International Urogynecology Journal (2025) 36:703–713
https://doi.org/10.1007/s00192-025-06079-0

ORIGINAL ARTICLE

Implantation of Autologous Skeletal Muscle-Derived Cells Combined with Electrical Stimulation in Patients with Stress Urinary Incontinence

Achim Rose[1] · **Herbert Rübben**[1]

Received: 24 May 2024 / Accepted: 9 July 2024 / Published online: 20 February 2025

Abstract

Introduction and Hypothesis Intrasphincteric injection of autologous skeletal muscle-derived cells (aSMDCs) is a minimally invasive treatment for stress urinary incontinence (SUI). This study investigated two cell counts (high/low dose) for functional urethral sphincter regeneration in combination with electrical stimulation, treatment safety and efficacy, and its potential superiority to duloxetine-placebo or duloxetine.

Methods This phase II, placebo-controlled trial randomised women with SUI to cell implantation (low or high cell number) and to control groups (duloxetine-placebo or duloxetine), each treatment combined with electrical stimulation. The primary efficacy endpoint was the mean reduction of incontinence episode frequency (IEF) at 12 weeks post-treatment compared with baseline. Secondary efficacy parameters included 1-h pad test, visual analogue scale (VAS), Incontinence Quality of Life questionnaire, clinical global impression score and frequency of responders based on IEF. Adverse events were analysed for safety evaluation. Additional follow-up data on IEF and selected secondary efficacy variables were obtained in a subpopulation of patients after 12 and 48 months.

Results The mean reduction ± SD in IEF after 12 weeks was: low cells: −16.4 ± 13.3 (61 patients), high cells: −18.5 ± 18.7 (56), placebo: −9.7 ± 13.7 (68), duloxetine −11.2 ± 19.6 (32). Cell treatments were significantly superior over placebo regarding IEF reduction and all secondary endpoints except for VAS. No safety issues were observed following cell implantation. Improvements were sustained over 12 and 48 months, with no difference between low and high cell implantation groups.

Conclusions Therapy for SUI with aSMDCs in combination with electrical stimulation is safe, effective and sustained over at least 48 months.

Keywords Stress urinary incontinence · Autologous skeletal muscle-derived cells · Duloxetine · Electrical stimulation · Randomized controlled trial

Introduction

Stress urinary incontinence (SUI) is characterised by urine leakage caused by insufficient closure of the urethra in situations of transiently increased abdominal pressure, as may be observed during physical activity or when sneezing or coughing [1]. Epidemiological estimates indicate that up to 45% of women may be affected by incontinence at some point in life and that about half of these cases are due to SUI [2]. The main causes underlying SUI are urethral hypermobility, causing inadequate pressure transmission to the urethra and intrinsic sphincter deficiency, resulting in dysfunctional urethral closure control, with most patients showing elements of both disorders. In general, urethral closure is controlled by urethral smooth and striated muscle tone combined with the supportive properties of the urethral mucosa and submucosa, especially the vascular submucosal layer. The rhabdosphincter appears of particular importance, being part of the external urethral sphincter and forming a striated muscular coating surrounding the urethra ventrally and laterally [3, 4]. Damage of the urethral sphincter may occur during childbirth, or as a consequence of surgical procedures or be the result of an age-dependent loss of striated muscle cells [5], and all of these effects may ultimately cause intrinsic sphincter deficiency.

Handling Editor: G. Alessandro Digesu
Editor in Chief: Maria A. Bortolini

✉ Achim Rose
Achim.Rose@helios-gesundheit.de

[1] Urologische Klinik, Neurourologie und Kontinenz, Helios Marien Klinikum Duisburg, Grunewaldstr. 96, 47053 Duisburg, Germany

A wide range of physical, pharmacological and surgical treatments has been used to manage SUI, the optimal choice of which may depend on the aetiology of the disease. Pelvic floor muscle training (PFMT) via biofeedback or electrical stimulation (ES) for at least 3 months is a first-line treatment of SUI recommended by the European Association of Urology [6]. Although these treatments have been examined in numerous studies, a Cochrane review expressed uncertainty regarding the difference between ES and sham treatment in terms of subjective cure and found no difference in cure or improvement for ES versus PFMT [7]. A widely prescribed therapeutic drug to treat moderate to severe SUI is duloxetine, a selective serotonin and norepinephrine reuptake inhibitor [8, 9]. The therapy method considered most efficacious is surgical treatment. However, although up to 80% continence can be observed 1 year after the surgery [10], therapeutic efficacy decreases over time and a relatively high number of post-operative complications has been reported [11–13]. Less invasive methods employed comprise the injection of bulking agents such as bovine collagen, silicone particles, or carbon beads, and more recently, polyacrylamide hydrogel, but these interventions have mostly yielded only short-term success or required multiple (up to 14) injections [14] and are also associated with numerous adverse events [15–17].

Cell-based therapy for potential cure of the sphincter deficiency in SUI is at the forefront of incontinence research, aimed at not merely reducing SUI symptoms but also ideally at restoring sphincter function and thus control over micturition [18, 19]. Animal models showed that myoblasts injected into the urethral sphincter can form functional myotubes [20, 21], confirmed the long-term engraftment of human smooth muscle progenitor cells into the receiving rat urethral sphincter [22] and the integration into existing muscle layers of GFP-labelled muscle precursor cells in a non-human primate model [23], suggesting that similar processes occur upon injection of autologous skeletal muscle-derived cells (aSMDCs) into the human urethral sphincter. Accordingly, besides its use in the treatment of myocardial infarction [24] and muscular dystrophies [25], aSMDC therapy has also been clinically applied to treat SUI [26–28] and the functionally related condition of fecal incontinence [29]. It has also been shown that the combination of aSMDCs and electrical stimulation leads to improved integration [23]. To our knowledge, there are no reports of severe side effects caused by aSMDC therapy, whereas most of these studies reported rather promising results on the efficacy of the cell treatment of SUI, despite somewhat conflicting results regarding the dose dependency of the effects [30–32].

Accordingly, we set out to test whether aSMDC therapy is an efficient treatment for SUI as evaluated through assessing incontinence episode frequency and additional efficacy parameters and tested the hypothesis that its impact on SUI would exceed that of a placebo treatment. In addition, we tested its potential superiority to the currently used therapeutic drug duloxetine and we evaluated if injection of a high number of aSMDCs is more efficient than injecting a low number of cells.

