Autologous myoblasts and fibroblasts versus collagen for treatment of stress urinary incontinence in women: a randomised controlled trial

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Summary

Background Preclinical studies have suggested that transurethral injections of autologous myoblasts can aid in regeneration of the rhabdosphincter, and fibroblasts in reconstruction of the urethral submucosa. We aimed to compare the effectiveness and tolerability of ultrasonography-guided injections of autologous cells with those of endoscopic injections of collagen for stress incontinence.

Methods Between 2002 and 2004, we recruited 63 eligible women with urinary stress incontinence. 42 of these women were randomly assigned to receive transurethral ultrasonography-guided injections of autologous myoblasts and fibroblasts, and 21 to receive conventional endoscopic injections of collagen. The first primary outcome measure was an incontinence score (range 0–6) based on a 24-hour voiding diary, a 24-hour pad test, and a patient questionnaire. The other primary outcome measures were contractility of the rhabdosphincter and thickness of both the urethra and rhabdosphincter. Analysis was by intention to treat. This trial is registered with Controlled-Trials.com, number CCT-NAPN-16630.

Findings At 12-months' follow-up, 38 of the 42 women injected with autologous cells were completely continent, compared with two of the 21 patients given conventional treatment with collagen. The median incontinence score decreased from a baseline of 6·0 (IQR 6·0–6·0; where 6 represents complete incontinence), to 0 (0–0) for patients treated with autologous cells, and 6·0 (3·5–6·0) for patients treated with collagen (p<0·0001). Ultrasonographic measurements showed that the mean thickness of the rhabdosphincter increased from a baseline of 2·13 mm (SD 0·39) for all patients to 3·38 mm (0·26) for patients treated with autologous cells and 2·32 mm (0·44) for patients treated with collagen (p<0·0001). Contractility of the rhabdosphincter increased from a baseline of 0·58 mm (SD 0·32) to 1·56 mm (0·28) for patients treated with autologous cells and 0·67 mm (0·51) for controls (p<0·0001). The change in the thickness of the urethra after treatment was not significantly different between treatment groups. No adverse effects were recorded in any of the 63 patients.

Interpretation Long-term postoperative results and data from multicentre trials with larger numbers of patients are needed to assess whether injection of autologous cells into the rhabdosphincter and the urethra could become a standard treatment for urinary incontinence.

Introduction

The most common causes of urinary incontinence are excessive activity of detrusors (neurogenic and idiopathic), which is known as urge incontinence, and incompetence of the urethral sphincter complex, which causes stress incontinence.[1] Because 78% of incontinent women suffer from stress or mixed urinary incontinence,[2] the target of treatment in most women is the urethral sphincter complex—ie, the urethra and striated rhabdosphincter.

Factors that affect closure of the urethra include urethral smooth and striated muscle tone and the supportive properties of the urethral mucosa and submucosa, especially the vascular submucosal layer. Poor mucosal function, due to atrophy and reduced vascularisation, might contribute to stress incontinence, especially in elderly women with low oestrogen.[1] The role of the striated urinary sphincter, the rhabdosphincter, is pivotal. The rhabdosphincter is a muscular coat that surrounds the urethra at its ventral and lateral aspects.[3,4]

and is integral to the urethral closure mechanism.[1,5] The resting tone and contractility of the rhabdosphincter are noticeably reduced in incontinent patients, and the urethra does not close completely.[1,5,6] Damage to this muscle can result from maternal injury during childbirth or from surgical injury. Spontaneous apoptosis also contributes to an age-dependent loss of the striated muscle cells of the rhabdosphincter.[7,8]

Conventional surgical procedures to treat urinary incontinence produce a mean rate of up to 80% continence 1 year after surgery, but this continence rate has been shown to decrease with time. Injection of a bulking agent, such as collagen, into the periurethral tissue to compress the urethra and thus occlude the urethral lumen is one of the standard techniques for treatment of urinary incontinence. Injection of bulking agents is less invasive than other surgical treatments, but overall success rates are poor.[9,10] Implantation or injection of material into the lower urinary tract can also cause severe side-effects.[9,11–20]
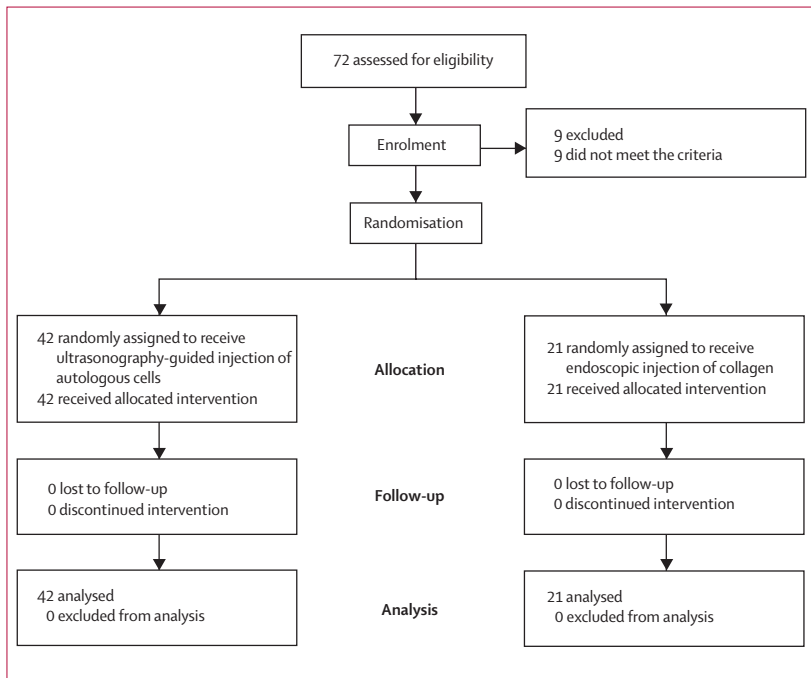
Figure 1: Trial profile

