

Original Article

Effects of human umbilical cord mesenchymal stem cells on intrauterine adhesions in a rat model

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Abstract: Objective: The aim of this study is to investigate the effects of human umbilical cord mesenchymal stem cells (hUCMSCs) transplantation on a rat model with intrauterine adhesions (IUA). Methods: 30 healthy female Sprague-Dawley rats were randomly divided into a sham operated group, a PBS group and a hUCMSCs group. The hUCMSCs were injected into uterines of the rat models in hUCMSCs group, and the glandular count, the endometrial fibrosis ratio, proliferation of endometrial cells and the location of hUCMSCs were observed. Moreover the gene expressions of cytokines relating to fibrosis and regeneration were tested after transplantation. Results: hUCMSCs transplantation can effectively reduce endometrial fibrosis area, increase glandular count and promote proliferation of endometrial cells. The transplanted hUCMSCs were also observed in uterines one week after surgery. In addition, there were significant differences in the expressions of cytokines among three groups. Conclusions: hUCMSCs transplantation is able to reduce fibrosis and improve the endometrial regeneration in IUA rats. It may become a promising strategy for the therapy of IUA in the future.

Keywords: Umbilical cord mesenchymal stem cells, intrauterine adhesions, transplantation, rat model

Introduction

Intrauterine adhesions (IUA) result from deep trauma involving the basal layer of endometrium with subsequent scarring [1]. It is reported that nearly 90% of IUA is related to postpartum or post-abortion dilatation and curettage [2]. The presence of IUA leads to variable symptoms such as amenorrhea, infertility, recurrent miscarriage and so on. The goal of treatment is to rebuild the normal shape of uterine cavity, prevent re-adhesions, improve the endometrium regeneration and eventually restore uterine function. However, the current comprehensive approach doesn't work effectively especially in severe cases. In fact, due to loss of most endometrial cells, severe endometrial damages were found to be irreparable. So new treatments are in great demand.

Recently, the substantial evidences indicate that adult stem cells have been identified in the continuously regenerative human endometrium and were considered to be involved in endo-

metrial scar-free regeneration [3-5]. The scarcity and abnormality of local endometrium stem cells may explain the failure of the endometrial functional layer to regenerate in IUA [6-8]. Such hypothesis suggested the potential use of stem cells therapy in treating disorders associated with inadequate endometrium. Currently, stem cell-based therapy for IUA has been adopted in several studies showing a satisfactory treatment effect in reducing endometrial fibrosis and repairing damaged endometrium [9-12]. Nagori et al reported a woman with refractory intrauterine adhesions became pregnant after receiving autologous bone marrow derived mesenchymal stem cells transplantation [13]. However, this new therapy faces many difficulties, like the optimal stem cell line is unresolved and the mechanism has not been fully understood. Accordingly, an alternative source of stem cells and further studies are imperatively needed, and Wharton's jelly mesenchymal stem cells derived from umbilical cord (hUCMSCs) are regarded as a most promising candidate for cell transplantation therapy.

Unlike bone marrow and other stem cells, hUCMSCs appear to offer the best clinical utility since they are non-controversial, easy accessing, can be harvested by non-invasive procedures in abundance and have faster self-renewal properties [14, 15]. More importantly, human leukocyte antigen 1 are not detectable or weakly expressed in hUCMSCs, demonstrating the beneficial effect on allograft transplantation without immunological suppression [16, 17]. It has been reported that hUCMSCs could regenerate other tissues loss and treat various diseases [18-21]. However, there is no report on repairing the damaged endometrium using hUCMSCs in IUA.

The aim of our study was to investigate the therapeutic effects of hUCMSCs transplantation on IUA rat models, and verify whether it could reduce the endometrial fibrosis and promote the regeneration of endometrium. hUCMSCs have provided new possibilities for the treatment of endometrial degenerative diseases.

Materials and methods

Animals

Female Sprague-Dawley rats (9 weeks old) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and raised in the Chao-Yang Hospital Medical Research Center Animal Department. All rats were sexually mature and nulliparous. Rats were housed five per cage in a room with a 12 hr light/dark cycle (7:00 am-7:00 pm) with free access to food and water. The room temperature is (23±2°C); humidity is 45-55%. All rats were treated in accordance with Guidelines for the Care and Use of Laboratory Animals of Beijing Obstetrics and Gynecology Hospital, and the protocol was approved by the Institutional Review Board of Beijing Obstetrics and Gynecology Hospital. Rats were anaesthetized and killed painless after tissues obtained.

Preparation of hUCMSCs

The hUCMSCs were given by Dr. Wang Jing (Newish Technology Co., Ltd., Beijing, China). These cells derived from umbilical cord of informed, healthy mothers in local maternity hospitals after cesarean deliveries. The isolation and culture methods were adopted as pre-

viously described [22]. In brief, once isolated, cells were cultured in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12; Gibco, USA) with 10% fetal bovine serum (FBS; Gibco), and 100 U/mL penicillin/streptomycin (Gibco) at 37°C in a 5% CO₂ incubator. After reaching 80% confluency cells, cells were passaged with a 1:3 split. Then the cells of passage 3 were stained with fluorescein-conjugated monoclonal antibody against CD29, CD34, CD73, CD90, CD105, CD106, HLA-ABC and HLA-DR (Becton, Dickinson and company, USA) for 30 minutes, then analyzed by flow cytometry (FACSscan, Becton, Dickinson and company, USA). hUCMSCs of passages 3-5 were trypsinized and dissociated into single cell PBS suspensions and used for the following transplantation.