Here, we present the results of a multicentre randomised, parallel-group, placebo-controlled phase IIb clinical study on women suffering from SUI. These patients were treated with either a low or a high dose of injected aSMDCs, or with duloxetine, or with a placebo, each treatment combined with electrical stimulation. The aim of this trial was to determine the effective and safe dose of aSMDCs for regeneration of urethral sphincter function and to compare its efficacy with that of a placebo treatment. In addition, a group of 43 patients was also investigated at 48 months post-injection, providing information on long-term efficacy and safety.

Materials and Methods

Study Population

This multinational phase IIb clinical study, registered in the EU clinical trial register under the number EudraCT No. 2014–001656-34, was carried out in 32 centres in Bulgaria, the Czech Republic, Germany and Romania; the follow-up study included only 5 Bulgarian centres (Supplementary Table S1 and S2). The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines and the World Medical Association Declaration of Helsinki 1964 and its amendments and subsequent clarifications. Ethical approval was obtained from all relevant local ethics committees, and written informed consent was obtained from all patients involved in this trial.

Patients enrolled in the study were women aged 18 to 75 years suffering from SUI of mild to moderate severity (pad test 2–50 ml) according to the classification based on the short-pad test of Hahn and Fall as adapted by Klingler [33]. The diagnosis of SUI was based on urodynamic testing at screening and the patient's medical history. A detailed list of inclusion and exclusion criteria is provided in Supplementary Table S3.

Treatment

Randomisation was performed centrally by an external service provider. Patients were first randomised in an open manner to cell implantation and control groups and subsequently, in a double-blind manner, to the low cell count or the high cell count implantation group or to the duloxetine-placebo and the duloxetine group in a ratio of 2:2:2:1.

From the patients assigned to the cell groups, skeletal muscle cells were obtained from a biopsy sample of approximately 1 cm^3 taken from the biceps muscle or pectoral muscle under local anaesthesia. Subsequent isolation, culturing and propagation of the cells from biopsy samples and cell preparation for implantation were conducted according to standard manufacturing procedures of the institution conducting the study as described in the Supplementary Methods. Cell implantation was conducted under general anaesthesia, injecting cells at either a low dose (Cell implantation group I, CI1, cell count of 0.2×10^6 cells) or a high dose (CI2, cell count of 10×10^6 cells) into the external sphincter, delivering a total volume of 2 ml with depots of 50 µl each by means of an ultrasound-directed special injector directed through the urethra (Supplementary Fig. 1).

Control patients received either duloxetine or placebo as oral capsules titrated with weekly increments from 20 mg/day to 80 mg/day. Based on drug tolerability, the highest tolerated dose was then applied for 12 weeks followed by a gradual down-titration over the next 2 weeks. Medication compliance was monitored by inventory of individually returned medication packs.

To provide simultaneous and standardised pelvic floor muscle rehabilitation, patients from all groups underwent concomitant therapy of pelvic floor electrical stimulation (PFES), which was performed for 20 min twice dayly for 12 weeks. Although this treatment alone has no to only a moderate impact on SUI [7], we previously found it to be an indispensable addition to support the efficacy of cell injection [26].

Outcome Measures

The primary efficacy endpoint was the mean reduction in the Incontinence Episode Frequency (IEF) score at 12 weeks after cell implantation or of treatment with duloxetine or placebo. The IEF score was assessed before cell implantation and the start of the drug treatment, and 6 and 12 weeks after treatment initiation. The IEF was calculated as the number of incontinence episodes (IEs) experienced over 7 days prior to each assessment, based on a micturition diary recorded by the patients, comprising entries on time of voiding, urgency and leakage, and volume of excreted urine. The micturition diary was designed in accordance with EMA recommendations [34].

Secondary efficacy endpoints included the pairwise comparison of IEF score changes between treatment groups not considered in the primary analysis, the change from screening in the short pad-test results, the change from baseline in the visual analogue scale score (VAS) and the I-QoL score, responder rates reflecting the share of patients with defined reductions of the IEF score, and the change from baseline in the Clinical Global Impression (CGI) score. All outcome measures were assessed at baseline and 6 and 12 weeks after treatment start, details on these assessments are provided in the Supplementary Methods.

Safety Assessment

Concomitant medication and adverse events (AEs) were recorded at each study visit (Supplementary Methods). Separate analyses were performed for baseline AEs observed after enrolment but before any treatment, biopsy-emergent AEs occurring after biopsy and prior to cell implantation, and treatment-emergent AEs (TEAEs) seen after first intake of trial medication or after biopsy until trial end. Safety examinations including routine laboratory measures, a physical examination and assessment of vital signs were performed at screening and after 12 weeks of study, and the frequency, type, intensity and seriousness of AEs were documented.

Follow-Up

Follow-up investigations were performed at 12 and 48 months post-implantation and included only patients of five Bulgarian centres and only patients of the two cell implantation groups. The primary aim was to determine the long-term efficacy and safety of the cell injection treatment and to evaluate whether there were differences observable between the CI1 and CI2 group.

Statistical Analyses

The primary endpoint was analysed through confirmatory statistical analysis with a two-sample *t* test and 95% two-sided confidence intervals, which tests whether the pre-post difference of the IEF score shows a higher reduction in the cell implantation groups in comparison with the placebo control group. A hierarchical test scheme first analysed whether CI2 was superior to the C1 (duloxetine-placebo) group, and subsequently compared the CI2 group with the CI1 group. Secondary endpoints were analysed in an exploratory manner. The pairwise comparison of the change from baseline in the IEF score between the C2 (duloxetine) group and the other treatment groups was performed analogously to the analysis of the primary endpoint. All other continuous endpoints were analysed by calculating summary statistics and comparing groups by means of the Wilcoxon rank-sum test on a two-sided α-level of 5%. For categorical endpoints, absolute and relative frequencies by treatment groups were analysed and the groups were compared using the two-sided Fisher's exact test on a α level of 5%.

Details on statistics regarding sample size calculation, an interim analysis and imputation of missing values are outlined in the Supplementary Methods.

Results

Patient Characteristics

Figure 1 presents an overview on the disposition of patients enrolled in the study. Out of 319 patients screened, 263 were randomised to the treatment groups. A total of 36 patients were excluded from the safety analysis set (SAS), as they did not receive any treatment, and 10 patients were excluded from the intention-to-treat (ITT) set prior to analysis as they were not eligible for analysis. Baseline demographic data of the participants are summarised in Table 1 and show no significant differences between treatment groups. Other characteristics reflecting disease severity established before treatments are summarised in Table 2.

