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Original Article

Validation of human umbilical cord mesenchymal stem cell therapy in treating stress urinary incontinence in rat models

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Abstract: Objective: To investigate the therapeutic efficacy and underlying mechanisms of human umbilical cord mesenchymal stem cell (HUCMSC) transplantation in rats with stress urinary incontinence (SUI). Methods: SUI rat models were established through ovariectomy combined with vaginal dilation. The model rats were randomly divided into two groups: the HUCMSC treatment group and the model control group. The treatment group received periurethral injections of approximately 5×10^6 HUCMSCs, while the model control group received an equal volume of normal saline. A separate group of normal rats injected with normal saline served as the normal control group. Leak point pressure (LPP) and maximum bladder capacity (MBC) were measured at two time points: 1 week post-modeling and 2 weeks post-transplantation. In addition to LPP and MBC assessments, hematoxylin-eosin (HE) and Masson staining were performed to evaluate inflammatory cell infiltration, as well as damage and recovery of muscle and collagen fibers in the urethral sphincter tissue. Immunohistochemical staining was used to analyze the expression of myosin heavy chain (MHC) and protein gene product 9.5 (PGP9.5) in the rat urethral sphincters. Results: One week after SUI modeling, the model group exhibited significantly lower levels of LPP and MBC compared with the normal control group ($P < 0.05$), respectively. Two weeks following HUCMSC intervention, urodynamic assessments demonstrated that the treatment group had significantly higher LPP and MBC values than the model control group ($P < 0.05$), and with no significant difference observed relative to the normal control group ($P > 0.05$). Hematoxylin-eosin (HE) staining revealed reduced inflammatory cell infiltration and relatively intact urethral lumen architecture in the treatment group, consistent with morphological features of the normal control group. Masson staining indicated recovery and proliferation of muscle and collagen fibers in the treatment group, in contrast to severe structural damage and depletion of these fibers in the model group. Immunohistochemical analysis showed that the expression levels of MHC and PGP9.5 in the treatment group were significantly higher than those in the model control group ($P < 0.05$), while no significant difference was noted compared with the normal control group ($P > 0.05$). Conclusion: HUCMSC transplantation effectively ameliorates SUI in rats by repairing injured urethral sphincters and peripheral nerves, thereby improving urinary control function.

Keywords: Stress urinary incontinence (SUI), rat models, human umbilical cord mesenchymal stem cells (HUCMSCs), urine leak point pressure, maximum bladder capacity

Introduction

SUI significantly impairs the quality of life in middle-aged and elderly women, with its incidence increasing alongside global population aging. The global prevalence of SUI varies substantially across studies due to differences in survey methodologies and study populations, ranging from 10% to 70% [1]. Specifically, in the United States, the prevalence of SUI among adult women and adult men with prostate or bladder cancer was reported as 46% and 31%, respectively, between 2017 and 2020 [2, 3]. In China, the prevalence of SUI in women aged 20 years and older reaches 18.9% [4]. To date, over 200 therapeutic approaches for SUI have been documented. For instance, obstetrician-gynecologists commonly employ periurethral filler injections to repair damaged urethral sphincters; however, such treatments are not widely adopted clinically due to the limited tissue regenerative capacity, poor integration with

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host tissues, and rapid degradation of filler materials. Consequently, there is a growing demand for effective, safe, and regenerative injectable materials for urethral intervention in SUI management [5].

In recent years, mesenchymal stem cells (MSCs) have exhibited promising therapeutic potential in treating urinary incontinence in both clinical and preclinical studies. Nevertheless, most clinical trials are constrained by small sample sizes, short follow-up durations, and undefined mechanisms of action, hindering their widespread clinical translation. HUCMSCs represent an ideal candidate for urinary incontinence treatment [6], characterized by multiple advantages: ready availability, self-renewal and differentiation capacities, stable genetic profiles, favorable safety profiles, low immunogenicity, immunomodulatory properties, robust hematopoietic support, and broad clinical applicability for treating diverse diseases [7].

In this study, urodynamic parameters including LPP and MBC were measured to evaluate urethral function. Hematoxylin-eosin (HE) staining and Masson staining were used to assess inflammatory cell infiltration, urethral lumen integrity, and the damage/recovery status of muscle and collagen fibers in the urethral sphincter. Immunohistochemical analysis was performed to detect the expression of MHC and PGP9.5, a pan-neuronal marker, is widely used to evaluate axonal density and nerve repair [8], while MHC reflects muscle fiber maturation status; both markers are critical for assessing the functional recovery of neural and muscular components [9].

This study aimed to investigate the therapeutic effects of HUCMSC transplantation in a rat model of SUI induced by urethral injury, via local injection of HUCMSCs into the urethral wall. Specifically, we evaluated the amelioration of urethral tissue damage and the survival and functional recovery of transplanted HUCMSCs in injured tissues.

The results demonstrated that HUCMSC transplantation reduced inflammatory cell infiltration, promoted muscle and collagen fiber proliferation, and upregulated MHC and PGP9.5 expression in the treatment group. HUCMSC transplantation effectively ameliorated SUI in model rats by repairing injured urethral sphincters and peripheral nerves, thereby improving urinary control function. This study provides a novel therapeutic strategy for SUI management.

