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Research Report

Umbilical cord mesenchymal stem cell transplantation significantly improves neurological function in patients with sequelae of traumatic brain injury

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ABSTRACT

The aim of this study was to investigate the effects of transplantation with umbilical cord mesenchymal stem cells in patients with sequelae of traumatic brain injury (TBI). The study hypothesis was that umbilical cord mesenchymal stem cell transplantation could safely and effectively improve neurological function in patients with sequelae of traumatic brain injury. Forty patients with sequelae of TBI were randomly assigned to the stem cell treatment group or the control group. The patients in the stem cell treatment group underwent 4 stem cell transplantations via lumbar puncture. All patients of the group were also evaluated using Fugl-Meyer Assessments (FMA) and Functional Independence Measures (FIM) before and at 6 months after the stem cell transplantation. The patients in the control group did not receive any medical treatment (i.e., neither surgery nor medical intervention), and their FMA and FIM scores were determined on the day of the visit to the clinic and at 6 months after that clinical observation. The FMA results demonstrated an improvement in upper extremity motor sub-score, lower extremity motor sub-score, sensation sub-score and balance sub-score in the stem cell transplantation group at 6 months after the transplantation ($P<0.05$). The FIM results also exhibited significant improvement ($P<0.05$) in the patient self-care sub-score, sphincter control sub-score, mobility sub-score, locomotion sub-score, communication sub-score and social cognition sub-score. The control group exhibited no improvements after 6 months ($P>0.05$). All in all,

Abbreviations: TBI, traumatic brain injury; FMA, Fugl-Meyer Assessment; FIM, Functional Independence Measure; UCMSCs, umbilical cord mesenchymal stem cells; MSCs, mesenchymal stem cells; PT, physical therapy; OT, occupational therapy; WHO, World Health Organization; GCS, Glasgow Coma Scale; DMEM, Dulbecco's modified Eagle's medium; GDNF, glial cell line-derived neural factor; BDNF, brain-derived neurotrophic factor; SDF-1, stromal-derived factor 1

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the study results confirmed that the umbilical cord mesenchymal stem cell transplantation improved the neurological function and self-care in patients with TBI sequels. Umbilical cord mesenchymal stem cell transplantation may be a potential treatment for patients with sequelae of TBI. Further research, including a multicenter and large sample size prospective randomized clinical trial, will be required to define definitively the role of umbilical cord mesenchymal stem cell transplantation on sequelae of TBI.

1. Introduction

Umbilical cord mesenchymal stem cells (UCMSCs) display strong self-renewal and differentiation abilities. When induced by chemical and neurotrophic factors, UCMSCs can differentiate into bone, cartilage, fat, muscle and vascular endothelial cells or even neural cells and glial cells with secretory functions (Fan et al., 2011; Koh et al., 2008; Secco et al., 2008; Troyer and Weiss, 2008; Wu et al., 2007; Zhang et al., 2010). The major cell types used in animal experiments and in clinical treatment are neural stem cells (Itoh et al., 2011; Pardal and Lopez-Barneo., 2012), bone marrow mesenchymal stem cells (Cheng et al., 2010; Chernykh et al., 2011), umbilical cord mesenchymal stem cells (Liao et al., 2009; Yang et al., 2008; Zanier et al., 2011), embryonic stem cells (Cui et al., 2011; Palmgren et al., 2012; Ronaghi et al., 2010) and umbilical cord blood stem cells (Ali et al., 2011; Dasari et al., 2009). UCMSCs have many advantages compared with other cell types, including the following: (1) the wide range of sources and the ease of their collection, storage and transport; (2) no risk of allograft rejection; (3) no ethical controversy (Romanov et al., 2003).

Therefore, mesenchymal stem cells (MSCs) are used in a wide range of applications, such as the treatment of traumatic brain injury, Parkinson's disease, neuromyelitis optica or diabetic renal injury among others (Lu et al., 2012; Park et al., 2012; Shi et al., 2012; Xiong et al., 2011).