A rat model of intrauterine adhesions

IUA were induced by scraping the uterine horn in rats, mimicking the cause of IUA in human. Briefly, SD female rats were used to create IUA animal model during diestrus. After administration of 5% pentobarbital sodium solution (5 mg/kg) by intraperitoneal injection, a vertical incision (3-4 cm) was made in the abdominal wall and the two uterine horns were exposed. A small incision was made in the right uterine horn at the utero-tubal junction and the uterine horn traumatized in a standardized fashion using self-made mini-endometrial curette inserted two-thirds of the way through the lumen, rotated and withdrawn many times until the uterine wall became rough and feel granular sensation. Then, the uterine lumen was washed with saline to prevent the residual of endometrial cells and establish hemostasis. Later on, the uterine small incision was closed by stitching with 6-0 vicryl sutures (ETHICON, W9981, Coated polyglactin 910 sutures, Johnson & Johnson, USA). Interrupted 3-0 silk non-absorbable sutures (ETHICON) were used to close the abdominal incision. After modeling, rats were placed in temperature controlled incubation chambers until they awoke and returned to their housing facilities.

Animal grouping and transplantation

A total of 30 rats were randomly divided into three groups as follows: a sham operated (Sham-O) group (n=10), a PBS group (n=10) and a hUCMSCs group (n=10). For the sham operated group, after abdominal surgical inci-

Table 1. Specific primers used in qRT-PCR analysis

Gene	Primer	Sequence (5'→3')
GAPDH	FW	TACCCACGGCAAGTTCAACG
	RV	CACCAGCATCACCCATTG
COL-1	FW	CCTCCCAGAACATCACCTAT
	RV	CATCCACAAGCGTGCTGTAG
FGF2	FW	GATCCCAAGCGGCTCTACTG
	RV	TCGCACACACTCCCTTGATG
CTGF	FW	GCTGGAGAAGCAGAGTCGTC
	RV	CACACCCACAGAACTTAGC
TGF-β1	FW	CAACAATTCCTGGCGTTACC
	RV	TGGGACTGATCCATTGATT
Ki67	FW	AAGACGAAGATCAGGCAGGA
	RV	CAACAATCAGGTTGGCTTCA
VEGF	FW	TACTGCTGTACCTCCACCAT
	RV	GGGTACTCCTGGAAGATGTC
VIM	FW	CACCCTGCAGTCATTGAGACA
	RV	GTGAGGTCAGGCTTGGAACG
CK	FW	TCCTCAGCCATGTCTTCTATG
	RV	CTAAACTTCCACCGCGTCCT

Abbreviation: GAPDH, glyceraldehyde phosphate dehydrogenase; COL-1, collagen I; FGF2, fibroblast growth factor-2; CTGF, connective tissue growth factor; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor; VIM, vimentin; CK, cytokeratin.

sion, the normal uterine horns were exposed without any injuries to endometrium and any interventions. For the PBS group, after modeling, the uterine horn was injected with 500 µl PBS. For the hUCMSCs group, 500 µl hUCMSCs suspensions (1×10^7 cells) were locally injected into injured side of uterine horn at multiple points intramuscularly. In each group, ten rats were sacrificed one week after surgery. All uterine horns were collected and fixed in formalin liquid or liquid nitrogen for further tests.

Hematoxylin-eosin and masson staining

The formalin-fixed paraffin-embedded uterine tissues were sectioned at 4 µm and the sections on slides were immersed in xylene (10 minutes, twice) and rehydrated in a series of ethanol dilutions (100%, 100%, 95%, 95%, 75%, 0%, 1 minute each). The sections were rinsed in deionized water, stained in hematoxylin for 45 seconds, rinsed in deionized water, and stained in eosin for 1 second. After the color reaction, sections were dehydrated in graded ethanol and xylene. Finally, the morphology of endometrium and the mean number

of glands were observed and evaluated in high-power fields (HPF) under light microscope (DP70, Olympus, Japan) among three groups.

Masson's staining was applied to evaluate the endometrial fibrosis area. After being rehydrated as described previously, sections on slides were performed by procedures in Masson trichrome staining kit (Zhongshan Golden Bridge Biotechnology, Co., Ltd., Beijing, China). Finally, mount the slides with mounting media. The fibrosis area was measured with Image-Pro Plus 6.0 software on photos taking from slides.

Immunohistochemistry

Briefly, all sections (4 µm) were de-paraffinized using xylene, rehydrated in a series of decreasing concentrations of ethanol. Antigen retrieval was performed using pressure cooker with enough retrieval solution. Endogenous peroxidase activity was inhibited by incubating slides in 0.3% H_2O_2 for 30 minutes at room temperature. After blocking for one hour in PBS supplemented with 10% normal goat serum, the slides were incubated with primary antibodies: rabbit monoclonal antibodies to CK18 (1:200; Abcam, Cambridge, UK) and Ki67 (1:200; Abcam, Cambridge, UK) overnight at 4°C. Sections were incubated with second antibody goat-anti-rabbit IgGs for 30 minutes at room temperature. Slides were then briefly counterstained (1 min) with hematoxylin solution and observed using a microscope (Olympus Company, Tokyo, Japan). The expressions of Ki67 and CK18, the markers of the regeneration of endometrial cells were analyzed using Image-Pro Plus 6.0 software by average optical density (AOD).