Materials and methods

Human umbilical cord mesenchymal stem cells (HUCMSCs)

HUCMSC suspension was provided by Qingdao Aoke Biological Development Co., Ltd. (Specification: 3×10^7 cells/30 mL per bag; Lot Number: y01-m-201902 P5 20200220) and stored in liquid nitrogen prior to use.

Antibodies

Primary antibodies used for immunohistochemical staining, including myosin heavy chain (MHC) antibody and protein gene product 9.5 (PGP9.5) antibody, were purchased from Abcam (Cambridge, UK).

Equipment

The BL-410 Biological Functional Experimental System, utilized for urodynamic testing, was obtained from Chengdu Taimeng Technology Co., Ltd. (Chengdu, China).

Animal experiments

Twenty-five 6-week-old inbred female Sprague-Dawley (SD) rats under specific pathogen-free (SPF) conditions were purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. (Production License No.: SCXK (Beijing) 2016-0006). All animal handling and care procedures were performed in accordance with the guidelines of the Chinese National Institutes of Health and were approved by the Institutional Animal Care and Use Committee (IACUC) of Women's & Children's Health Care Hospital of Linyi, Shandong, China (Approval Number: IACUC-2021-IAEC-003). Rats were housed in a temperature-controlled environment (21 ± 2°C) with a relative humidity of 55 ± 10% and a strict 12-hour light/dark cycle (lights on at 07:00). Housing conditions included open-top polysulfone cages with autoclaved corn cob bedding (changed twice weekly) and paper-based nesting materials. Environmental enrichment was provided via PVC tunnels. Rats had ad libitum access to autoclaved LabDiet 5053

rodent chow and reverse osmosis water supplied through an automated watering system. Individual animal identification was performed via ear punching.

Construction of SUI rat models

Twenty-five rats were randomly assigned to three groups: the human umbilical cord mesenchymal stem cell (hUC-MSC) treatment group (n = 10), the model control group (n = 10), and the normal control group (n = 5).

For rats in the model control group and hUC-MSC treatment group, SUI models were established as follows: (1) Anesthesia was induced using 10% chloral hydrate. (2) A 2 cm longitudinal abdominal incision was made, followed by bilateral ovariectomy; the incision was then sutured. (3) A catheter balloon was inserted into the vaginal cavity, and 5 mL of sterile water was injected into the balloon to expand it. (4) The catheter was fixed at the vaginal orifice, and a 140 g weight was suspended from the external end of the catheter to maintain vaginal dilation for 4 hours. (5) In the normal control group, only a longitudinal abdominal incision was made without ovariectomy or vaginal dilation, and the incision was directly sutured after sham operation.

Validation of SUI rat models using LPP and MBC

One week post model establishment, the SUI models were validated through urodynamic assessment of the LPP and MBC. The validation protocol was executed as follows: First, rats were anesthetized by intraperitoneal injection of 10% chloral hydrate at a dosage of 0.25 mL/100 g body weight. Subsequently, each rat was secured in a supine position on a wooden board. After the perineal region was thoroughly disinfected, a lubricated catheter was carefully inserted into the bladder via the urethra to a depth of 2.5-3 cm and then anchored to the tail to ensure complete bladder emptying.

The distal end of the catheter was connected to a three-way stopcock: one port was interfaced with the BL-410 Biological Functional Experimental System, while the other was attached to a 20 mL syringe. The three-way system was flushed with methylene blue solution to remove any air bubbles. Once the urodynamic detector was calibrated, *HUCMSC* mL/min using the syringe. The injection was continued until the first drop of blue fluid was observed at the urethral orifice. At this moment, two critical parameters were recorded using the BL-410 system software: the MBC, defined as the bladder volume at the onset of leakage, and the LPP, measured as the detrusor pressure at the leakage event. Each rat underwent three independent urodynamic tests, and the mean values of LPP and MBC were calculated for subsequent statistical analysis.

HUCMSC intervention in SUI model rats

One week after successful model establishment, rats assigned to the HUCMSC treatment group received local injections of 5×10^6 HUCMSCs into the middle-upper segment of the urethral wall. In contrast, rats in the model control group and normal control group received equivalent volumes of normal saline at the same anatomical location. Two weeks post-intervention, urodynamic assessments, including LPP and MBC measurements, were repeated to evaluate the therapeutic efficacy of the HUCMSC treatment.

Hematoxylin-eosin (HE) staining of urethral tissue sections

Two weeks after the HUCMSC intervention, the kinetic detection of urine was conducted first, and then the rats were euthanized. The method of euthanasia is excessive use of pentobarbital sodium (150 mg/kg, IP), followed by cervical dislocation to confirm death. Urethral tissues were removed and fixed in 4% paraformaldehyde for 24 hours, dehydrated through a graded ethanol series, and embedded in paraffin using a paraffin embedding machine. Serial 5 μm-thick sections were prepared with a paraffin microtome, and every fifth section was subjected to HE staining. Urethral morphological features were observed and photographed under a light microscope. Inflammatory cell counting was performed on HE-stained sections using Image J software.