Primary Outcomes: Changes in IEF

Analysis of the primary efficacy endpoint revealed a reduction in the mean IEF score after 12 weeks of study,

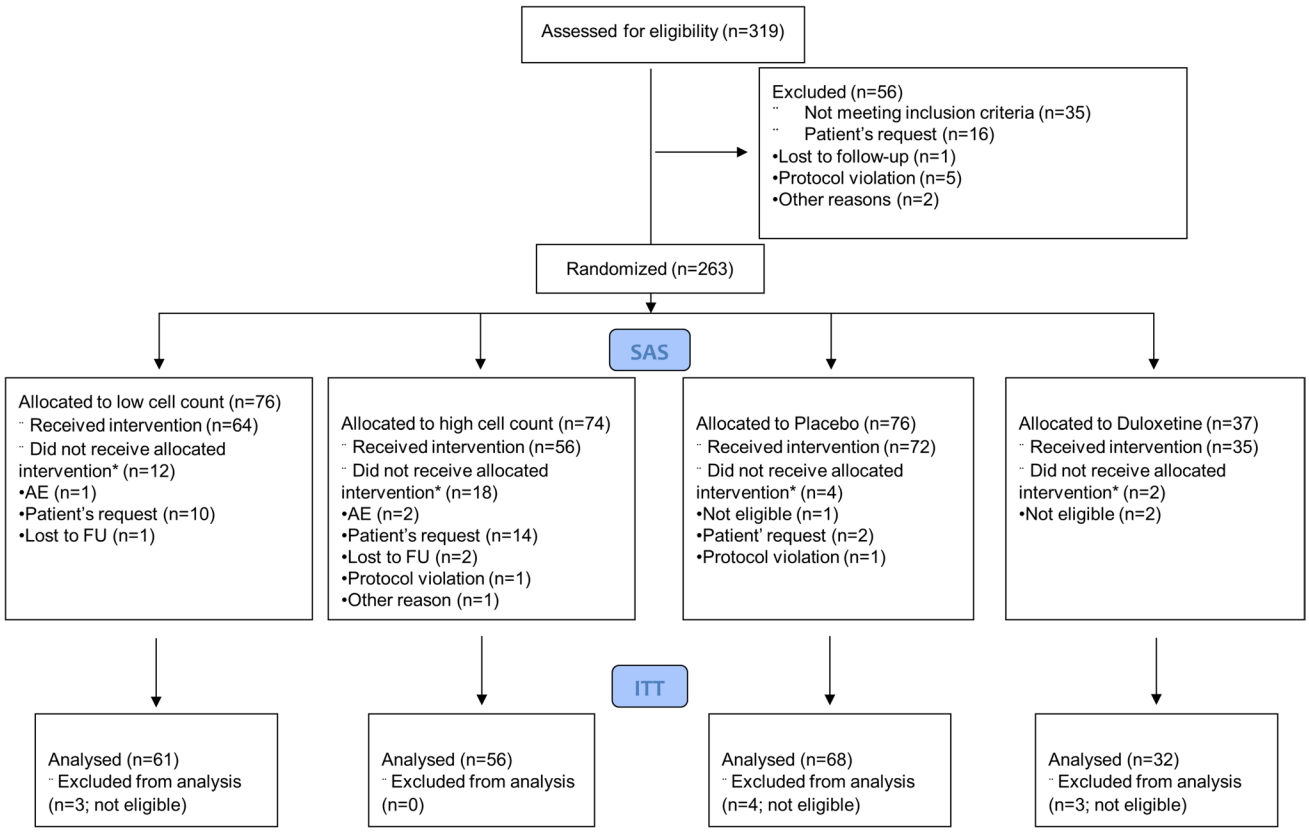

Fig. 1 Flow chart of study participants involved in the initial 12-week study. *multiple reasons possible, *AE* adverse event, *FU* follow-up, *SAS* safety analysis set, *ITT* intention to treat

Table 1 Demographic data and screening measurements (intention to treat, $N=217$)

Variable	Low cell count, $N=61$	High cell count, $N=56$	Placebo, $N=68$	Duloxetine, $N=32$
Age (years)	55.0 (12.97)	55.2 (11.07)	58.2 (11.85)	61.7 (11.73)
Height (cm)	162.0 (7.31)	162.1 (5.34)	161.7 (6.02)	160.6 (7.83)
Weight (kg])	70.91(13.01)	76.91 (17.02)	72.73 (17.16)	71.0 (14.90)
BMI (kg/m^2)	27.05 (4.98)	29.28 (6.40)	27.79 (6.11)	27.52 (5.09)
Race (%), white	100	100	100	100
Disease duration (months)	72.0 (66.15)	66.4 (65.6)	64.7 (57.02)	57.8 (53.74)

Values are mean (standard deviation)

BMI body mass index

Table 2 Summary statistics of incontinence and quality-of-life parameters at baseline and 12 weeks after treatment/start of therapy, and objective cure rates observed after 12 weeks (intention to treat, $N=217$)

Variable	Low cell count (CI1), $N=61$	High cell count (CI2), $N=56$	Placebo, $N=68$	Duloxetine, $N=32$
IEF (episodes/week)				
Pre-treatment, mean (SD)	25.2 (12.4)	28.6 (18.3)	25.1 (15.5)	26.8 (17.1)
Post-treatment, mean (SD)	8.8 (12.5)	10.2 (15.7)	15.2 (17.5)	13.8 (18.8)
Difference, mean (SD)	−16.4 (13.3)	−18.5 (18.7)	−9.7 (13.7)	−11.2 (19.6)
IEF objective cure rate, *n* (%)	21 (34.4)	10 (17.9)	7 (10.3)	5 (15.6)
Pad test (g)				
Pre-treatment, mean (SD)	15.4 (10.2)	16.0 (10.4)	14.6 (9.5)	17.4 (10.9)
Post-treatment, mean (SD)	4.4 (6.8)	4.8 (5.6)	7.6 (6.7)	10.3 (11.7)
Difference, mean (SD)	−11.0 (9.0)	−11.3 (8.2)	−7.8 (10.0)	−8.0 (12.9)
Pad test objective cure rate, *n* (%)	34 (55.7)	20 (35.7)	10 (14.7)	7 (21.9)
VAS (cm)				
Pre-treatment, mean (SD)	4.1 (2.0)	4.1 (2.3)	4.1 (1.9)	3.8 (1.9)
Post-treatment, mean (SD)	2.1 (2.2)	2.2 (2.1)	2.7 (2.0)	2.1 (2.1)
Difference, mean (SD)	−2.0 (2.5)	−1.9 (2.4)	−1.5 (2.2)	−1.7 (2.0)
I-QoL (total scores)				
Pre-treatment, mean (SD)	43.5 (21.2)	41.7 (21.9)	43.6 (18.0)	42.4 (17.2)
Post-treatment, mean (SD)	74.8 (26.0)	74.8 (21.4)	58.9 (24.0)	65.6 (22.9)
Difference, mean (SD)	31.3 (25.3)	33.0 (21.4)	14.6 (22.0)	22.1 (18.3)