Immunofluorescence

Rat uterine horns were removed at day 7 and embedded into OCT (SAKURA, Japan) and froze at -30°C for 10 min. Then the frozen tissues were continuously sectioned to 6 µm thickness (Tissue-Tek Cryo; Sakura, Japan). The sections were stained with mouse anti-human nuclear antigen antibody (HN, 1:100; Chemicon, Millipore, California, USA), mouse anti-vimentin (VIM, 1:100, Boster Biological Technology Co., Ltd, Wuhan, China) and pan-cytokeratin (CK, 1:100, Santa Cruz Biotechnology, USA). Then fluorescence-tagged secondary antibodies (1:100; Zhongshan Golden Bridge Biotechnology, Co., Ltd., Beijing, China) were used to amplify signal

hUCMSCs improve recovery of intrauterine adhesions

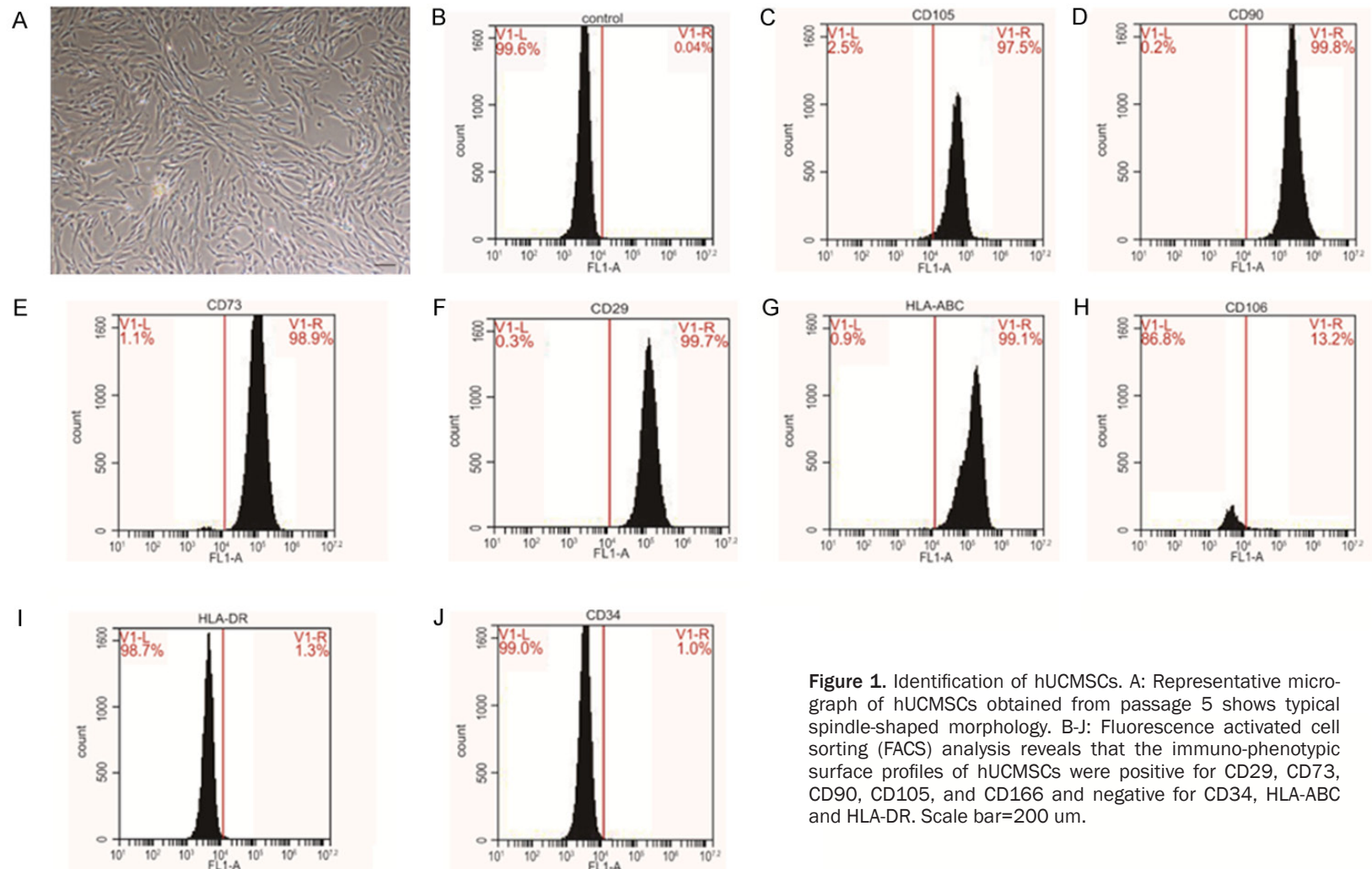


Figure 1. Identification of hUCMSCs. A: Representative micrograph of hUCMSCs obtained from passage 5 shows typical spindle-shaped morphology. B-J: Fluorescence activated cell sorting (FACS) analysis reveals that the immuno-phenotypic surface profiles of hUCMSCs were positive for CD29, CD73, CD90, CD105, and CD166 and negative for CD34, HLA-ABC and HLA-DR. Scale bar=200 μ m.