Masson staining of urethral tissue sections

Following urodynamic assessment, urethral tissues were harvested for Masson trichrome staining. The staining procedure was performed manually as follows: dewaxing, hematoxy-

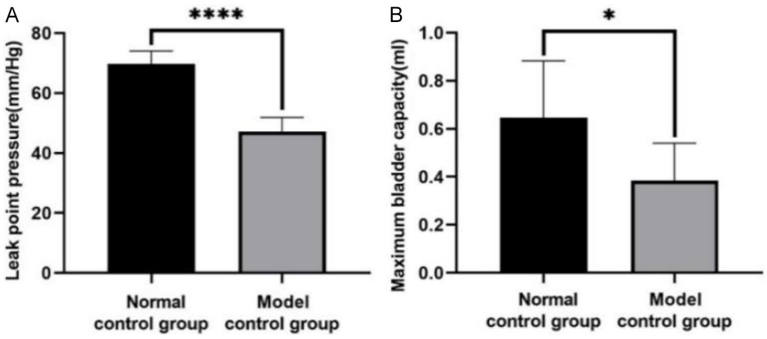

Figure 1. Measurement of Urine flow dynamics in rats by LPP and MBC. A. LPP values in the model control group and normal control group after one week of modeling. B. MBC in the model control group and normal control group after one week of modeling. All data were analyzed using Prism (GraphPad, Diego, USA), presented as the mean ± SEM, each test value was performed in triplicate. The t-test was used for comparison between groups. * indicates $P < 0.05$, **** indicates $P < 0.0001$.

lin staining, ethanol differentiation, bluing, washing, acid fuchsin staining, rinsing with 1% phosphomolybdic acid solution, aniline blue staining, dehydration, clearing, and mounting. After staining, muscle fibers were visualized as red, and collagen fibers as green or blue. The extent of tissue lesions was quantified by calculating the percentage of positively stained area relative to the total field area using Image J software.

Immunohistochemical staining and analysis

Urethral tissue sections were deparaffinized in xylene and rehydrated through a graded ethanol series. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, and nonspecific binding was inhibited using 2% bovine serum albumin (BSA). Antigen retrieval was performed by heating sections in sodium citrate buffer. Subsequently, sections were incubated overnight at 4°C with primary antibodies against MHC and PGP9.5 at a dilution of 1:200. After washing with phosphate-buffered saline (PBS), sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Protein expression was visualized using 3,3'-diaminobenzidine (DAB) chromogen, followed by counterstaining of nuclei with hematoxylin. After washing, sections were mounted with neutral balsam and dried in a fume hood.

Positive staining was observed and evaluated using a light microscope equipped with a digital imaging system. The expression levels of target proteins were quantified based on staining intensity and distribution area. Using Image J software, the integrated optical density (IOD) and total area of each image were measured. The mean density, reflecting target protein concentration per unit area, was calculated as: mean density = IOD/total area. For each sample, the average mean density from five randomly selected fields was used as the representative value.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, USA). All results are presented as mean ± standard error of the mean (SEM). For multiple group comparisons, two-tailed t-tests with Tukey's post hoc test were applied according to the experimental design. A *P*-value < 0.05 was considered statistically significant.

Results

Establishment of the SUI rat model

One week after SUI model induction, urodynamic parameters (LPP and MBC) were measured using the BL-410 Biological Functional Experimental System. As shown in **Figure 1A**, the LPP mean in the model control group was significantly lower than that in the normal control group (47.24 ± 1.445 mmHg vs. 69.74 ± 1.926 mmHg, $P < 0.0001$, **Figure 1A**). Similarly, the MBC mean in the model control group was significantly reduced compared with the normal control group (0.385 ± 0.156 mL vs. 0.646 ± 0.236 mL, $P < 0.05$, **Figure 1B**). These results confirmed the successful establishment of the SUI rat model (**Figure 1**).

LPP and MBC measurements for validation of therapeutic effects of HUCMSC treatment

Following validation of the SUI model (characterized by low LPP and MBC), rats in the HUCMSC treatment group received urethral sphincter injections of 5×10^6 HUCMSCs, while the model control group and normal control group received equivalent volumes of normal saline at the same site. Urodynamic assess-

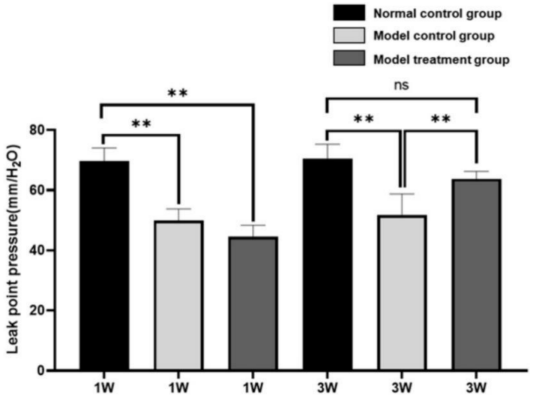

Figure 2. The LPP of rats in each group were detected in the first and third week after modeling. Inter-group comparison of the LPP levels in the first week and the third week after modeling, respectively. All data were analyzed using Prism (GraphPad, Diego, USA), presented as the mean ± SEM, each test value was in triplicate. Comparisons between groups were performed using a t-test. **P < 0.01, ns indicates no significance. ** indicates P < 0.01, ns. indicates no significance.