Traumatic brain injury (TBI) is one of the many serious diseases that threaten human life and health. TBIs are caused primarily by traffic accidents, collisions with hard objects and falling from high places (Hu et al., 2012; Wu et al., 2008; Zhao and Wang, 2001). With improving medical technology, the survival rate of patients with TBIs has increased significantly. However, the majority of those who survive suffer from varying types of disabilities, such as body motor dysfunction, language and communication difficulties, mental problems and psychological and social cognitive defects. All of these disabilities affect the patients' studies, work and daily life seriously (Andelic et al., 2009; Jaracz and Kozubski, 2008). The current typical treatment for TBI includes surgery or conservative symptomatic treatment during early stages and physical therapy (PT) and occupational therapy (OT) during late stages. Stem cell therapy for TBI remains at the stage of animal experimentation (Chuang et al., 2012; Tu et al., 2012). Studies have indicated that active rehabilitation exercises during the first year after TBI can restore in part the damaged nerve function (Al-Jarrah et al., 2009). However, the current typical rehabilitation protocols have little benefit for patients with TBIs that have existed for more than one year. This study investigated the clinical treatment effects of transplantation of UCMSCs in patients with sequelae of TBI that had been sustained more than one year previously. The study aims to certify the additional compensation of neurological recovery providing by the migration and differentiation of stem cells or the neurotrophic factors.

2. Results

2.1. FMA scoring

2.1.1. Baseline FMA scores of the patients in both groups

The upper extremity motor sub-score of the stem cell transplantation group and the control group were 16.60±11.70 and 15.95±9.63, respectively, and the lower extremity motor sub-score were 12.75±6.25 and 14.80±8.54, respectively. The sensation sub-score were 15.75±7.25 and 14.15±9.22, respectively. The balance sub-score were 5.40±3.19 and 6.05±3.89, respectively, and the total scores of the two groups were 50.50±21.80 and 50.95±25.48, respectively. The differences between the two groups were not statistically significant ($P>0.05$; Table 1).

2.1.2. Comparison of the FMA scores before and at 6 months after stem cell transplantation

The motor sub-scores of upper and lower extremity, the sensation and balance sub-scores at baseline and 6 months after stem cell transplantation are presented in Table 1. Statistically significant improvements after transplantation were observed in the upper extremity motor sub-score ($P<0.001$), the lower extremity motor sub-score ($P<0.05$), the sensation sub-score ($P<0.05$), the balance sub-score ($P<0.001$), and the total FMA score ($P<0.001$; Table 1).

2.1.3. Comparison of the FMA scores in the control group at baseline and at 6 months

The motor sub-scores of upper and lower extremity, the sensation and balance sub-scores at baseline and at 6 months in the control group are presented in Table 1. Statistical analyses revealed that, in the control group, there were no significant differences between timepoints in the upper extremity motor sub-score, lower extremity motor sub-score, sensation sub-score, balance sub-score or in the total scores ($P>0.05$). In addition, there were no changes in the lower extremity motor sub-score or in the sensation sub-score between baseline and 6 months (Table 1).

Table 1 – FMA scores of the two groups.

Group	Time	Patients	The sub-scales scores and total scale scores of the FMA				
			Upper extremity motor subscore	Lower extremity motor subscore	Sensation subscore	Balance Subscore	Total score
Stem cell transplantation group	Baseline	20	16.60±11.70	12.75±6.25	15.75±7.25	5.40±3.19	50.50±21.80
	Six months after transplantation	20	19.15±12.42[a]	13.90±6.52[a]	16.55±7.35[a]	6.50±3.12[a]	56.10±23.10[a]
	Delta value of FMA scores	20	2.55±1.85	1.15±1.31	0.80±1.51	1.10±0.79	5.60±3.15
Control group	Baseline	20	15.95±9.63	14.80±8.54	14.15±9.22	6.05±3.89	50.95±25.48
	Six months after baseline	20	16.10±9.55	14.80±8.54	14.15±9.22	6.15±3.86	51.20±25.45
	Delta value of FMA scores	20	0.15±0.49[b]	0.00±0.00[b]	0.00±0.00[b]	0.10±0.45[b]	0.25±0.64[b]

[a] Scores at six months after treatment or observation vs. baseline, $P<0.05$.
[b] Scores in control group vs. stem cell transplantation group, $P<0.05$.