Results of primary (IEF) and secondary efficacy variables before and 12 weeks after cell implantation or start of control treatment

Pad test: weight of urine, lost during a standardised pad test

Objective cure rates after 12 weeks defined by the amount of lost urine in a standardised 1-h pad test, or by self-reported incontinence episodes per week. Patient was regarded as cured if the short-pad test result was < 2 g or the IEF was < 2

SD standard deviation, *IEF* incontinence episode frequency documented by the patients over a 1-week prior to each visit, *VAS* visual analogue scale ranging from 0 (no complaints) to 100 (worst complaints), *I-QoL* Incontinence Quality of Life questionnaire, sum scores (maximum score = 100)

both in the cell implantation groups and in the control groups. The reduction was most pronounced in the Cl2 group, with a reduction of 18.5, and slightly less pronounced in the Cl1 group, with a mean reduction of 16.4. A reduction that was 40% and 48% lower was seen in the duloxetine and the placebo group respectively (Table 2). Pairwise comparisons demonstrated the superiority of Cl1 ($p=0.0019$, CI −12.5, −2.3) and Cl2 ($p=0.002$, CI −15.9, −2.9) over placebo, but no significant difference versus duloxetine.

Pad Tests

In the pad tests, the mean reduction in both cell implantation groups amounted to 71%, which compares with 46% in the placebo group and 39% in the duloxetine group (Table 2). The changes in the pad weight from baseline to week 12 showed the superiority ofCl1 and Cl2 over both placebo ($p=0.0005$, CI −7, −2; $p<0.0001$, CI −8, −3) and the duloxetine treatment ($p=0.0051$, CI −7.5, −1; $p=0.0023$, CI −9, −2] respectively).

Objective Cure Rates

Considering patients with a threshold of IEF < 2 or a pad test result < 2 g as objectively cured, we found a higher objective cure rate in the cell implantation groups (IEF criterion: 18% and 34%; pad test criterion: 36% and 56%) than in the duloxetine and the control group (IEF criterion: 10% and 16%; pad test criterion: 15% and 22%). Statistical post hoc analysis revealed the superiority of Cl1 over placebo for the IEF criterion ($p=0.0012$, CI 6.9%; 40.3%), and based on the pad test results, Cl1 showed superiority over Cl2 ($p=0.0421$, CI −36.8%, −1.1%), over duloxetine ($p=0.0036$, CI 11.2%, 52%) and over placebo ($p<0.001$, CI 23.9%; 55.7%). In addition, Cl2 proved to be superior to placebo in this regard ($p=0.0106$, CI 3.2%, 38.2%).

VAS Scores

Visual analogue scale scores showed a decrease from baseline in disease complaints in all groups (Table 2) and indicated a tendency, but no statistical significance, towards a

larger improvement in the cell implantation groups than in the control groups.

I-QoL Scores

The more pronounced improvement in the I-QoL score of patients treated with cells by more than 30 points, which compared with an improvement by 15 or 22 points with placebo and duloxetine treatment respectively (Table 2), was statistically significant. Thus, pairwise comparisons indicated superiority of the Cl1 and Cl2 group over both placebo (Cl1: $p = 0.0002$, CI 7.9, 26.1, Cl2: $p \leq 0.0001$, CI 10.2, 27.4) and duloxetine (Cl1: $p = 0.0438$, CI 0, 20.8; Cl2 $p = 0.0094$, CI 2.3, 22.6) at 12 weeks after treatment initiation.

Responders

Applying pre-defined response criteria regarding IEF reduction, the responder analysis showed higher rates of responders in the cell implantation groups than in the control groups 12 weeks after treatment (Table 3). Pairwise comparisons of all treatment groups in the case of the 50% definition of response showed superiority of both cell implantation groups over placebo (Cl1: $p = 0.0003$, CI 14.2%, 46.9%; Cl2: $p < 0.0001$, CI 19%, 51.8%) and over duloxetine (Cl1: $p = 0.0369$, CI 0.9%, 42.7%; Cl2: $p = 0.0141$, CI 5.4%, 47.3%). Similarly, both cell implantation groups were also superior to placebo in the case of a 75% reduction in IEF (Cl1: $p = 0.0002$, CI 17.2%, 49.6%; Cl2: $p = 0.0090$, CI 5.9%, 40%), and the Cl1 group was also significantly superior to duloxetine ($p = 0.0043$, CI 11%, 51.8%). Finally, consistent results were also obtained for the 90% definition of response, with both cell implantation groups being superior to placebo (Cl1: $p = 0.0001$, CI 13.8%, 46.6%; Cl2: $p = 0.0229$, CI −1%, 33.8%) and Cl1 being superior to duloxetine ($p = 0.0235$, CI 2.5%, 44%). In contrast, pairwise comparison of responders of any type between both cell implantation groups and between the control groups did not indicate any significant differences ($p > 0.05$).

CGI Scale

Investigator assessment of treatment-induced changes and treatment efficacy determined after 12 weeks post-injection based on the CGI scale indicated that most patients in the cell implantation groups had improved. In the Cl1 group shares were 47.5% (very much), 23.0% (much) and 13.1% (minimal) of the patients, in the Cl2 group these were 37.5% (very much), 39.3% (much) and 14.3% (minimal). In the control groups, improvement was reported for fewer patients. For placebo 11.8% (very much), 17.6% (much) and 19.1% (minimal) of the patients showed improvements, for duloxetine 15.6% (very much), 21.9% (much) and 21.9% (minimal). Pairwise comparisons showed the superiority of Cl1 and Cl2 over placebo ($p = 0.001$, $p = 0.0001$ respectively). The comparison of the cell implantation with duloxetine treatment did not show superiority ($p > 0.05$).

Extension Study

Patients included in the 12- and 48-month follow-up comprised 23 and 19 patients of the CI1 and CI2 group, these patients being slightly older, showing a slightly higher BMI on average, but a disease duration preceding the study comparable with that of the overall population (Supplementary Table S4).

The significant decrease in IEF from baseline to week 12 observed in these patients was sustained at both the 12-month and the 48-month follow-up (Fig. 2). The decrease from baseline to 48 months of 19.21 ± 11.89 in the CI1 group exceeded that of 13.95 ± 10.78 in the CI2 group, but this was not statistically significant.

The VAS scores remained reduced (Supplementary Table S5) and I-QoL scores remained elevated (Supplementary Table S6) in both groups, with changes in the CI1 group exceeding those in the CI2 group, but these differences were not significant either.