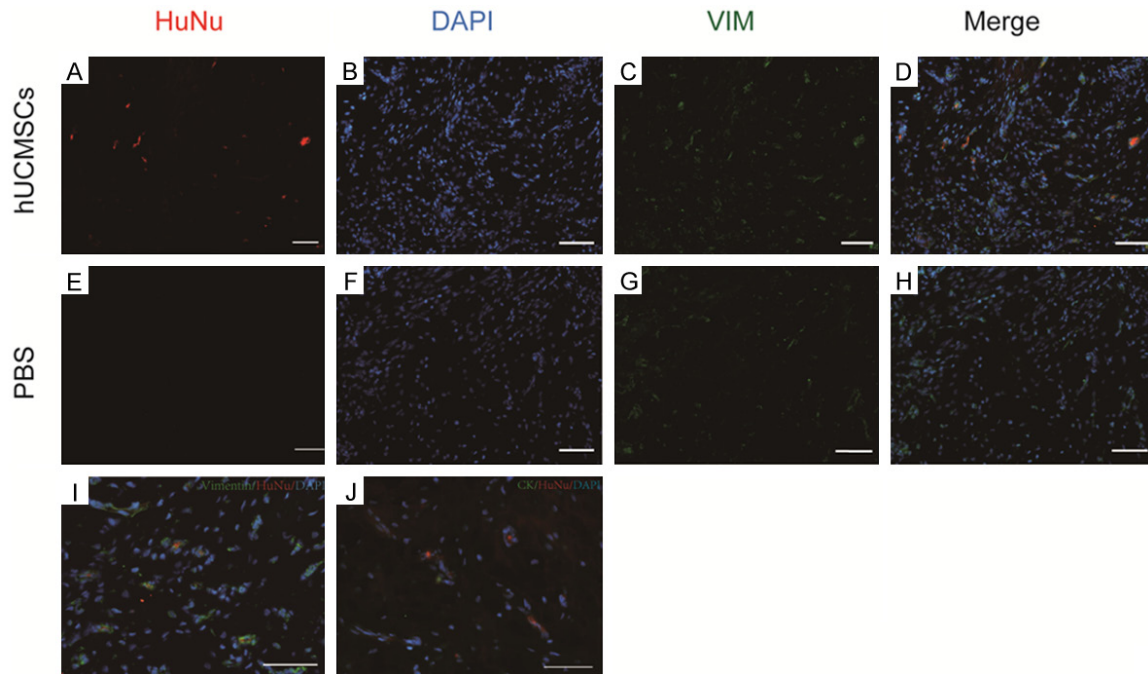


Figure 2. Location of hUCMSCs in uterine. A-D: One week after transplantation, representative images for HN (red), DAPI (blue) and vimentin (green) immunofluorescence staining in uterine of hUCMSCs group. E: There was no red fluorescence within uterine of PBS group. F-H: There are only micrographs for DAPI (blue) and vimentin (green) immunofluorescence staining in uterine of PBS group. I: Vimentin (green) staining co-localized with human nuclear antigen (red) in uterines of hUCMSCs group reveals that the transplanted cells expressed the endometrium stromal cell marker vimentin. J: While double-staining with CK and HN shows that hUCMSCs did not differentiate into endometrium epithelial cells after transplantation. Scale bar=50 μ m.

of the primary antibodies. Cell nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). Secondary antibodies were detected using fluorescence microscopy (Nikon ECLIPSE TI-SR, Japan) instantly for visualization to locate and observe the hUCMSCs. Negative controls were generated using PBS instead of primary antibodies.

Quantitative real-time polymerase chain reaction

Total RNA samples were extracted from uterine tissues using RNAiso Plus (Takara Bio Inc., Otsu, Shiga, Japan) and quantified using NanoDrop 2000 spectrophotometry (NanoDrop Technologies, Thermo Scientific, Waltham, USA). cDNA was synthesized from 1 μ g RNA employing the TransScript First-Strand cDNA synthesis SuperMix kit (TransGen Biotech). Then, quantitative real-time PCR was performed on an Applied Biosystems 7500 Real-time PCR system (Applied Biosystems, Life Technologies, California, USA) using TransStart Top Green qPCR SuperMix SYBR (TransGen Biotech). The primer sequences are shown in

Table 1. The cycling parameters for the qRT-PCR were as follows: an initial denaturation at 95°C for 15 min followed by 40 cycles of 10 s at 95°C and 32 s at 60°C. The relative mRNA abundance was performed as fold-changes relative to Sham-O controls. Glyceraldehyde phosphate dehydrogenase (GAPDH) was used as an internal control to quantify mRNA expression and mRNA expressions were analyzed according to the $2^{-\Delta\Delta(CT)}$ method.

Statistical analysis

Data and statistical analysis were performed using GraphPad prism5. Data were presented as mean \pm SD. We analyzed the differences between the groups using one-way ANOVA and student's t-test. For all statistical tests, *P*-values were set at 0.05 (*P* < 0.05) for significant differences.

Results

Characterization of hUCMSCs

The hUCMSCs displayed two distinct morphological features: spindle-shaped and whirlpool-