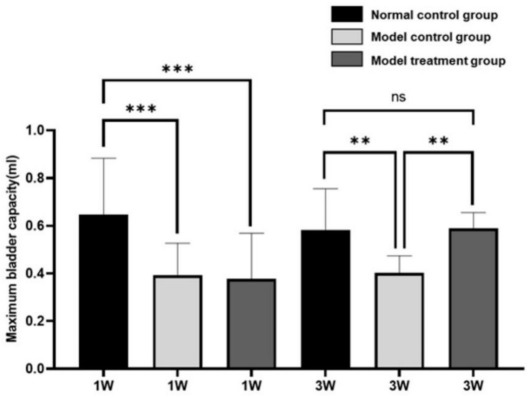

Figure 3. MBC of rats in each group was detected in the first and third week after modeling. Inter-group comparison of the MBC levels in the first week and the third week after modeling, respectively. All data were analyzed using Prism (GraphPad, Diego, USA), presented as the mean ± SEM, each test value was in triplicate. Comparisons between groups were performed using a t-test. ** indicates P < 0.01, *** indicates P < 0.001, ns indicates no significance.

ments were repeated two weeks post-intervention (i.e., three weeks after initial model induction).

One week after SUI modeling, the LPP values were as follows: the normal control group exhibited baseline levels (as shown in **Figure 1**), while the model control group and HUCMSC treatment group (before intervention) showed significantly reduced LPP at 47.24 ± 1.445 mmHg and 43.23 ± 12.56 mmHg, respectively. Statistical analysis revealed that both the model control group and the pre-treatment HUCMSC group had significantly lower LPP compared with the normal control group (both P < 0.01).

Three weeks after modeling (i.e., two weeks post-intervention), the LPP values were measured as follows: 69.74 ± 1.926 mmHg in the normal control group, 47.24 ± 1.445 mmHg in the model control group, and 63.79 ± 1.13 mmHg in the HUCMSC treatment group. Statistical analysis demonstrated a significant increase in LPP in the HUCMSC treatment group compared with the model control group (P < 0.01), with no statistically significant difference from the normal control group (P > 0.05, ns), as shown in **Figure 2**.

While for MBC detection results, the MBC values at the first week were as follows: the normal control group exhibited baseline levels (as shown in **Figure 1**), while the model control group and HUCMSC treatment group (before intervention) showed significantly reduced MBC at 0.385 ± 0.156 mmHg and 0.377 ± 0.87 mmHg, respectively. Statistical analysis revealed that both the model control group and the pre-treatment HUCMSC group had significantly lower MBC compared with the normal control group (both P < 0.001). Three weeks after modeling (i.e., two weeks post-intervention), the MBC values were measured as follows: 0.646 ± 0.236 mL (normal control group), 1.101 ± 0.248 mL (model control group), and 0.661 ± 0.383 mL (HUCMSC treatment group).

Statistical analysis demonstrated a significant increase in the HUCMSC treatment group compared with the model control group (P < 0.01), with no statistically significant difference from the normal control group (P > 0.05, ns), as shown in **Figure 3**. The results indicated that the LPP and MBC of the HUCMSC treatment group was close to the level of the normal control group. This proves that HUCMSC intervention is effective in the treatment of SUI.

Reduction of inflammatory cell infiltration in urethral tissues following HUCMSC treatment in SUI models after HE staining

Two weeks after HUCMSC injection, pathological analysis via hematoxylin-eosin (HE) staining revealed that the number of inflammatory cells