Responder rates with IEF reduction of at least 50, 75 or 90% remained comparable after 48 months with those seen after 12 weeks post-implantation, amounting to

Table 3 Frequency of responders 12 weeks after treatment/start of therapy by response definition [50%, 75%, 90%] and treatment group (ITT, LOCF)

Definition of response	Responder rate, % (n_r) after 12 weeks			
	Low cell count (CI1), $N = 61$	High cell count (CI2), $N = 56$	Placebo, $N = 68$	Duloxetine, $N = 32$
[50%]	75.4 (46)	80.4 (45)	44.1 (30)	53.1 (17)
[75%]	60.7 (37)	50.0 (28)	26.5 (18)	28.1 (9)
[90%]	42.6 (26)	28.6 (16)	11.8 (8)	18.8 (6)

n number of patients, n_r number of responders

approximately 70–80%, 60% and 40% in both cell groups respectively (Supplementary Table S7).

The CGI-based assessments of improvements at 48 months indicated that in the CI1 group 30.4%, 13.0% and 26.1% were very much, much and minimally improved compared with baseline respectively; in the Cl2 group, these numbers were 26.3% (very much), 10.5% (much) and 31,6% (minimal). Although these rates were lower than those observed in the overall population at 12 weeks, the average CGI values of the follow-up patients had improved from 3.53 at baseline (CI1 and CI2) to 2.57 (CI1) and 2.84 (CI2) after 48 months.

Safety

Treatment-emergent adverse events occurred in 27, 36, 46 and 43% of the patients in the CI1, CI2, placebo and the duloxetine group respectively (Table 4), being either mild or moderate in at least 90% of all cases. Pairwise comparisons of TEAE frequency during the initial 12 weeks of study revealed a statistically significantly higher rate of TEAEs with placebo than with injection of a low cell count ($p = 0.0355$), whereas all other comparisons showed no significant differences between groups ($p > 0.05$).

Adverse events with at least a possible relationship to the treatment occurred at a significantly higher rate in control groups than in cell implantation groups (Cl1 vs placebo: $p = 0.0108$; Cl1 vs duloxetine: $p = 0.0241$; Cl2 vs placebo: $p = 0.0010$; Cl2 vs duloxetine: $p = 0.0036$). In the cell implantation groups ($N = 120$) the most common AEs with at least a possible relation to treatment were urethral or post-procedural haemorrhage with a frequency of 7 episodes of mild intensity. Urgency or frequency symptoms of mild intensity were observed in 3 cases and urinary tract infections in 2 cases.

The 23 biopsy-emergent AE episodes observed in the patients with cell injection ($N = 133$) occurred in 20 patients (15.0%), and 2 episodes (8.7%) of post-procedural bleeding were assessed as probably related to treatment.

No patient died during the trial. Of the 7 observed treatment-emergent SAE episodes in 6 patients, 4 episodes were assessed as "not related" to treatment and 3 episodes in the control groups were assessed as at least probably related.

Assessment of safety variables at 48 months post-implantation showed neither related nor non-related AEs in any of the 43 patients investigated.

Discussion

The present study confirms and extends previous investigations showing the beneficial effects of the injection of aSMDCs to treat SUI [26, 27, 31, 32] in combination with electrical stimulation. Evaluating the reduction of IEF after 12 weeks compared with baseline as the primary endpoint and additionally assessing pad-test results, VAS, I-QoL and CGI scale, we observed the superiority of both a low and a high cell concentration over placebo regarding all endpoints except for VAS. In addition, cell implantation showed superiority over duloxetine treatment in pad testing and I-QoL improvement, higher responder rates in the low cell count group for all responder definitions, and for the 50% response when comparing a high cell count with duloxetine. Overall, cell injection as a treatment of SUI was thus superior to placebo and in many aspects also to an established treatment option, the application of duloxetine.

In addition, post-hoc analysis of the 12-week data revealed higher objective cure rates, defined as a pad test result of < 2 g or an IEF score of < 2, in the cell implantation groups compared with the control groups. Although pad testing may appear as an artificial simulation, with exercises not truly reflecting daily lives, the IEF score is obtained under everyday conditions that may also include episodes of extreme physical activity. Thus, a share of 18% (Cl2) and 34% (Cl1; IEF) and 36% (Cl2) and 56% (Cl1; pad test) of the patients achieved an objective cure following cell injection, which compares with only 10 and 16% (IEF) and 15 and 22% (pad test) with placebo and duloxetine respectively, which appears impressive and meaningful in a real-life context.

Importantly, in the extension study comprising 43 patients with cells injected, the reduction of IEF was largely sustained over 12 and 48 months, as were improvements in I-QoL, responder rates and CGI. Thus, different from only transient improvements observed with bulking agents [15, 16] or, in the context of faecal incontinence, with a placebo injection [29], the beneficial effects did not vanish over time.

Besides comparing cell injection with placebo, we also tested the potential superiority of a high (CI2) over a low dose (CI1) of cells injected but did not detect any difference in this regard. This agrees with the study by Blaganje et al. [30], but contradicts two studies where higher cell numbers produced better results [31, 32], whereas yet other studies with very high cell numbers yielded no effect at all beyond that of the placebo treatment [27, 35]. A tentative explanation for this discrepancy is that with the transurethral ultrasound-guided method of cell application used here and by Blaganje et al. [26, 30] a higher proportion of cells can reach the sphincter, enabling a required minimum number of potent cells to fuse with existing cells and trigger muscular restoration processes. A simple bulking effect exerted by the injection appears unlikely given the small cell injection volume of only 2 ml with a viscosity comparable with saline solution. A study comparing the impact of injecting autologous fat showed no superiority to saline placebo injection and both treatments were far less effective than cell therapy