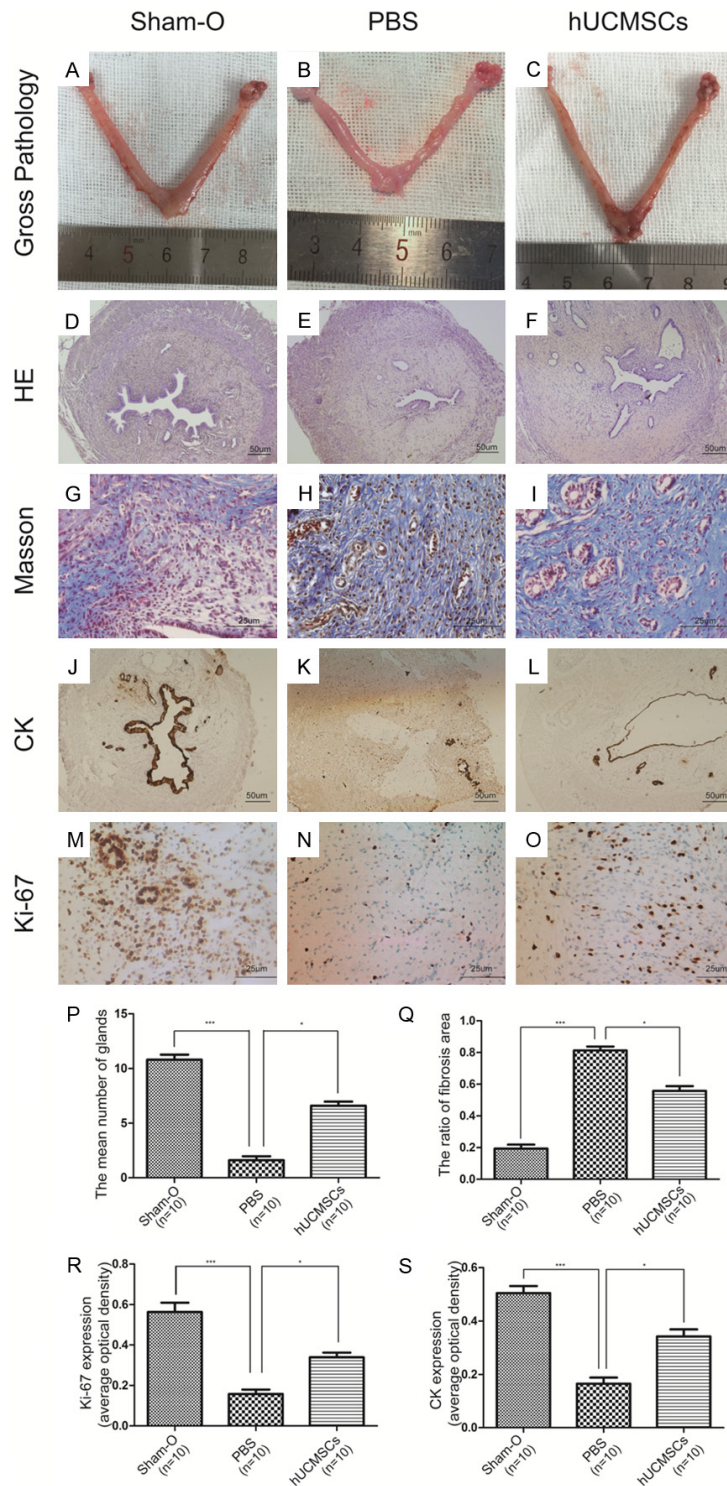


Figure 3. Uterine pathology of rats in different groups. A-C: Gross pathology. D-F: Histological structures in the uterine horns. G-I: Masson trichrome staining of endometrium. J-L: Immuno-staining of CK. M: Immuno-staining of Ki67 in sham operated group. N: Immuno-staining of Ki67 in PBS group. O: Immuno-staining of Ki67 in hUCMSCs group. P: Statistical analysis of the glandular count of endometrium. Q: The ratios of fibrotic area in the endometrium. R: Immunohistochemically expression of Ki67. S: Immunohistochemically expression of CK. Data were presented as mean \pm SEM. * $P < .05$ and *** $P < .001$.

like growth. Cells resembled fibroblasts and formed bipolar spindle-like cells with parallel or whirlpool-like arrangement (**Figure 1**). Flow cytometry analysis showed that over 95% cells at passage 3 expressed CD105, CD73, CD90, CD106 and CD29 while they did not exhibit expression of the hematopoietic makers CD34, and HLA-DR, HLA-ABC (**Figure 1**), indicating that the majority cells used in the present study were hUCMSCs.

Engraftment of hUCMSCs in the injected rats

One week after transplantation, anti-human nuclear antigen (HN) was used to detect the presence of human cells. Immunofluorescence staining of HN (red) showed that hUCMSCs localized in the damaged endometrium of rats and no cytokeratin-positive cells double staining with HN were observed. A few hUCMSCs may differentiate into endometrial stromal cells which were both positive for vimentin (green) and HN staining. As expected, we did not observe any HN immunostaining in the PBS control group, only DAPI (blue) and Vimentin (green) staining (**Figure 2**).

Therapeutic effects after transplantation

Gross pathology: As we can see, the uterus of Sham-O group is smooth and supple, compared with normal self-control uterus, there is no difference; and after endometrial scraping, the uterus of PBS group is thin and covered with coagulation and exudate; the transplantation group is better than PBS after the surgery.

Histopathology: H&E staining and Masson's staining show that hUCMSCs transplantation exert-

ed favorable effects. The transplantation group tends to have more glands and less collagen deposition. H&E staining shows the absence and irregular structure of endometrial gland in PBS group (1.6 ± 1.2) while an increased number of glands in stem cell treated horns (6.6 ± 1.2) ($P < 0.05$) (**Figure 3**). The ratio of fibrosis area is (0.19 ± 0.08) in Sham-O group, while in PBS and transplantation group is (0.81 ± 0.08) and (0.56 ± 0.09) respectively ($P < 0.05$) (**Figure 3**). When we look at the immuno-histochemical staining of CK18, Ki67, we can see that the expression of CK18 is higher in transplantation group (0.34 ± 0.08) than in PBS group (0.17 ± 0.07) significantly ($P < 0.05$); Compared to the PBS group (0.158 ± 0.07), the expression of Ki67 is higher (0.339 ± 0.07) in hUCMSCs group ($P < 0.05$) (**Figure 3**).

mRNA expressions of cytokines: One week after surgery, qRT-PCR analysis revealed decreased mRNA levels of Col-1, TGF- β 1, FGF2, CTGF; and increased levels of VEGF, Ki67, VIM, CK in the hUCMSCs group (**Figure 4**). Compared to the PBS group, the Col-1, TGF- β 1, FGF2 and CTGF mRNA expressions are decreased significantly in the hUCMSCs group ($P=0.0016$, $P < 0.0001$, $P=0.0018$, $P=0.0383$). Meanwhile, VEGF, Ki67, VIM, CK mRNA expressions are increased when compared with PBS group ($P=0.0005$, $P=0.0006$, $P < 0.00018$, $P=0.0087$) (**Figure 4**).