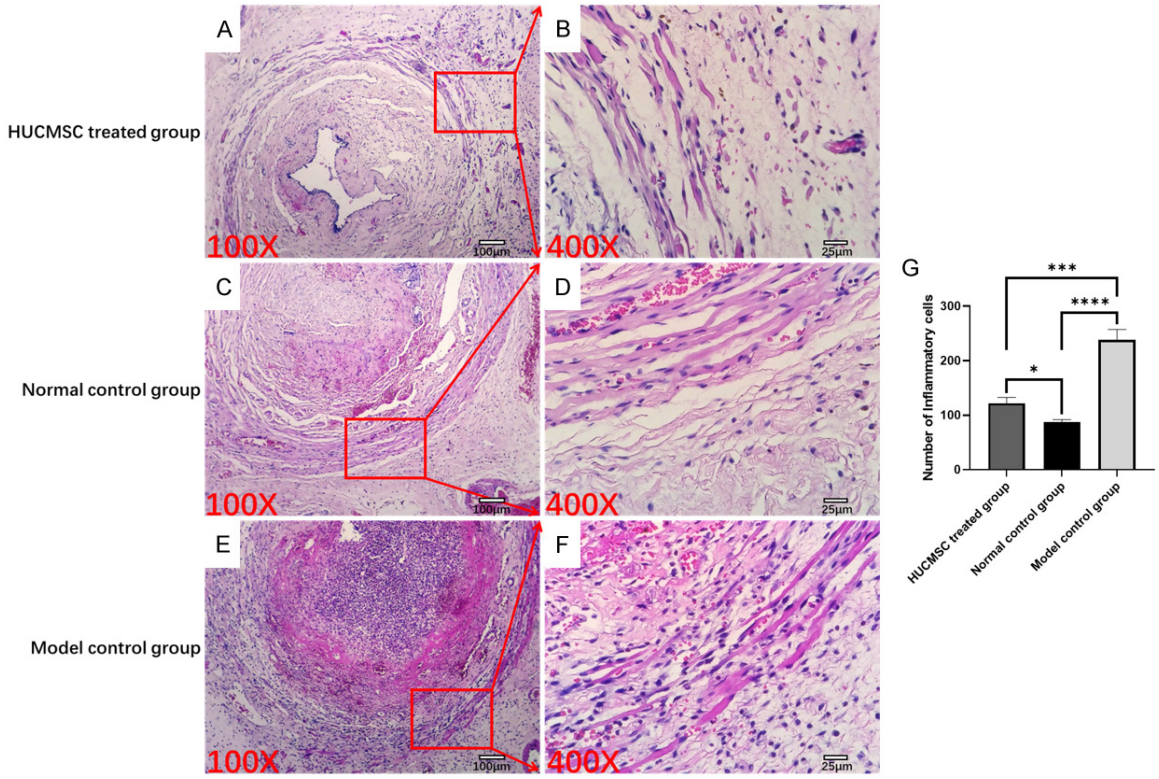

Figure 4. Inflammatory cell infiltration in urethral tissue after HUCMSC treatment. Representative results of HE staining. (A and B) Inflammatory cells in the urethral tissues of the HUCMSC treatment group. (C and D) Inflammatory cells in the normal control group. (E and F) Inflammatory cells in the model control group. Bar = 100 μm and Bar = 25 μm. (G) All data (A-F) were analyzed using Prism (GraphPad, Diego, USA), presented as the mean ± SEM. For multiple comparisons, a two-tailed t-test with Tukey post hoc tests was used to analyze differences depending on the experiment design. A $P < 0.05$ was considered statistically significant difference. * indicates $P < 0.05$, *** indicates $P < 0.001$, **** indicates $P < 0.0001$.

infiltrating the urethral tissues in the HUCMSC treatment group was significantly reduced. However, compared with the normal control group, there were still statistical differences ($P < 0.05$). In contrast, the model control group exhibited a marked increase in inflammatory cell infiltration compared with the treatment group ($P < 0.001$) and the normal control group ($P < 0.0001$). These findings indicate that HUCMSC therapy effectively alleviates urethral tissue inflammation in SUI model rats (**Figure 4**).

Masson staining analysis of collagen fibers and muscle fibers in the urethral wall

Urethral collagen fibers and muscle fibers are critical structural components that maintain normal urethral morphology and function. Masson staining, which differentiates nuclei, collagen fibers (stained blue/green), and muscle fibers (stained pink), was used to evaluate tissue integrity.

Masson staining results showed that the urethral surrounding tissues in the normal control group maintained intact collagen fibers and muscle fibers with normal morphology. In the model control group, extensive muscle fiber destruction and collagen fiber depletion were observed. Notably, the HUCMSC treatment group exhibited increased muscle fiber and collagen fiber content in urethral tissues, suggesting that HUCMSC injection promotes the repair of urethral muscle fiber injury and effectively ameliorates SUI caused by urethral tissue damage (**Figure 5**).

Immunohistochemical staining analysis of PGP9.5 and MHC expression

Immunohistochemical staining was performed to detect the expression of PGP9.5 and MHC in urethral tissues. Regarding PGP9.5 expression, positive staining was primarily localized in the cytoplasm of striated muscle cells and their adjacent regions, particularly around