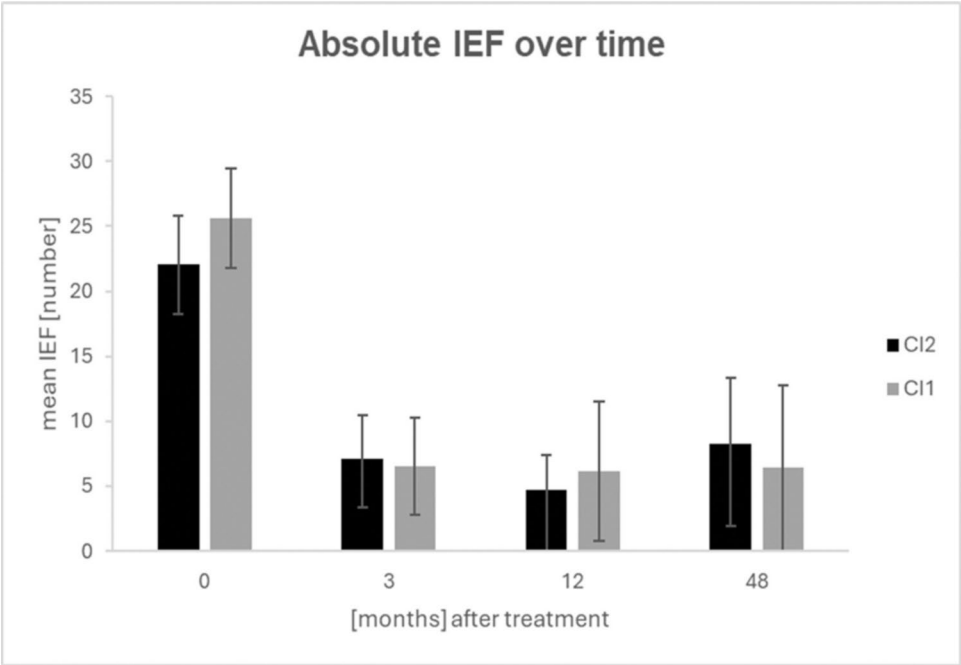

Fig. 2 Change in incontinence episode frequency (*IEF*) score over time in the follow-up population. Data are means and 95% confidence intervals of 23 and 19 patients in the CI1 and CI2 groups respectively

[36]. In a recent trial examining MDSC injection into the anal sphincter to treat faecal incontinence, cell-free solution also produced significant but only transient benefits clearly exceeded by those elicited by cell injection [29].

A more plausible factor explaining differences between failed previous investigations and the more successful present study is the concomitant use of PFES, which has been found to be crucial for the therapeutic success in a recent study applying our cell injection technique [26]. In line, in a mouse dystrophic muscle model, electrical stimulation clearly enhanced the engraftment of injected muscle-derived stem cells and the recruitment of motor units to dystrophic muscle, resulting in significantly improved muscle strength, whereas cell injection alone was largely ineffective [37]. Improved formation of neuromuscular junctions could also be demonstrated in a myoblast-neuron co-culture cell model exposed to electric stimulation [38], and electrical stimulation of the anal sphincter in a rat model elicited increased width of mucosal, submucosal and muscle layers, internal sphincter hyperplasia, and external sphincter hypertrophy [39]. Alternatively, improved integration of injected muscle-derived stem cells into existing muscle tissue can also be evoked through muscle training, as shown in mice subjected to forced swimming [40] or to treadmill running [41].

Table 4 Summary of treatment-emergent adverse events (TEAEs) in the treatment groups (safety set, $N=227$)

	Low cell count (CI1), $N=64$	High cell count (CI2), $N=56$	Placebo, $N=72$	Duloxetine, $N=35$
N_{TEAE} (%)	17 (26.6)	20 (35.7)	33 (45.8)	15 (42.9)
n_e	28	28	82	32
Intensity based on n_e, *n* (%)				
Mild	23 (82.1)	23 (82.1)	41 (50.0)	20 (62.5)
Moderate	4 (14.3)	5 (17.9)	33 (40.2)	10 (31.3)
Severe	1 (3.6)	0 (0.0)	8 (9.8)	2 (6.3)
Causal relation to IMP based on n_e, *n* (%)				
Related	5 (17.9)	2 (7.1)	7 (8.5)	8 (25.0)
Probable	4 (14.3)	4 (14.3)	41 (50.0)	20 (62.5)
Possible	2 (7.1)	1 (3.6)	12 (14.6)	1 (3.1)
Not related	15 (53.6)	21 (75.0)	20 (24.4)	3 (9.4)
Not assessable	2 (7.1)	0 (0.0)	2 (2.4)	0 (0.0)

N number of patients in treatment group, N_{TEAE} number of patients with TEAE; n_e number of episodes, *IMP* investigational medicinal product

Altogether, these studies and our own observations confirm meta-studies indicating the general benefits of electrical stimulation [7, 42] and, more generally, of combined therapeutic approaches [43, 44] in the treatment of urinary and faecal incontinence disorders.

Regarding the safety aspects of our trial, we showed that injection of aSMDC is a safe and well-tolerated treatment that did not produce any unexpected adverse events. Local complications after biopsy or cell implantation were of mild intensity and did not require any re-interventions. Also, severe voiding dysfunction was not observed, which is in line with other observations [30, 32, 45].

Limitations of the Study

A limitation of the study is that it did not include an injection regime as placebo treatment, as it appears conceivable that the tissue injury resulting from injection could by itself have induced regenerative processes, which could also have blurred the differences between injections with low and high cell numbers. However, as pointed out above, both the injection of saline and fat cells [29, 36] had either no or only minor effects and these were very transient, whereas those observed here persisted up to 48 months. The latter points to another limitation, i.e. a comparatively small patient group in the long-term follow-up, and clearly larger patient numbers per treatment arm should be included in future studies.

Future Approaches

In addition to combining cell injection with PFES as applied here, future therapies may include further treatments, such as, for example, pelvic floor muscle training or the injection of bulking agents [18, 46]. By potentially exerting currently unknown synergistic effects, such approaches could lead to even better long-term stabilisation, extend the share of patients benefitting from cell injection therapy, and increase the number of eventually fully cured patients. Finally, as our study misses an objective measure of muscle regeneration, a future trial should include assessment of maximum urethral closure pressure and/or other measures of muscle function.

Conclusions

In this study, dose-independent safety and both short- and long-term effectiveness of aSMDC implantation in combination with electrical stimulation for the treatment of female SUI was demonstrated. Future research should investigate additional therapeutic combination approaches and examine a larger patient group over an extended follow-up period.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00192-025-06079-0

Acknowledgements The authors would like to thank the trial participants and investigators, without whom this work could not have been performed. The clinical trial was conducted in collaboration with Pierrel Research Europe GmbH, Essen, Germany. The authors thank Gerhard Krumschnabel (www.medical-writing.at) for editorial and medical writing support for this manuscript.

Authors' Contributions A. Rose: manuscript writing, protocol development; H. Rübben: coordinating investigator, protocol development.

Funding This research was funded by Innovacell GmbH, Innsbruck, Austria. Duloxetine and duloxetine-placebo capsules were supplied by Pierrel Research IMP S.r.l.

Data Availability The data that support the findings of this study are available from the corresponding author, AR, upon reasonable request.

