflammation, have been reported to date following the implantation of

Characteristics of patients treated with autologous myoblasts and fibroblasts

	Preop	Postop Followup (1 yr)	p Value (Wilcoxon test)
Mean pt age ± SD	68 ± 6.4		
Median incontinence score (range)	6 (5–6)	1 (0–5)	<0.001
Median quality of life instrument score (range)	52 (31–69)	101 (59–110)	<0.001
Mean mm urethra thickness	3.5 ± 0.7	5 ± 1	<0.001
Mean mm rhabdosphincter thickness ± SD	2.2 ± 0.4	3.3 ± 0.4	<0.001
Mean contractility of rhabdosphincter ± SD	0.7 ± 0.3	1.2 ± 0.3	<0.001
Mean ml max residual urine ± SD	49.5 ± 130.0	12.5 ± 67.0	<0.001
Mean ml/sec max urinary flow ± SD	16.6 ± 6.7	18.3 ± 5.9	<0.001
Mean ml max bladder capacity ± SD	420.0 ± 125	446.5 ± 128.0	<0.570
Mean cm H_2O max detrusor pressure ± SD	64.4 ± 11	56 ± 14.2	<0.001
Mean cm H_2O max urethral closure pressure at rest ± SD	42.9 ± 16	62.8 ± 11	<0.001
Mean cm H_2O max urethral closure pressure at contraction ± SD	95.6 ± 18.6	112.7 ± 8.5	<0.001
Mean cm H_2O Valsalva leak point pressure ± SD	46.3 ± 17.1	68.2 ± 24.3	<0.001

autologous myoblasts and fibroblasts. Concerning the safety of autologous myoblast and fibroblast injection, previous studies have shown that these cells do not proliferate excessively due to contact inhibition and the absence of growth medium after injection.[6,11,18–20] In 1 patient who had undergone radiation therapy after radical prostatectomy and prior injections of bulking agents, a perforation of the bladder occurred during application of cells in January 2006, but no other intraoperative complication was observed, demonstrating that the procedure is safe. Furthermore, no side effects were observed postoperatively.

The present data, especially preoperative and postoperative ultrasonographic and urodynamic investigations, strongly support experimental findings that the ultrasound guided injection of autologous cells leads to regeneration of the urethral submucosa and the rhabdosphincter and not to passive obstruction of the lower urinary tract. That the postoperative effect cannot be simply due to a bulking effect is also underlined by the fact that normally the amount of injected material of bulking agents working through obstruction is higher.[12] It has to be pointed out that a preoperative thorough investigation to choose the patients that are right for this new treatment modality is important for the postoperative success of the treatment. Preoperative strictures, scars and fibrotic areas in the membranous urethra, prior injection of bulking agents or internal urethrotomy as well as radiation therapy negatively influence success rates. That these findings represent contraindications for this new treatment modality is supported by the fact that the only patient with a major intraoperative complication has undergone radiation therapy as well as several injections of bulking agents before the therapy.

With regard to application technique, the ultrasound guided application has a key role in this new treatment.[11] It is not only crucial to inject the right types of cells, it is equally important to inject the cells exactly into the right target structures. From a technical point of view, ultrasound guided injection of autologous myoblasts and fibroblasts can be compared with brachytherapy (fig. 2). In both treatment modalities modern endosonographic techniques are used. In addition, the transurethral application device for the cells is also mounted on a tripod to avoid any unintentional displacement of the applicator. Therefore, the cells can be injected step by step in many small depots all along the rhabdosphincter and the urethra (fig. 3). Ultrasonography allows accurate and precise injection of cells, which cannot be performed using standard endoscopic guidance.

In this article the 1-year results of 63 patients without control group are presented. Further no data about intraobserver and interobserver variability are available because all injections were performed by the same surgeon. Despite these limitations the present study evaluates and shows the feasibility of ultrasound guided transurethral application of autologous myoblasts and fibroblasts. It is planned to continue yearly control investigations of the patients who took part in this 1-year followup study to assess the long-term results of the treatment. Long-term postoperative results as well as data of multicenter trials involving larger numbers of patients are evaluated to prove if the application of autologous cells into the rhabdosphincter and the urethra may become a standard treatment modality for urinary incontinence in the future.

CONCLUSIONS

This study shows that autologous myoblasts and fibroblasts cure post-prostatectomy stress incontinence through regeneration of the urethra and rhabdosphincter. This new treatment modality represents a minimally invasive, effective and promising therapeutic approach.

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