Some injected materials have been shown to migrate into the brain and other organs,[18] and injection of bulking agents and implantation of synthetic slings can cause chronic inflammation, foreign body giant-cell responses, periurethral abscesses, erosions, passive occlusion of the urethra, obstruction of the lower urinary tract, urinary retention, and severe voiding dysfunction.[9,20] Conventional operations to treat incontinence also need to be repeated in many cases.[9,11–20] Standard treatments for incontinence do not address the pathophysiological causes of the disorder. For example, the recent finding that the female urethra has no direct ligamentous fixation to the pubic bone[21] means that the use of suspension or sling procedures should be reviewed.

Myoblasts taken from skeletal muscle have been shown to have the same physiological properties as those from the rhabdosphincter.[22] Myoblasts have adult tissue stem-cell potential, because they can proliferate or form new muscle tissue.[23] Injected autologous myoblasts and fibroblasts have been used to aid the regeneration of skeletal muscle tissue,[24–26] and can be effective for treatment of muscle defects, myocardial infarction, muscular dystrophies, and facial rhytids.[24–28] No serious side-effects (such as development of hyperplasia, tumours, or inflammation) have been reported after implantation of such cells. Autologous myoblasts and fibroblasts have been proven to be effective for reconstruction of the lower urinary tract in animals, and transurethral application of such cells has been shown to restore the normal morphology and function of the rhabdosphincter and urethral submucosa in animals.[29–32] A new injection device, which uses transurethral

ultrasonography, has been designed to allow more precise injection of fibroblasts and myoblasts into the urethra and rhabdosphincter than traditional endoscopic injection techniques.[32]

We aimed to investigate whether transurethral ultra-sonography-guided injections of autologous myoblasts and fibroblasts could promote regeneration of the rhabdosphincter and combat atrophy of the urethral submucosa. Our hypothesis was that treatment with transurethral ultrasonography-guided injections of auto-logous myoblasts and fibroblasts would be more effective and tolerable than treatment with standard endoscopic injections of collagen.

Methods
Patients
From September, 2002, until the end of 2004, we recruited 72 adult female volunteers at the Department of Urology of the Medical University of Innsbruck (figure 1). 63 patients met the eligibility criteria: intrinsic sphincter insufficiency or stress urinary incontinence with only mild hypermobility of the urethra and the urinary bladder; age 36 to 84 years; good state of health; and provision of signed informed consent. Exclusion criteria were urge incontinence and pronounced hypermobility of the urethra.

Procedures
After approval from the Ministry of Health of the Federal Government of Austria, we intentionally allocated unequal numbers of patients to the two interventions. On the basis of preclinical findings, our rationale was that injection of autologous cells was sufficiently promising that it should be offered to the greatest possible number of patients, and that valuable experience with use of the treatment should be maximised. Therefore, a randomisation ratio of 2:1 was implemented by use of a computer-generated randomisation list.

The list consisted of seven permuted blocks, each of which contained nine randomly selected and unsorted combinations of the numbers 1, 2, and 3. Patients allocated numbers 1 and 2 were assigned to receive treatment with autologous cells, and those allocated number 3 were assigned to receive treatment with collagen (controls). Patients were sequentially allocated to these randomised numbers in the order in which they were enrolled.

After randomisation, we revealed the allocation, since patients needed to be prepared for different surgical treatments. However, the investigators who did the randomisation and who collected and analysed data were unaware of the treatment allocated to each patient.

We made small incisions under local anaesthesia in the left or right upper arms of the 42 patients who were allocated to the cell treatment group. For each patient, a muscle biopsy (of about 0·5 cm³) and blood for autologous serum (about 250 mL) were taken, and were immediately

transported to one of two facilities certified to good manufacturing practice standards, and authorised to produce myoblasts and fibroblasts (IGOR, Institut fuer Gewebe- und Organrekonstruktion, Wels; Innovacell Biotechnologie, Innsbruck, Austria).