Declarations

Ethics Statement The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines and the World Medical Association Declaration of Helsinki 1964 and its amendments and subsequent clarifications. Ethical approval was obtained from the Ethics Committee for Multicentre Clinical Trials/Bulgarian Drug Agency (Bulgaria), the Ethik-Kommission der Ärztekammer Nordrhein/Paul-Ehrlich-Institut—Bundesamt für Sera und Impfstoffe (Germany), the Bioethics Committee-Romanian College of Physicians (Bucharest, Timisiora, Cara-Severin, Cluj), National Medicines and Medical Devices Agency (Romania), and the Multicenter Ethics Committee Hospital Brno/State/Institute for Drug Control and the Ethics Committee Hospital Hradec Králové/State/Institute for Drug Control (Czech Republic). Written informed consent was obtained from all patients involved in this trial.

Conflicts of Interest Achim Rose and Herbert Rübben are Consultants for Innovacell Biotechnology AG.

References

1. Haylen BT, De Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J, et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Neurourol Urodyn. 2010;29:4–20.
2. Abrams P, Andersson KE, Apostolidis A, Birder L, Bliss D, Brubaker L, et al. 6th International Consultation on Incontinence. Recommendations of the International Scientific Committee: evaluation and treatment of urinary incontinence, pelvic organ prolapse and faecal incontinence. Neurourol Urodyn. 2018;37:2271–2.
3. Colleselli K, Stenzl A, Eder R, Strasser H, Poisel S, Bartasch G. The female urethral sphincter: a morphological and topographical study. J Urol. 1998;160:49–54. https://doi.org/10.1016/S0022-5347(01)63025-8.
4. Frauscher F, Helweg G, Strasser H, Enna B, Klauser A, Knapp R, et al. Intraurethral ultrasound: diagnostic evaluation of the striated urethral sphincter in incontinent females. Eur Radiol. 1998;8:50–3. https://doi.org/10.1007/s003300050336.

5. Strasser H, Tiefenthaler M, Steinlechner M, Eder I, Bartasch G, Konwalinka G. Age dependent apoptosis and loss of rhabdosphincter cells. J Urol. 2000;164:1781–5. https://doi.org/10.1016/S0022-5347(05)67106-6.
6. Nambiar AK, Arlandis S, Bø K, Cobussen-Boekhorst H, Costantini E, de Heide M, et al. European Association of Urology guidelines on the diagnosis and management of female non-neurogenic lower urinary tract symptoms. I. Diagnostics, overactive bladder, stress urinary incontinence, and mixed urinary incontinence. Eur Urol. 2022;82:49–59.
7. Stewart AF, Berghmans B, Bø K, Glazener CMA. Electrical stimulation with non-implanted devices for stress urinary incontinence in women. Cochrane Database Syst Rev. 2017;12:CD012390.
8. Cardozo L, Lange R, Voss S, Beardsworth A, Manning M, Viktrup L, Zhao YD. Short-and long-term efficacy and safety of duloxetine in women with predominant stress urinary incontinence. Curr Med Res Opin. 2010;26:253–61. https://doi.org/10.1185/03007990903438295.
9. Rodriguez-Amorim D, Olivares JM, Spuch C, Rivera-Baltanás T. A systematic review of efficacy, safety, and tolerability of duloxetine. Front Psychiatry. 2020;11:554899. https://doi.org/10.3389/FPSYT.2020.554899.
10. Fusco F, Abdel-Fattah M, Chapple CR, Creta M, La Falce S, Waltregny D, Novara G. Updated systematic review and meta-analysis of the comparative data on colposuspensions, pubovaginal slings, and midurethral tapes int he surgical treatment of female stress urinary incontinence. Eur Urol. 2017;72:567–91. https://doi.org/10.1016/j.eururo.2017.04.026.
11. Ellington DR, Erekson EA, Richter HE. Outcomes of surgery for stress urinary incontinence in the older woman. Clin Geriatr Med. 2015;31:487–505.
12. Marcelissen T, Van Kerrebroeck P. Overactive bladder symptoms after midurethral sling surgery in women: risk factors and management. Neurourol Urodyn. 2018;37:83–8.
13. Gomelsky A, Steckenrider H, Dmochowski R. Urgency and urgency incontinence following stress urinary incontinence surgery: a review of evaluation and management. Indian J Urol. 2022;38:268–75.
14. Hoe V, Yao HH, Gough K, O'Connell HE. Long-term outcomes of polyacrylamide hydrogel treatment in women with stress urinary incontinence. BJU Int. 2022;130:22–4.
15. Kirchin V, Page T, Keegan PE, Atiemo KO, Cody JD, McClinton S, Aluko P. Urethral injection therapy for urinary incontinence in women. Cochrane Database Syst Rev. 2017;2017:1–67. https://doi.org/10.1002/14651858.CD003881.pub4.
16. McGowan S, Campbell P, Dolan L. The use of urethral bulking agents in the treatment of stress urinary incontinence. Obstet Gynaecol. 2020;22:137–46. https://doi.org/10.1111/TOG.12647.
17. Hoe V, Haller B, Yao HH, O'Connell HE. Urethral bulking agents for the treatment of stress urinary incontinence in women: a systematic review. Neurourol Urodyn. 2021;40:1349–88. https://doi.org/10.1002/NAU.24696.
18. Hillary CJ, Roman S, MacNeil S, Aicher WK, Stenzl A, Chapple CR. Regenerative medicine and injection therapies in stress urinary incontinence. Nat Rev Urol. 2020;17:151–61.
19. Schmid FA, Koudy Williams J, Kessler TM, Stenzl A, Aicher WK, Andersson KE, Eberli D. Treatment of stress urinary incontinence with muscle stem cells and stem cell components: chances, challenges and future prospects. Int J Mol Sci. 2021;22:3981. https://doi.org/10.3390/ijms22083981.
20. Chancellor MB, Yokoyama T, Tirney S, Mattes CE, Ozawa H, Yoshimura N, 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. https://doi.org/10.1002/(SICI)1520-6777(2000)19:3%3c279::AID-NAU9>3.0.CO;2-M.
21. Mitterberger M, Pinggera GM, Marksteiner R, Margreiter E, Plattner R, Klima G, Strasser H. Functional and histological changes after myoblast injections in the porcine rhabdosphincter. Eur Urol. 2007;52:1736–43. https://doi.org/10.1016/j.eururo.2007.05.007.
22. Li Y, Wen Y, Wang Z, Wei Y, Wani P, Green M, et al. Smooth muscle progenitor cells derived from human pluripotent stem cells induce histologic changes in injured urethral sphincter. Stem Cells Transl Med. 2016;5:1719–29. https://doi.org/10.5966/SCTM.2016-0035.
23. Badra S, Andersson KE, Dean A, Mourad S, Koudy Williams J. Long-term structural and functional effects of autologous muscle precursor cell therapy in a nonhuman primate model of urinary sphincter deficiency. J Urol. 2013;190:1938–45. https://doi.org/10.1016/J.JURO.2013.04.052.
24. Campos de Carvalho AC, Kassi-Brunswick TG, Bastos Carvalho A. Cell-based therapies for heart failure. Front Pharmacol. 2021;12:641116. https://doi.org/10.3389/fphar.2021.641116.
25. Biressi S, Filareto A, Rando TA. Stem cell therapy for muscular dystrophies. J Clin Invest. 2020;130:5652–64. https://doi.org/10.1172/JCI142031.
26. Blaganje M, Lukanović A. The effect of skeletal muscle-derived cells implantation on stress urinary incontinence and functional urethral properties in female patients. Int J Gynaecol Obstet. 2022;157:444–51. https://doi.org/10.1002/IJGO.13853.
27. Ismail S, Morin M, Tu LM. Assessment of the effects of autologous muscle-derived cell injections on urethral sphincter morphometry using 3D/4D ultrasound. World J Urol. 2020;38:2881–9. https://doi.org/10.1007/S00345-020-03076-4.
28. Seval MM, Koyuncu K. Current status of stem cell treatments and innovative approaches for stress urinary incontinence. Front Med. 2022;9:1073758. https://doi.org/10.3389/FMED.2022.1073758.
29. Frudinger A, Gauruder-Burmester A, Graf W, Lehmann JP, Gunnarsson U, Mihov M, et al. Skeletal muscle-derived cell implantation for the treatment of fecal incontinence: a randomized, placebo-controlled study. Clin Gastroenterol Hepatol. 2023;21:476-486.e8. https://doi.org/10.1016/J.CGH.2022.07.039.
30. Blaganje M, Lukanović A. Intrasphincteric autologous myoblast injections with electrical stimulation for stress urinary incontinence. Int J Gynecol Obstet. 2012;117:164–7. https://doi.org/10.1016/j.ijgo.2011.11.029.
31. Carr LK, Robert M, Kultgen PL, Herrschorn S, Birch C, Murphy M, Chancellor MB. Autologous muscle derived cell therapy for stress urinary incontinence: a prospective, dose ranging study. J Urol. 2013;189:595–601. https://doi.org/10.1016/j.juro.2012.09.028.
32. Peters KM, Dmochowski RR, Carr LK, Robert M, Kaufman MR, Sirls LT, et al. Autologous muscle derived cells for treatment of stress urinary incontinence in women. J Urol. 2014;192:469–76. https://doi.org/10.1016/j.juro.2014.02.047.
33. Klingler H, Maderbacher H, Primus G, Fischer M, Lüftenegger W, Wachter J, et al. Leitlinien Blasenfunktionsstörungen. J Urol Urogynäkol. 2007;14:4–27.
34. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on the clinical investigation of medicinal products for the treatment of urinary incontinence. 2002. London.
35. Jankowski RJ, Tu LM, Carlson C, Magali R, Carlson K, Quinlan D, et al. A double-blind, randomized, placebo-controlled clinical trial evaluating the safety and efficacy of autologous muscle derived cells in female subjects with stress urinary incontinence. Int Urol Nephrol. 2018;50:2153–65. https://doi.org/10.1007/s11255-018-2005-8.
36. Lee PE, Kung RC, Drutz HP. Periurethral autologous fat injection as treatment for female stress urinary incontinence: a randomized