2.1.4. *Comparison of the FMA delta value between the two groups*

The delta values (difference) of the upper and lower extremity, the sensation and balance sub-scores between the 6 months and baseline timepoints in the stem cell transplantation group and the control group were presented in Table 1. Statistically significant improvements were observed in the upper extremity motor sub-score ($P<0.001$), lower extremity motor sub-score ($P<0.001$), the sensation sub-score ($P<0.05$), the balance sub-score ($P<0.001$) and the total FMA score ($P<0.001$) in the stem cell transplantation group compared with the control group (Table 1).

2.2. *FIM scoring*

2.2.1. *Baseline FIM scores of both groups*

The self-care sub-score of the stem cell transplantation and the control groups were 19.90±10.26 and 20.50±12.43, respectively, and the sphincter control sub-score were 9.75±3.65 and 9.80±4.58, respectively. The mobility sub-score were 8.80±5.57 and 9.35±6.83, respectively, and the locomotion sub-scores were 7.30±4.76 and 7.05±5.12, respectively. The communication sub-score were 9.75±3.31 and 10.25±3.55, respectively, and the social cognition sub-score were 11.65±4.46 and 12.80±5.33, respectively. The total scores of the two groups were 67.15±25.05 and 69.75±34.49, respectively. The differences between the two groups at baseline were not statistically significant ($P>0.05$; Table 2).

2.2.2. *Comparison of the neurological function scores of the stem cell transplantation group at baseline and at 6 months*

The sub-scores of self-care and sphincter control, the mobility and locomotion, the communication and social cognition sub-scores at baseline and 6 months after stem cell transplantation are presented in Table 2. Statistical analyses revealed that the self-care sub-score, mobility sub-score, locomotion sub-score, communication sub-score and the total score of the stem cell transplantation group were significantly improved at 6 months after transplantation compared with the scores prior to transplantation ($P<0.05$). The sphincter control sub-score and the social cognition sub-score were improved at 6 months but the differences were not statistically significant ($P>0.05$; Table 2).

2.2.3. *Comparison of the neurological scores of the control group at baseline and at 6 months*

The sub-scores of self-care and sphincter control, the mobility and locomotion, the communication and social cognition sub-scores at baseline and at 6 months in the control group are presented in Table 2. Statistical analyses showed that at the 6-month follow-up, the patients in the control group did not exhibit any significant difference in self-care sub-score, sphincter control sub-score, mobility sub-score, locomotion sub-score, communication sub-score or social cognition sub-score or in the total score ($P>0.05$). In addition, there were no changes in sphincter control sub-score, mobility sub-score or locomotion sub-score (Table 2).

2.2.4. *Comparison of the FIM delta values between the groups*

The delta values (difference) of self-care and sphincter control, the mobility and locomotion, the communication and social cognition sub-scores between the 6 months and baseline timepoints in the stem cell transplantation group and the control group were presented in Table 2. Statistical analyses revealed that the self-care sub-score, mobility sub-score, locomotion sub-score and the total score in the stem cell transplantation group were significantly increased at 6 months after transplantation compared with the control group ($P<0.05$). The sphincter control sub-score, communication sub-score and the social cognition sub-score were also increased at 6 months after transplantation in the stem cell transplantation group compared with the control group but the differences were not statistically significant ($P>0.05$; Table 2).