Muscle tissue was separated from connective tissue. The muscle tissue was minced into small pieces, and after centrifugation (1200 revolutions per min [rpm] for 10 min, at room temperature), was treated overnight at 37°C with a digestion medium containing type I collagenase. We washed the myoblasts with phosphate-buffered saline and centrifuged them at 1200 rpm for 10 min. The resulting pellet was resuspended and cultured in Ham's F10 medium (PAA, Pasching, Austria), supplemented with 20% autologous serum. The myoblasts were plated in standard tissue-culture flasks and incubated at 37°C with 5% CO_2. Cells were passaged when they reached 80% confluence.

Fibroblasts were isolated from the connective tissue and resuspended, after digestion with type I collagenase, in Dulbecco's modified Eagle medium (DMEM) and Ham's F12 medium (1:1; PAA, Pasching, Austria). Fibroblasts were then supplemented with 10% autologous serum, plated in standard tissue-culture flasks and incubated at 37°C with 5% CO_2. We passaged cells when they reached 80% confluence. After 6 to 8 weeks in culture, fibroblasts and myoblasts were harvested separately by trypsinisation, and washed three times by centrifugation.

Figure 2 shows assessment of the quality of the cells by use of immunohistochemistry, immunofluorescence, and a fluorescence-activated cell sorter. Antibodies against desmin, vimentin, CD56, CD34, and AS02 were used to differentiate myoblasts from fibroblasts. We measured the fusion competence of myoblasts in differentiation medium (Cambrex, East Rutherford, NJ, USA) without serum to test their viability (figure 2). We counted the number of cells in each culture with a Neubauer chamber.[33]

On the basis of previous preclinical studies[22,32] we estimated that about 3.5×10^7 fibroblasts and 2.5×10^7 myoblasts should be used for each injection. We transferred the appropriate numbers of cells into separate sterile syringes. Myoblasts were suspended in 1·4 mL DMEM/F12 with 20% autologous serum, and fibroblasts in 1 mL DMEM/F12 with 20% autologous serum. Fibroblasts were also mixed with 2·5 mL of collagen (Contigen, Bard, Murray Hill, NJ, USA) as a carrier material to prevent migration of cells from the site of injection.

For the 42 patients randomly assigned to treatment with autologous cells, the ultrasonography transducer was carefully inserted into the urethra to visualise the urethral wall and rhabdosphincter. A specially designed device was used to inject 50–100 μL of the myoblast suspension directly into the omega-shaped rhabdosphincter in 15 to 18 portions at two different levels in the middle third of the urethra (figure 3). Then we injected 50–100 μL of the fibroblast and collagen suspension circumferentially into
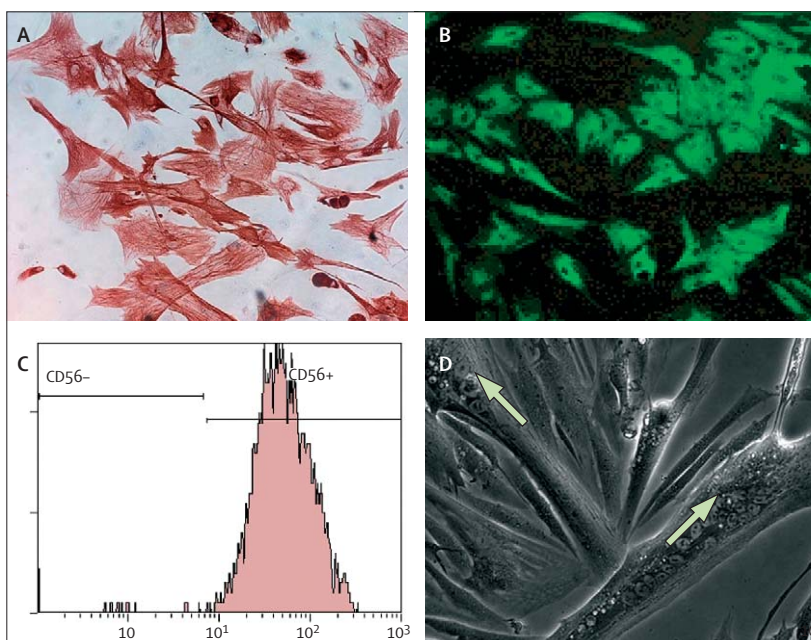
***Figure 2:* Characterisation of cells**
(A) Immunohistochemical image of human myoblasts stained with antidesmin antibodies. (B) Immunofluorescence image of human fibroblasts stained with antivimetin antibodies. (C) Fluorescent antibody cell sorter (FACS) analysis of myoblast cell culture showing that 97% of the myoblasts are positive for CD56 antibodies. (D) Phase-contrast microscope image of multinucleated myotubes (marked with arrows) that have formed after fusion of mononucleated myoblasts in differentiation medium.

the submucosa in 25 to 30 portions at three levels: slightly cranial to the injected myoblasts, slightly caudal to them, and between the two levels of myoblast injections.

For the 21 patients randomly assigned to receive conventional treatment, the endoscope was inserted into the urethra, and collagen was injected into the lower urinary tract by transurethral injection, under endoscopic guidance. The operating surgeons judged the position and number of deposits of collagen needed to ensure good filling and coaptation of the urethral wall as well as compression of the urethral lumen for each patient.