double-blind controlled trial. J Urol. 2001;165:153–8. https://doi.org/10.1097/00005392-200101000-00037.
37. Distefano G, Ferrari RJ, Weiss C, Deasy BM, Boninger ML, Kelley Fitzgerald G, et al. Neuromuscular electrical stimulation as a method to maximize the beneficial effects of muscle stem cells transplanted into dystrophic skeletal muscle. PLoS One. 2013;8:e54922. https://doi.org/10.1371/JOURNAL.PONE.0054922.
38. Lozano R, Gilmore KJ, Thompson BC, Stewart EM, Waters AM, Romero-Ortega M, Wallace GG. Electrical stimulation enhances the acetylcholine receptors available for neuromuscular junction formation. Acta Biomater. 2016;45:328–39. https://doi.org/10.1016/J.ACTBIO.2016.08.006.
39. de Sousa HA, da Glória da Silva M, de Paula de Barbosa K, Vianna LM, Pacheco YG, Pimenta de Godoy JR, Kuckelhaus SAS. Electrical stimulation structurally affects the tissues of the rectum and anus of nulliparous rats. J Anat. 2017;231:398–404. https://doi.org/10.1111/JOA.12635.
40. Bouchentouf M, Benabdallah BF, Mills P, Tremblay JP. Exercise improves the success of myoblast transplantation in mdx mice. Neuromuscul Disord. 2006;16:518–29. https://doi.org/10.1016/j.nmd.2006.06.003.
41. Ambrosio F, Ferrari RJ, Distefano G, Plassmeyer JM, Carvell GE, Deasy BM, et al. The synergistic effect of treadmill running on stem-cell transplantation to heal injured skeletal muscle. Tissue Eng Part A. 2010;16:839–49. https://doi.org/10.1089/TEN.TEA.2009.0113.
42. Sarmento ALC, Sá BS, Vasconcelos AG, Arcanjo DSR, Durazzo A, Lucarini M, et al. Perspectives on the therapeutic effects of pelvic floor electrical stimulation: a systematic review. Int J Environ Res Public Health. 2022;19:14035. https://doi.org/10.3390/IJERPH192114035.
43. Ayeleke RO, Hay-Smith EJC, Omar MI. Pelvic floor muscle training added to another active treatment versus the same active treatment alone for urinary incontinence in women. Cochrane Database Syst Rev. 2015;2015:CD010551. https://doi.org/10.1002/14651858.CD010551.PUB3.
44. Moritz CT, Ambrosio F. Regeneration rehabilitation: combining stem cell therapies and activity-dependent stimulation. Pediatr Phys Ther. 2017;20(Suppl 3):S10–1. https://doi.org/10.1097/PEP.0000000000000378.
45. Sèbe P, Doucet C, Cornu JN, Ciofu C, Costa P, Dies de Medina SG, et al. Intrasphincteric injections of autologous muscular cells in women with refractory stress urinary incontinence: a prospective study. Int Urogynecol J. 2011;22:183–9. https://doi.org/10.1007/s00192-010-1255-5.
46. Li H, Westney OL. Injection of urethral bulking agents. Urol Clin North Am. 2019;46:1–15. https://doi.org/10.1016/J.UCL.2018.08.012.