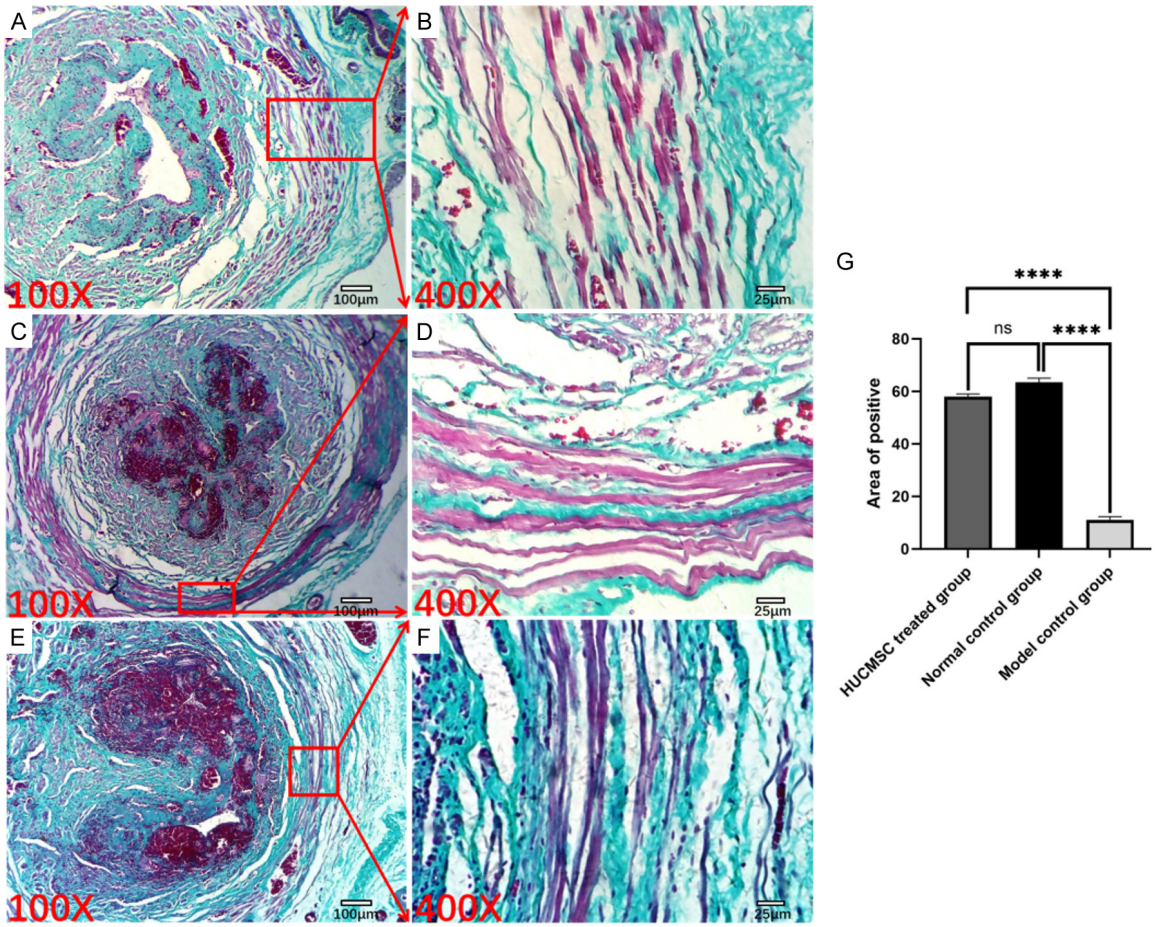

Figure 5. Urethral function validations after HUCMSC treatment by Masson staining. Muscle fibers are red and collagen fibers are green after Masson staining. (A and B) are representative results of the HUCMSC treated group. Regenerated muscle fibers (red) can be seen in urethral tissue. However, the arrangement of urethral tissue is sparse and discontinuous compared with normal urethral tissue. (C and D) are representative results of the normal control group. The muscle fibers of urethra tissue were closely arranged and were continuous. (E and F) are representative results of the model control group. The model control group presented muscle fibrosis and color is atypical, collagen fibrous hyperplasia, normal muscle fibers are less. Bar = 100 μm and Bar = 25 μm. (G) All data (A-F) were analyzed using Prism (GraphPad, Diego, USA), presented as the mean ± SEM. For multiple comparisons, a two-tailed t-test with Tukey post hoc tests was used to analyze differences depending on the experiment design. A $P < 0.05$ was considered statistically significant difference, **** indicates $P < 0.0001$, ns. indicates no significance.

blood vessels. The model control group displayed lower level of PGP9.5 expression (**Figure 6E**, **6F**), whereas the HUCMSC treatment group (**Figure 6A**, **6B**) showed significantly increased PGP9.5 expression compared with the model control group ($P < 0.0001$) and no significant difference from the normal control group ($P > 0.05$, ns.), (**Figure 6C**, **6D**). For MHC expression, the model control group (**Figure 6K**, **6L**) exhibited significantly lower levels in urethral tissues compared with the normal control group ($P < 0.0001$), (**Figure 6I**, **6J**). In contrast, the HUCMSC treatment group (**Figure 6G**, **6H**) showed significantly higher MHC expression than the model control group ($P < 0.001$), with levels approaching those of the normal control group ($P > 0.05$, ns.).