Discussion

Stem cell therapy has become a novel method for treatment of tissue damage and fibrosis in response to injury. In 2009, it was firstly reported an infertile IUA patient achieved pregnancy after autologous bone marrow mesenchymal stem cells transplantation [13]. As confirmed by many in vivo experiments, embryonic stem cells [23], mesenchymal stem cells (including bone marrow, fat) [11, 12] all showed a positive therapeutic effect on IUA model. However, considering the possibilities of future clinical application, Clearly, mesenchymal stem cells derived from umbilical cord Wharton's jelly constitute an attractive alternative because of many irreplaceable advantages, such as no ethical constraints, the collection procedure is non-invasively, easy preparation and amplified in abundance, lower risk of viral contamination and lower immunogenicity. To our knowledge, there has been no evaluation of the therapeutic

effect of hUCMSCs in IUA model until now [14]. So we preferred to use hUCMSCs, a more accessible source, to treat IUA rat model.

Our results showed that IUA rats who received intrauterine hUCMSCs transplantation had more endometrial glands, a higher expression of cytokeratin and vimentin, lower fibrosis area and expressions of fibrosis factors than control rats. This indicated that direct transplantation of hUCMSCs into the uterine cavities of IUA rats prevented against fibrosis process and cell damage, and promoted the regeneration of endometrial cells. Currently, there are many studies using different approaches to create IUA model, such as chemical aggression, mechanical and/or infectious injury, fibroblast implants and ovariectomy [24-26]. While, since endometrial curettage is the actual situation in routine clinical practice, our present study decided to create a rat model of IUA based on the endometrial curettage which is similar to the clinical situation. In fact, the histopathology evaluation showed that curettage was effective for endometrial destruction after one week.

Another finding of the present study was that hUCMSCs were not rejected by the immune system of rats, existed at least one week in damaged uterine horn and those transplanted rats lived well. Chen et al. reported that hUCMSCs maintain their biological character and function after long-term in vitro culturing (P15). So they can be safely expanded in vitro and maintain a stable immune phenotype and chromosome structure in serum-free medium [27]. In fact, the most intriguing feature of hUCMSCs is displaying low expression levels of HLA class I, no expression of HLA class II, and no expression of costimulatory molecules [28, 29], but expression of HLA-G6, HLA-G5 isoform can inhibit maternal alloreactivity [30-32]. It is reported hUCMSCs can be differentiated and engrafted with successful functional outcome in vivo in rat models for cerebral and myocardial ischemia, multiple sclerosis, retinal disease, type 1 and type 2 diabetes, and so on [33]. Immunity is always a hazard for allogeneic transplantation, however, hUCMSCs make it possible in the future clinical practice.

In order to observe the distribution of hUCMSCs more easily and precisely, we adopt a specific antibody (human nuclear antigen) for human cells rather than using labeled protein.

hUCMSCs improve recovery of intrauterine adhesions

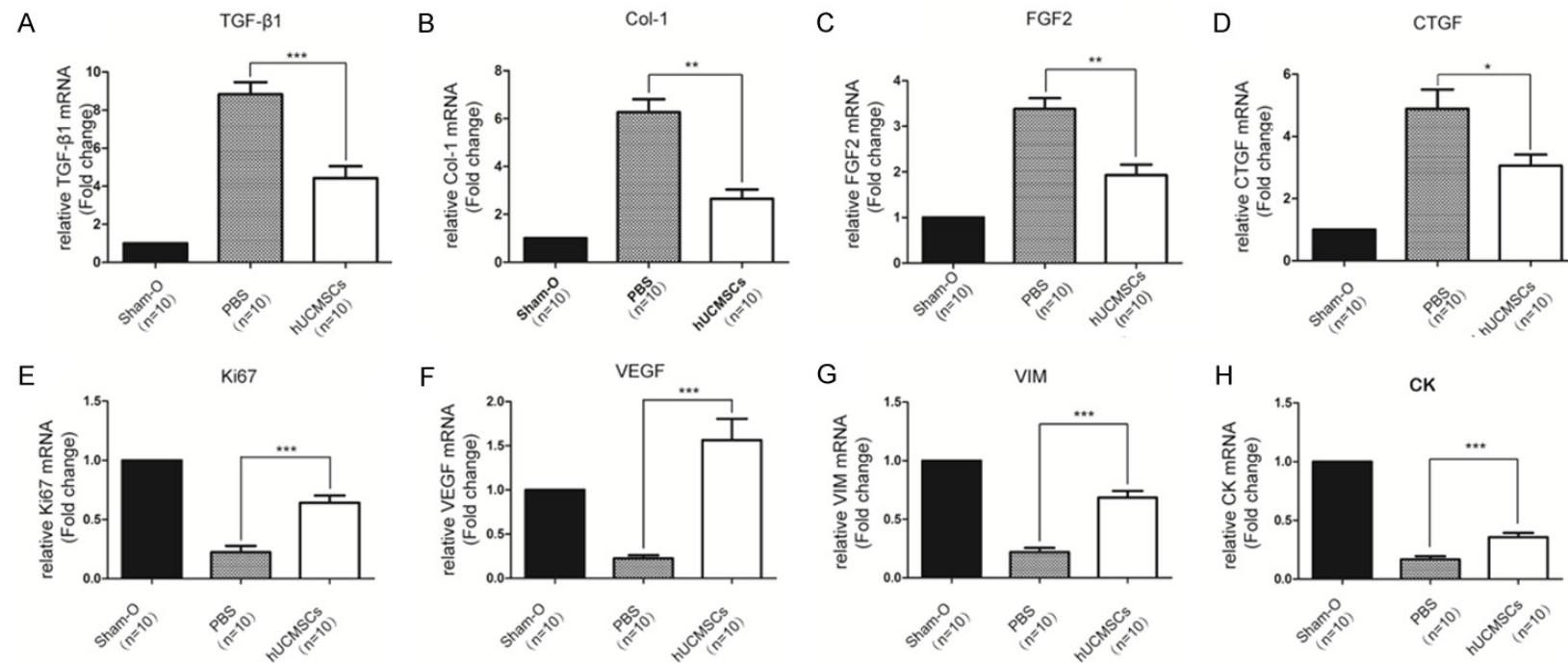


Figure 4. The mRNA expression of TGF-β1, Col-1, FGF2, CTGF, Ki67, VEGF, VIM and CK in rat uteri after one week transplantation was investigated by Quantitative Real-time PCR (A-H). Data are representative of the relative expression of mRNA normalized by GAPDH. Data were presented as mean ± SEM. *P < .05, **P < .01 and ***P < .001.