All 63 patients had done pelvic-floor exercises before the preoperative examination without improvement of their symptoms. Patients were instructed to continue regular training of the rhabdosphincter for 12 weeks and transvaginal electrical stimulation for 4 weeks after their operations.

Outcome measures

No standardised methods for recording outcomes after treatment of incontinence have been agreed.[34] One of our two primary outcome measures was an incontinence score that has been validated in previous clinical studies.[10] The score consisted of three different criteria: a 24-hour voiding diary, a 24-hour incontinence pad test, and a written questionnaire.[10] Possible scores ranged from 0 (representing continence) to 6 (representing complete incontinence).

The second set of primary outcome measures were contractility of the rhabdosphincter and thickness of both
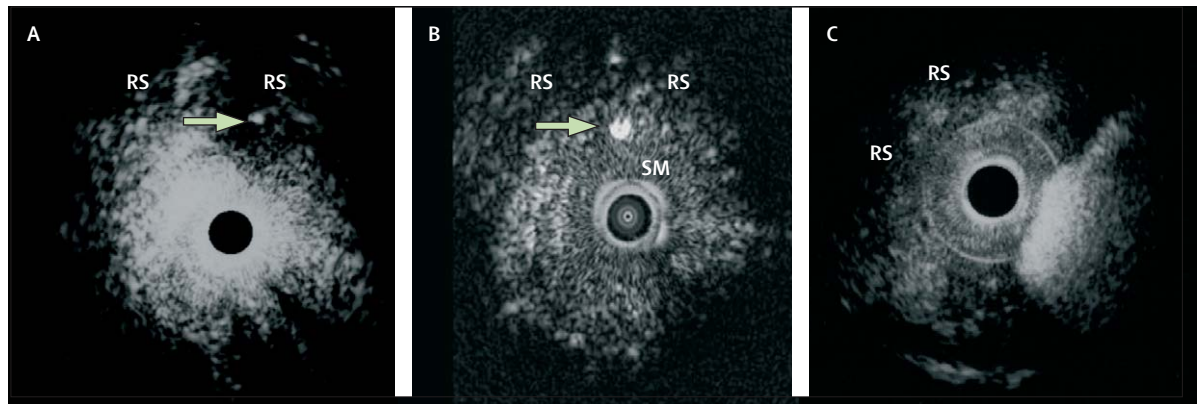
Figure 3: Cross-sectional ultrasonography images of the urethra and the rhabdosphincter

(A) The tip of the needle (marked with an arrow) is positioned at the inner aspect of the rhabdosphincter (RS) for injection of myoblasts. (B) The tip of the needle is placed at the outer aspect of the submucosa (SM) for injection of fibroblasts. (C) Transurethral ultraonography image after endoscopic injection of collagen. The hyperechoic deposit of collagen in the urethral wall compresses the urethral lumen.

the urethra and the rhabdosphincter. We used transurethral ultrasonography with high-frequency transducers (15–20 MHz) to assess the lower urinary tract; this is the only available imaging technique for routine investigation of the morphology and function of the urethra and rhabdosphincter.[4-6] Contractility of the rhabdosphincter was quantified by ultrasonographic measurement of the difference in position of the rhabdosphincter at rest and during voluntary contraction of the muscle (figure 4).[4-6] All ultrasonography measurements were made by skilled radiologists, who were unaware of treatment allocations.

Secondary endpoints included a quality-of-life score and a range of urodynamic and clinical tests. We used a prevalidated instrument to assess women's quality of life before and after treatment of incontinence.[35] Possible scores for all answered questions ranged from 22 (which indicates severely restricted quality of life) to 110 (which indicates no restrictions on quality of life). Urodynamic and clinical tests included cytoscopy, pressure-flow studies, and measurement of urethral closure pressure. We aimed to investigate the function of the lower urinary tract and identify any obstructions. We also used periurethral surface electrodes to take kinesiological electromyograph measurements of muscle activity of the rhabdosphincter, at rest and during voluntary contraction.[36]

Investigators asked all patients about side-effects both immediately after surgery and during control visits. We also did cystoscopy, urodynamics, and transurethral ultrasound to check for strictures, voiding dysfunction, urinary retention, de-novo urge incontinence, or formation of hyperplasias or tumours.

Statistical analysis

On the basis of previous studies[9,19,29–32] we estimated that we would need to treat 40 patients with autologous myoblasts and fibroblasts and 20 patients with collagen to detect a difference of three incontinence score points between the two groups at a statistical power of 95%, assuming a common standard deviation of three points and a two-sided α of 0·05. With the sample size of 42 patients allocated to cell treatment and 21 to collagen, the statistical power to reject the null hypothesis (based on the seven category probabilities of the incontinence score) with the Mann-Whitney U test was 99%, with a 0·05 two-sided significance level.