The statistical analysis results of the quantitative data on the expression levels of PGP9.5 protein among each group showed that there was no difference between the treatment group and the normal control group (ns., $P > 0.05$); Compared with the model control group, there was a significant difference ($P < 0.0001$), (**Figure 6M**). The statistical analysis results of the comparison of quantitative data on MHC protein expression levels among each group showed that there was no difference between the treatment group and the normal control

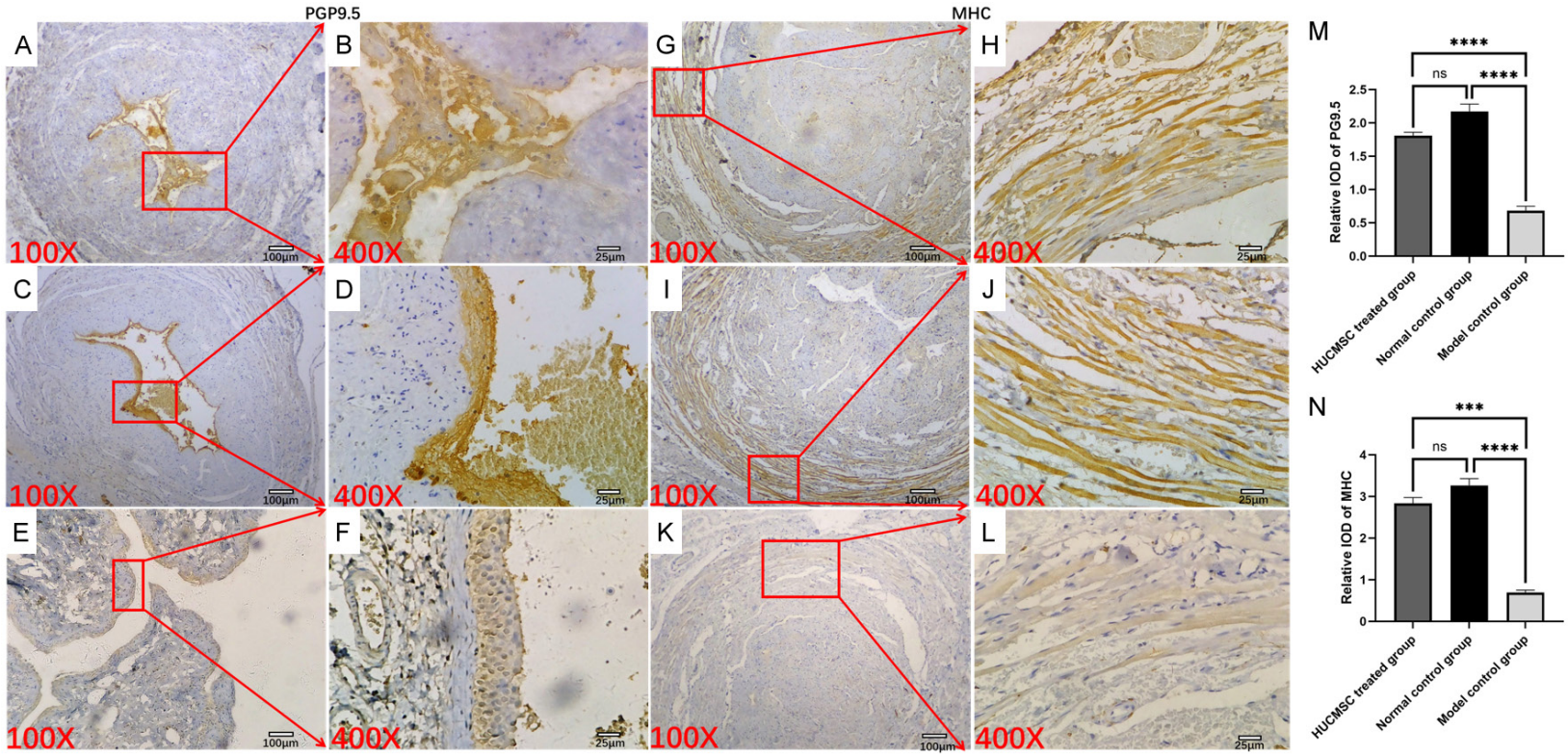

Figure 6. The expressions of PGP9.5 and MHC were detected by immunohistochemistry. Representative results expressed by PGP9.5. (A and B) are representative results from the HUCMSC treated group showing positive expression of PGP9.5. (C and D) are from the normal control group showing positive expression of PGP9.5. (E and F) are from the model control group showing low level expression of PGP9.5. Bar = 100 μm and Bar = 25 μm. Representative results expressed by MHC. (G and H) are representative results from the HUCMSC treated group showing positive expression of MHC. (I and J) are from the normal control group showing positive expression of MHC. (K and L) are from the model control group showing low level expression of MHC. Bar = 100 μm and Bar = 25 μm. (M, N) All data (A-F, G-L) were analyzed using Prism (GraphPad, Diego, USA), presented as the mean ± SEM. For multiple comparisons, a two-tailed t-test with Tukey post hoc tests was used to analyze differences depending on the experiment design. A $P < 0.05$ was considered statistically significant. *** indicates $P < 0.001$, **** indicates $P < 0.0001$, ns. indicates no significance.

group (ns., P > 0.05); Compared with the model control group, there was a significant difference (P < 0.001), (**Figure 6N**).

Consistent with morphological observations, the model control group exhibited minimal expression of both PGP9.5 and MHC, indicating severe damage to nerve fibers and muscle fibers. Notably, HUCMSC treatment restored the expression of both markers, suggesting effective repair of neural and muscular components (**Figure 6**).