The exact mechanism of how hUCMSCs promote endometrial regeneration is unknown. It is possible that compound action of hUCMSCs works. One study have described that some of male donor derived bone marrow cells were found to differentiate into epithelial cells in female recipients after bone marrow transplantation [34]. However, Jing Z showed that rat BMSCs could only differentiate into endometrial stromal cells [35]. Our result of double staining of HN and CK showed that hUCMSCs did not differentiate to epithelial cells. Since hUCMSCs is a kind of mesenchymal cells which could express vimentin protein originally. It is hard to say hUCMSCs can differentiate into endometrial stromal cells in vivo. In addition, several studies have shown that paracrine effect of MSCs plays an important role in tissues repair. After recruited to the sites of injury, they can produce various anti-inflammatory cytokines and growth factors, indirectly supporting revascularization, protecting tissue from apoptosis, and appropriately modulating inflammatory reaction [21, 36-38]. Endometrial repair after injury is a complex and dynamic process involving various cytokines. Many studies have shown that hUCMSCs possessed a paracrine capacity and could secrete multiple cellular factors which are benefit to wound healing [36, 39]. Our present study confirmed that several fibrosis factors can be ameliorated and some regenerative factors evaluated significantly by injection of hUCMSCs. Based on these reports and our results, we thought direct replacement of injured tissue cells may not be the major mechanism; hUCMSCs likely secreted a variety of factors that aid in endometrial repair and regeneration.

The limitations of our study can be the lack of hUCMSCs condition medium group and did not explain the exact mechanism. The observation time was not long enough. In addition, some problems remain to be elucidated before hUCMSCs could be utilized in clinical studies. The long term safety of hUCMSCs as a heterograft tissue, optimal and standard dosage, route of transplantation, the possible side effects are unclear yet.

In summary, the results suggested that hUCMSCs transplantation could significantly promote recovery of IUA rat. Taken together, the mechanism of action may be mediated by paracrine effects of hUCMSCs rather than direct differentiation. We hope these findings in the present study may shed some light into allogeneic hUCMSCs transplantation for their future clinical applications.

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Disclosure of conflict of interest

None.

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References

- [1] Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome-one century later. *Fertil Steril* 2008; 89: 759-779.
- [2] Deans R, Abbott J. Review of Intrauterine Adhesions. *J Minim Invas Gyn* 2010; 17: 555-569.
- [3] Mutlu L, Hufnagel D, Taylor HS. The endometrium as a source of mesenchymal stem cells for regenerative medicine. *Biol Reprod* 2015; 92: 138.
- [4] Gargett CE. Uterine stem cells: what is the evidence? *Hum Reprod Update* 2006; 13: 87-101.
- [5] Gargett CE, Masuda H. Adult stem cells in the endometrium. *Mol Hum Reprod* 2010; 16: 818-834.
- [6] Gargett CE, Healy DL. Generating receptive endometrium in Asherman's syndrome. *J Hum Reprod Sci* 2011; 4: 49-52.
- [7] Deane JA, Gualano RC, Gargett CE. Regenerating endometrium from stem/progenitor cells. *Curr Opin Obstet Gynecol* 2013; 25: 193-200.
- [8] Gargett CE, Ye L. Endometrial reconstruction from stem cells. *Fertil Steril* 2012; 98: 11-20.
- [9] Song T, Zhao X, Sun H, Li X, Lin N, Ding L, Dai J, Hu Y. Regeneration of uterine horns in rats us-