We processed data with SPSS statistical software (version 11.5.1). We compared the treatment groups by use of the Mann-Whitney U test. A p value of 0·05 or less was regarded as statistically significant. This trial is registered with Controlled-Trials.com, number CCT-NAPN-16630.
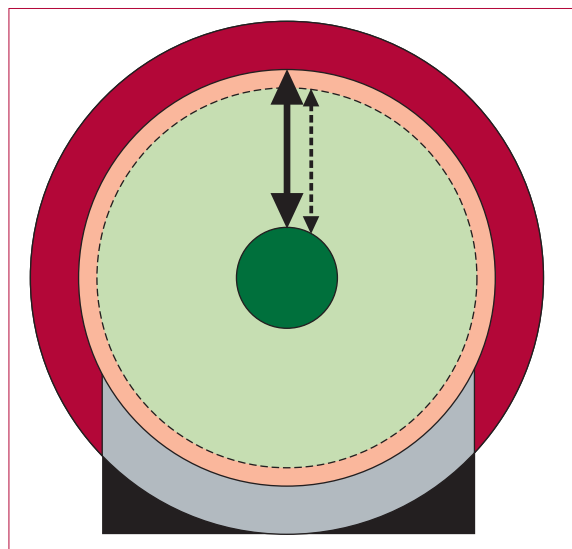
Figure 4: Measurement of contractility of the rhabdosphincter with transurethral ultrasonography

The red area represents the rhabdosphincter at rest, and the orange area the rhabdosphincter during voluntary contraction. The urethra is pale green. The dorsal connective tissue of the rhabdosphincter is grey. Contractility is defined as the reduction in distance between the ultrasound transducer (shown in dark green) and the inner boundary of the rhabdosphincter, measured at rest (black arrow) and during voluntary contraction (arrow with dotted line).

Role of the funding source

Neither Innovacell Biotechnologie nor IGOR (Institut fuer Gewebe- und Organrekonstruktion) had any role in study design, data collection, data analysis, data interpretation, or writing of the report. The first and corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

No infection or contamination arose during cell culture. Mean time of cell culture was 7·2 weeks. Figure 2 shows the characterisation and quantification of myoblasts and fibroblasts. Mononucleated myoblasts formed multi-nucleated and spontaneously contracting myotubes when cultured in differentiation medium (figure 2). On average, $3·8\times10^7$ fibroblasts (range $5·4\times10^6$–$6·0\times10^7$) and $2·8\times10^7$ myoblasts ($5·1\times10^6$–$3·6\times10^7$) were injected to patients in the cell treatment group, and 4·8 mL of collagen (2·5–7·5) to those in the collagen group.

Muscle biopsies, endoscopic injections of collagen, and transurethral injections of autologous cells were completed without complications in all patients (figure 3). Urethroscopy and transurethral ultrasonography immediately after endoscopic injection of collagen showed good adhesion of the mucosa, and allowed the size and location of the collagen deposits to be recorded (figure 3).

Table 1 shows the baseline characteristics in both groups, and in all enrolled patients. At baseline the median incontinence score was 6·0 (6·0–6·0), which is equivalent to complete incontinence. Table 2 shows that at a follow-up of 1 year, the median score for patients treated with autologous cells was 0 (IQR 0–0), compared with 6·0 (3·5–6·0) in patients treated with collagen (p<0·0001). 38 of the 42 patients given autologous cells were continent after 1 year of follow-up and did not need to wear pads during the day; three of these patients showed substantial improvement; and one only slight improvement (figure 5). By contrast, incontinence was cured in only two patients injected with collagen; one of these patients showed substantial improvement; and six only slight improvement.

Table 2 shows that the rhabdosphincter was more than 1 mm thicker in patients treated with autologous cells than in those given standard treatment (p<0·0001). Contractility of the rhabdosphincter was also improved for patients treated with autologous cells, compared with controls (p<0·0001). Changes in thickness could be seen in ultrasonographic images (figures 6 and 7 and webmovies). The thickness of the urethra after treatment was not significantly different between the two treatment groups (p=0·366). The change in the thickness of the urethra from baseline was 1·68 (0·88) for patients treated with autologous cells vs 0·77 (1·87) for patients treated with collagen (p=0·366).

Table 2 shows that two secondary outcome measures—quality-of-life score and electromyograph activity of the