Discussion

SUI, the most prevalent subtype of urinary incontinence, is defined as involuntary urine leakage caused by increased intra-abdominal pressure during physical activities, exercise, coughing, or sneezing, when bladder pressure exceeds urethral closure pressure [10]. Its prevalence increases with age, peaking in postmenopausal and pregnant women. Additional risk factors include childbirth, menopause, strenuous physical labor, smoking, obesity, and chronic cough [11]. SUI exerts significant negative impacts on physical, social, and psychological well-being, leading to reduced self-esteem, impaired quality of life, and social isolation. Globally, it imposes a substantial economic burden, with direct costs in the United States alone exceeding $12 billion annually [12]. As the global population ages, the prevalence of SUI and associated management costs are projected to rise in the coming decades.

Among the multiple pathogenic factors of SUI, urethral sphincter injury, sphincter dysfunction, and peripheral urethral nerve damage are fundamental contributors; effective resolution of these issues is critical for curative outcomes [13]. Stem cells have emerged as a promising tool in regenerative medicine due to their capacities for self-renewal, clonal expansion, and multi-lineage differentiation [14]. They can replace damaged or diseased tissues (e.g., regenerating urethral sphincters by replacing damaged smooth or striated muscle) and exert therapeutic effects through secretion of bioactive factors with angiogenic and cytoprotective properties [15]. Current research indicates that SUI treatment primarily focuses on inducing or promoting the formation of functional muscle fibers to replenish damaged or aged muscle cells, thereby improving muscle function.

Stem cells have demonstrated significant potential in SUI treatment and have been applied in clinical studies. Commonly used cell types include muscle-derived stem cells (MDSCs) and non-muscle-derived stem cells such as adipose-derived stem cells (ADSCs), bone marrow-derived stem cells (BMDSCs), amniotic fluid stem cells (AFSCs), and cord blood stem cells (CBSCs) [16]. Therapeutic strategies involve direct injection of stem cells after in vitro verification of their proliferation and differentiation capacity, transplantation of in vitro-differentiated muscle precursor cells, or co-injection with growth factors into the urinary system or peripheral target areas [13, 14]. Myogenic differentiation of stem cells around the damaged external urethral sphincter can enhance or repair striated muscle tone in SUI patients, thereby improving tissue function.

HUCMSCs have become indispensable seed cells in stem cell therapy due to their advantages of abundant sources, rapid expansion, high cell yield, and low immunogenicity [17]. Periurethral injection of HUCMSCs is hypothesized to induce directional differentiation into muscle and nerve cells while secreting trophic factors, potentially improving urethral sphincter contractile function and achieving SUI remission.

PGP9.5, a highly specific axonal marker, serves as a key indicator of nerve regeneration and reinnervation [18]. In this study, immunohistochemical analysis of rat urethral sections revealed significantly higher PGP9.5 expression in the urethral sphincter of the HUCMSC treatment group compared with the model control group, indicating increased nerve fiber content. This suggests that HUCMSCs promote recovery or partial restoration of nerve injury, potentially involving nerve regeneration. Consistent with previous reports, HUCMSCs can be induced to differentiate into neural cells and secrete neurotrophic factors that promote axonal growth [19-22].

Skeletal muscle MHC, a highly specific marker of skeletal (striated) muscle [23], constitutes approximately 25% of total protein content in striated muscle cells. Changes in urethral MHC expression thus reflect injury and regeneration of urethral smooth and striated muscles [24].

In this study, MHC expression in the HUCMSCs treatment group was significantly higher than in the model control group, indicating that HUCMSCs promote repair and regeneration of urethral sphincter muscle cells in SUI rats. These findings align with reports that HUCMSCs secrete insulin-like growth factor-1, which accelerates damaged muscle recovery, promotes myoblast proliferation and differentiation, enhances peripheral nerve regeneration, and inhibits skeletal muscle atrophy - collectively improving urethral sphincter function [23, 25-27].

Urodynamic assessments of LPP and MBC showed that the HUCMSC treatment group approached normal levels, with no statistically significant difference from the normal control group. In contrast, the model control group maintained low LPP and MBC two weeks post-modeling, with significant differences from both the normal control and treatment groups. The incomplete recovery of LPP and MBC at the 2-week time point may reflect the model's induction of severe subacute injury, which typically requires longer times for functional restoration.

Histopathological observations after periurethral allogeneic HUCMSC transplantation in rats showed local urethral muscle thickening and improved periurethral connective tissue integrity (from loose to compact) at 2 weeks, enhancing urethral support. Immunohistochemical analysis confirmed structural improvement of the urethral sphincter, while urodynamic indices (LPP and MBC) were significantly improved in the treatment group.

Conclusion

This study demonstrated that HUCMSC transplantation ameliorates urodynamic function (as indicated by LPP and MBC) in SUI model rats, repairs damaged urethral tissues, and upregulates MHC and PGP9.5 expression in muscle fibers and peripheral nerves. These findings confirm that HUCMSC transplantation effectively treats SUI in rats by repairing injured urethral sphincters and peripheral nerves, thereby improving urinary control function. Although further research is needed before clinical translation, this study provides a novel therapeutic strategy for SUI management.

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Disclosure of conflict of interest

None.

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