- ing collagen scaffolds loaded with human embryonic stem cell-derived endometrium-like cells. *Tissue Eng Part A* 2015; 21: 353-361.
- [10] Zhao J, Zhang Q, Wang Y, Li Y. Uterine infusion with bone marrow mesenchymal stem cells improves endometrium thickness in a rat model of thin endometrium. *Reprod Sci* 2015; 22: 181-188.
- [11] Kilic S, Yuksel B, Pinarli F, Albayrak A, Boztok B, Delibasi T. Effect of stem cell application on Asherman syndrome, an experimental rat model. *J Assist Reprod Genet* 2014; 31: 975-982.
- [12] Alawadhi F, Du H, Cakmak H, Taylor HS. Bone marrow-derived stem Cell (BMDSC) transplantation improves fertility in a murine model of Asherman's syndrome. *PLoS One* 2014; 9: e96662.
- [13] Nagori CB, Panchal SY, Patel H. Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. *J Hum Reprod Sci* 2011; 4: 43-48.
- [14] Ding DC, Chang YH, Shyu WC, Lin SZ. Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. *Cell Transplant* 2015; 24: 339-47.
- [15] Watson N, Divers R, Kedar R, Mehindru A, Mehindru A, Borlongan MC, Borlongan CV. Discarded wharton jelly of the human umbilical cord: a viable source for mesenchymal stromal cells. *Cytotherapy* 2015; 17: 18-24.
- [16] Bongso A, Fong C. The therapeutic potential, challenges and future clinical directions of stem cells from the wharton's Jelly of the human umbilical cord. *Stem Cell Rev Rep* 2013; 9: 226-240.
- [17] Chao YH, Wu HP, Chan CK, Tsai C, Peng CT, Wu KH. Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation. *J Biomed Biotechnol* 2012; 2012: 759503.
- [18] Fan CG, Zhang QJ, Zhou JR. Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. *Stem Cell Rev* 2011; 7: 195-207.
- [19] Lin SZ, Chang YJ, Liu JW, Chang LF, Sun LY, Li YS, Luo GH, Liao CH, Chen PH, Chen TM, Lee RP, Yang KL, Harn HJ, Chiou TW. Transplantation of human wharton's jelly-derived stem cells alleviates chemically induced liver fibrosis in rats. *Cell Transplant* 2010; 19: 1451-1463.
- [20] Gao LR, Chen Y, Zhang NK, Yang XL, Liu HL, Wang ZG, Yan XY, Wang Y, Zhu ZM, Li TC, Wang LH, Chen HY, Chen YD, Huang CL, Qu P, Yao C, Wang B, Chen GH, Wang ZM, Xu ZY, Bai J, Lu D, Shen YH, Guo F, Liu MY, Yang Y, Ding YC, Yang Y, Tian HT, Ding QA, Li LN, Yang XC, Hu X. Intracoronary infusion of Wharton's jelly-derived mesenchymal stem cells in acute myocardial infarction: double-blind, randomized controlled trial. *BMC Med* 2015; 13: 162.
- [21] Cheng Q, Zhang Z, Zhang S, Yang H, Zhang X, Pan J, Weng L, Sha D, Zhu M, Hu X, Xu Y. Human umbilical cord mesenchymal stem cells protect against ischemic brain injury in mouse by regulating peripheral immunoinflammation. *Brain Res* 2015; 1594: 293-304.
- [22] Wang X, Wu H, Xue G, Hou Y. Progesterone promotes neuronal differentiation of human umbilical cord mesenchymal stem cells in culture conditions that mimic the brain microenvironment. *Neural Regen Res* 2012; 7: 1925-1930.
- [23] Song T, Zhao X, Sun H, Li X, Lin N, Ding L, Dai J, Hu Y. Regeneration of uterine horns in rats using collagen scaffolds loaded with human embryonic stem cell-derived endometrium-like cells. *Tissue Eng Part A* 2015; 21: 353-361.
- [24] Liu F, Zhu Z, Li P, He Y. Creation of a female rabbit model for intrauterine adhesions using mechanical and infectious injury. *J Surg Res* 2013; 183: 296-303.
- [25] Polishuk WZ, Schenker JG. Induction of intrauterine adhesions in the rabbit with autogenous fibroblast implants. *Am J Obstet Gynecol* 1973; 115: 789-794.
- [26] Bazoobandi S, Tanideh N, Rahmanifar F, Tamaadon A, Keshtkar M, Mehrabani D, Kasraeian M, Koohi-Hosseinabadi O. Induction of asherman's syndrome in rabbit. *J Reprod Infertil* 2016; 17: 10-16.
- [27] Chen G, Yue A, Ruan Z, Yin Y, Wang R, Ren Y, Zhu L. Human umbilical cord-derived mesenchymal stem cells do not undergo malignant transformation during long-term culturing in serum-free medium. *PLoS One* 2014; 9: E98565.
- [28] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005; 105: 1815-1822.
- [29] Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, Deans RJ, McIntosh KR. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J Biomed Sci* 2005; 12: 47-57.
- [30] Selmani Z, Naji A, Gaiffe E, Obert L, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F. HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. *Transplantation* 2009; 87: S62-S66.
- [31] Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, Borg C, Saas P, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. *Stem Cells* 2008; 26: 212-222.

- [32] Prasanna SJ, Jahnavi VS. Wharton's Jelly Mesenchymal Stem Cells as Off-The-Shelf Cellular Therapeutics: a closer look into their regenerative and Immunomodulatory properties. *Open Tissue EngRegMedJ* 2011; 28-38.
- [33] Ding DC, Shyu WC, Chiang MF, Lin SZ, Chang YC, Wang HJ, Su CY, Li H. Enhancement of neuroplasticity through upregulation of beta1-integrin in human umbilical cord-derived stromal cell implanted stroke model. *Neurobiol Dis* 2007; 27: 339-353.
- [34] Ikoma T, Kyo S, Maida Y, Ozaki S, Takakura M, Nakao S, Inoue M. Bone marrow-derived cells from male donors can compose endometrial glands in female transplant recipients. *Am J Obstet Gynecol* 2009; 201: 601-608.
- [35] Jing Z, Qiong Z, Yonggang W, Yanping L. Rat bone marrow mesenchymal stem cells improve regeneration of thin endometrium in rat. *Fertil Steril* 2014; 101: 587-594.
- [36] Guo ZY, Sun X, Xu XL, Zhao Q, Peng J, Wang Y. Human umbilical cord mesenchymal stem cells promote peripheral nerve repair via paracrine mechanisms. *Neural Regen Res* 2015; 10: 651-658.
- [37] Huang S, Wu Y, Gao D, Fu X. Paracrine action of mesenchymal stromal cells delivered by microspheres contributes to cutaneous wound healing and prevents scar formation in mice. *Cytotherapy* 2015; 17: 922-31.
- [38] Guo ZY, Sun X, Xu XL, Zhao Q, Peng J, Wang Y. Human umbilical cord mesenchymal stem cells promote peripheral nerve repair via paracrine mechanisms. *Neural Regen Res* 2015; 10: 651-658.
- [39] Hu SL, Luo HS, Li JT, Xia YZ, Li L, Zhang LJ, Meng H, Cui GY, Chen Z, Wu N, Lin JK, Zhu G, Feng H. Functional recovery in acute traumatic spinal cord injury after transplantation of human umbilical cord mesenchymal stem cells. *Crit Care Med* 2010; 38: 2181-2189.