	Patients assigned to receive autologous cells (n=42)	Patients assigned to receive collagen (n=21)	All eligible patients (n=63)
Age (years)	60·4 (11·9)	66·9 (10·5)	62·5 (11·8)
Incontinence score (possible range 0–6)	6·0 (6·0–6·0)	6·0 (5·0–6·0)	6·0 (6·0–6·0)
Quality-of-life score (possible range 110–22)	49·0 (42·5–54·8)	59·0 (42·5–65·5)	52·0 (43·0–61·0)
Thickness of urethra (mm)	3·73 (0·89)	4·59 (1·62)	4·01 (1·24)
Thickness of rhabdosphincter (mm)	2·03 (0·35)	2·32 (0·40)	2·13 (0·39)
Contractility of rhabdosphincter	0·54 (0·20)	0·66 (0·47)	0·58 (0·32)
Maximum residual urine (mL)	49·29 (98·79)	6·43 (17·97)	35·00 (83·50)
Maximum urinary flow (mL/s)	21·62 (9·15)	19·7 (7·46)	20·98 (8·61)
Maximum detrusor pressure during flow (cm H_2O)	36·62 (25·78)	32·81 (11·39)	35·35 (22·01)
Maximum bladder capacity (mL)	441·69 (173·61)	399·95 (92·74)	427·78 (151·97)
Maximum closure pressure at rest (cm H_2O)	28·88 (12·37)	32·24 (10·66)	30·00 (11·25)
Maximum closure pressure during voluntary contraction of the rhabdosphincter (cm H_2O)	38·52 (16·55)	40·71 (11·59)	39·25 (15·02)
Periurethral EMG recording at rest (μV)	34·31 (11·07)	31·38 (11·56)	33·33 (11·23)
Periurethral EMG recording during voluntary contraction of the rhabdosphincter (μV)	45·10 (11·86)	41·76 (10·79)	43·98 (11·54)

EMG=electromyograph. Numbers are mean (SD) or median (IQR).

Table 1: Baseline characteristics

	Patients assigned to receive autologous cells (n=42)	Patients assigned to receive collagen (n=21)	p
Primary outcomes			
Incontinence score	0 (0–0)	6·0 (3·5–6·0)	<0·0001
Thickness of urethra (mm)	5·41 (0·86)	5·36 (2·12)	0·366
Thickness of rhabdosphincter (mm)	3·38 (0·26)	2·32 (0·44)	<0·0001
Contractility of rhabdosphincter (mm)	1·56 (0·28)	0·67 (0·51)	<0·0001
Secondary outcomes			
Number of incontinent patients	4	19	
Quality-of-life score	108 (106–109)	64 (57–90)	<0·0001
Maximum residual urine (mL)	13·83 (43·65)	8·81 (24·49)	0·651
Maximum urinary flow (mL/s)	25·29 (8·17)	19·63 (10·22)	0·052
Maximum detrusor pressure during flow (cm H_2O)	31·26 (16·78)	34·76 (12·21)	0·194
Maximum bladder capacity (mL)	469·86 (158·95)	404·05 (104·94)	0·153
Maximum closure pressure at rest (cm H_2O)	40·52 (15·81)	35·24 (10·94)	0·204
Maximum closure pressure during voluntary contraction of the rhabdosphincter (cm H_2O)	53·26 (23·06)	40·57 (12·72)	0·029
Periurethral EMG recording at rest (μV)	44·23 (15·01)	31·57 (10·86)	0·001
Periurethral EMG recording during voluntary contraction of the rhabdosphincter (μV)	56·47 (15·36)	41·62 (9·98)	<0·0001

EMG=electromyograph. Numbers are mean (SD) or median (IQR). p values for differences between the two groups were calculated with the Mann-Whitney U test.

Table 2: Primary and secondary outcomes of patients treated with autologous cells and collagen, 1 year after intervention

rhabdosphincter muscle—changed more in patients treated with autologous cells than in those given standard treatment (p<0·0001). Figure 8 shows that median quality-of-life scores increased from an average of just over 50 in all patients before treatment to 108 in patients
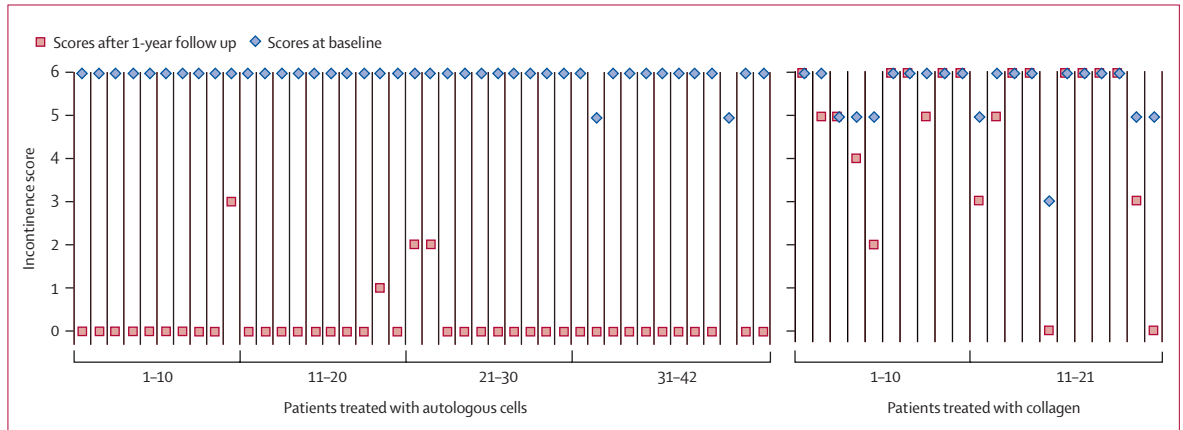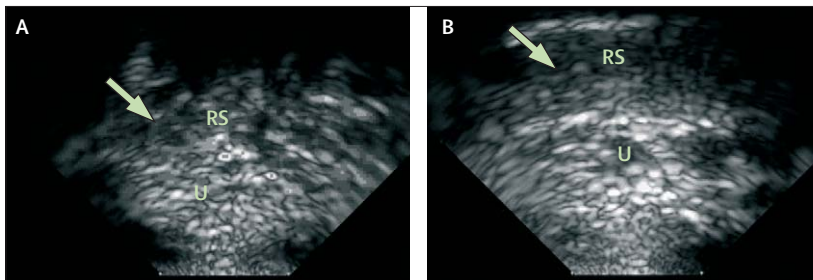
Figure 5: Incontinence score before and after treatment
Blue marks represent baseline scores and pink marks are scores after 1 year of follow-up.

treated with autologous cells, compared with 64 in patients given standard treatment with collagen (p<0·0001)

Transurethral ultrasonography and cytoscopy after treatment did not reveal any scars, strictures or bulks of cells (figures 6 and 7 and webmovies). Postoperative urodynamic assessment showed no obstruction of the lower urinary tract in either group. Coaptation of urethral submucosa was noticeably improved after treatment with autologous cells, as shown by endoscopy (figure 9). By contrast, in the patients treated with collagen, coaptation of urethral submucosa and urethral closure pressure at rest improved only slightly.

Three patients treated with autologous cells and two patients given collagen needed to have a catheter inserted after treatment until the first postoperative day. However, neither treatment was associated with severe side-effects such as pelvic pain, inflammation, or de-novo urgency.

Discussion

Our data accord with other results that suggest success rates for injection of bulking agents such as collagen to treat urinary incontinence are poor.[9,10] We show that continence improved more in patients injected with autologous myoblasts and fibroblasts than in those injected with collagen. Ultrasonographic measurements showed increased thickness and improved contractility of the rhabdosphincter after 12 months of follow-up in the patients treated with autologous cells, compared with those treated with collagen.

Periurethral electromyography also revealed increased activity of the rhabdosphincter, resulting in increased resting tone and voluntary contractile force. This finding suggests that additional muscle tissue was formed in the rhabdosphincter. Although we did not include micturition as an outcome measure, the slight improvement recorded in patients treated with injection of cells could have been due to increased bladder volume. No postoperative obstructions were recorded in either group.

Although collagen was used as a carrier material for fibroblasts, the total amount of injected material was much smaller than the volumes used to treat the 21 patients injected with collagen (figure 3). Therefore, results in patients treated with autologous cells cannot be attributed to the small amounts of collagen in these injections. Moreover, myoblasts and fibroblasts were

Figure 6: Transurethral ultrasonography in a female patient before and after injection of autologous cells
Longitudinal scan showing the rhabdosphincter (RS) (also marked with arrows) and the urethral wall (U).
(A) Before treatment. (B) 1 year after treatment, showing thickened urethral wall and rhabdosphincter.
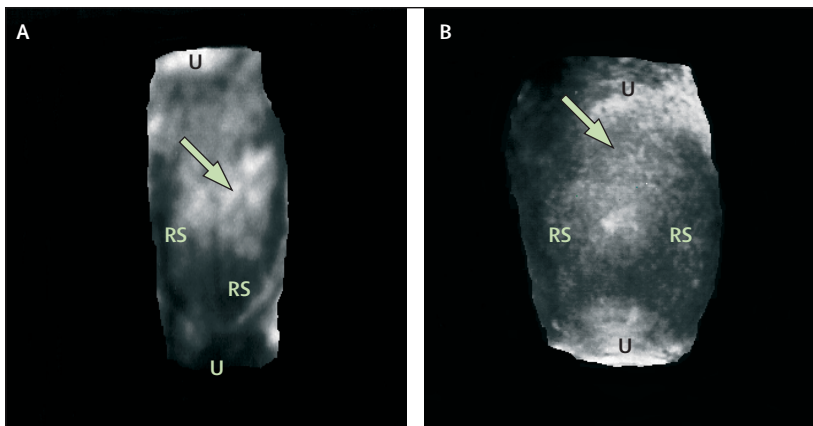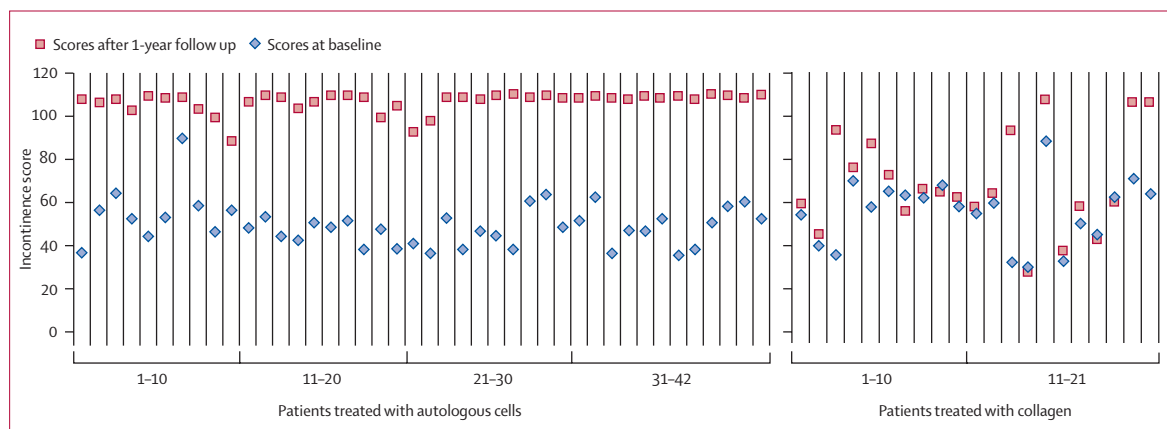

Figure 7: Three-dimensional ultrasonography in a female patient before and after injection of autologous cells
Reconstruction of ventral view of urethra (U) and rhabdosphincter (RS) of an incontinent woman. (A) Before treatment. (B) 1 year after treatment, showing increased thickness of urethra and rhabdosphincter. The arrows mark the ventral atrophic area of the rhabdosphincter, which is filled with new muscle tissue after treatment.

Figure 8: Quality-of-life scores before and after treatment
Blue marks represent baseline scores and pink marks are scores after 1 year of follow-up.

suspended in autologous serum that would have been absorbed immediately after injection, and were injected in several small deposits, rather than in large quantities. Therefore, postoperative effects after injection of autologous cells cannot be explained by a bulking effect, since the volumes were not sufficient to compress the urethra. Ultrasonographic, electromyographic, and clinical results in the group treated with autologous cells suggest that the injection of myoblasts and fibroblasts, unlike that of collagen, led to regeneration of the rhabdosphincter and submucosa.

Would the injection of either myoblasts or fibroblasts alone be sufficient to treat incontinence? Our findings, of increases in the thickness of the urethra and rhabdosphincter, the contractility of the rhadbosphincter, urethral closure pressure, and activity of the rhabdosphincter muscle, suggest not. Combined regeneration of both the rhabdosphincter and the urethra seem to be needed for improved closure of the urethral lumen.

Guidance of injections with ultrasonography was crucial to our results, since endoscopic injection of bulking agents is not sufficiently accurate for injection of cells into the lower urinary tract.[32] Collagen was injected under endoscopic guidance, as is standard practice for treatment of incontinence. To date, transurethral ultrasound guidance has not been used to apply collagen or other bulking agents into the lower urinary tract. A good postoperative result after a standard endoscopic injection of collagen is completely dependent on the surgeon's skill, whereas ultrasound-guided application could be more easily standardised. However, use of the ultrasonography-guided technique to inject large volumes of collagen into the urethra might not improve postoperative results, since collagen has not been shown to stimulate regeneration of musculature. Furthermore, injection of large quantities of collagen or other foreign material directly into the lower urinary tract could have severe side-effects such as damage to the rhabdosphincter or the urethra.
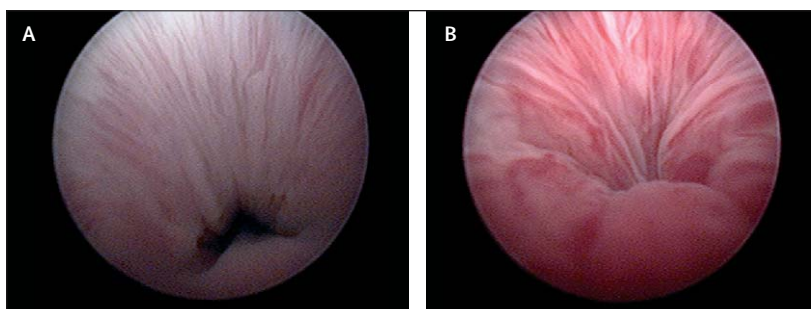
Figure 9: Endoscopic view of the urethral mucosa
(A) Before treatment, showing atrophic urethral mucosa, with weak adhesion, or coaptation. (B) 1 year after treatment, showing improved coaptation due to regeneration of urethral mucosa and rhabdosphincter. Both images created with the same endoscope and identical conditions.

One limitation of our study was that we could not do a classic prospective randomised double-blinded drug trial, because the modes of action and the underlying therapeutic concepts of the two treatments were completely different. Doctors and patients could not remain unaware of assignment, since the biopsy, injection technique, injected volume, and injected substances differed widely. To avoid any possible systematic error, the doctors who did randomisation, data collection, and analysis of data were unaware of the treatment allocation of each patient.

After a median follow-up of 3 years no severe side-effects or scars have been reported, and postoperative results have not changed. We will continue to do yearly control investigations of patients to assess longer-term postoperative results. Data from multicentre trials with larger numbers of patients are also needed to assess whether the application of autologous cells into the rhabdosphincter and the urethra could become a standard treatment for urinary incontinence.

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