Table 2 – FIM scores of the two groups.

Group	Time	Patients	The sub-scales scores and total scale scores of the FIM						
			Self-care subscore	Sphincter control subscore	Mobility subscore	Locomotion subscore	Communication subscore	Social cognition subscore	Total score
Stem cell transplantation group	Baseline	20	19.90±10.26	9.75±3.65	8.80±5.57	7.30±4.76	9.75±3.31	11.65±4.46	67.15±25.05
	Six months after transplantation	20	21.80±11.01[a]	9.80±3.61	9.60±5.77[a]	7.95±4.33[a]	10.25±2.99[a]	12.10±4.56	71.35±26.21[a]
	Delta value of FIM scores	20	1.90±2.17	0.05±0.22	0.80±1.24	0.65±0.99	0.50±0.61	0.45±1.00	4.20±3.58
Control group	Baseline	20	20.50±12.43	9.80±4.58	9.35±6.83	7.05±5.12	10.25±3.55	12.80±5.33	69.75±34.49
	Six months after baseline	20	20.80±12.22	9.80±4.58	9.35±6.83	7.05±5.12	10.45±3.43	12.95±5.13	70.40±33.87
	Delta value of FIM scores	20	0.30±0.66[b]	0.00±0.00	0.00±0.00[b]	0.00±0.00[b]	0.20±0.52	0.15±0.67	0.65±1.42[b]

[a] Scores at six months after treatment or observation vs. baseline, $P<0.05$.
[b] Scores in control group vs. stem cell transplantation group, $P<0.05$.

2.3. *Adverse reactions during treatment and during the follow-up period in the stem cell transplantation group*

Four patients (4/20) experienced low intracranial pressure reactions within 48 h of the lumbar puncture injection. The symptoms included mild dizziness and headache that were rarely accompanied by nausea and vomiting. These symptoms worsened when the patients got out of bed and moved and were relieved when the patients lay in bed. All of these symptoms were relieved or disappeared when the patients lay in bed in a supine position without a pillow and were treated with intravenous saline infusions. During treatments, all patients' body temperatures, heart rates, blood pressures, oxygen saturations and respiratory rates were monitored, and no obvious abnormalities were found. At 6 months after stem cell transplantations, both a head and spinal cord MR were performed for each patient. No abnormalities that were related to the stem cell transplantations were found.

3. Discussion

TBI can destroy neurons, glial cells, nerve fibers and blood vessels directly. Ischemia, edema and other factors secondary to brain injury can cause new damage to the surrounding intact tissue (Kurland et al., 2012) thereby causing varying neurological dysfunction in surviving patients. Determining how to effectively treat neurological impairment has been a widespread and difficult area of neuroscience research. Dogma suggested that the central nervous system had no ability to renew or regenerate after injury (Jackson and Alvarez-Buylla, 2008) but with intensive study in fields of stem cells and neuroregeneration, this viewpoint has gradually been corrected. The neurological recovery of patients with TBI depends primarily on compensation provided by uninjured neurons and on the migration and differentiation of neural stem cells, which are predominantly located in the subependymal zone and the parahippocampal gyrus (Johanson et al., 2011). However, the quantity of a patient's own stem cells is limited; and their capability of enabling self-recovery is weak. Therefore, exogenous stem cell transplantation provides a novel method of promoting the recovery of neurological function in patients with TBI.

The safety of allogenic MSC transplantation has been studied and confirmed in many species and even in non-human primate models. MSCs did not induce immune rejection or graft versus host reactions after transplantation even when no immunosuppression was administered. Indeed, MSCs can reduce alloimmune responses and promote tolerance in allograft animal models. Feng et al. generated a model of intracerebral hematoma in *Macaca fascicularis* monkeys and then injected human-derived MSCs into the brain tissue near the hematomas. The efficacy of the treatment was evaluated using serial ^{18}F-FDG PET scans, scoring for neurologic deficits and pathologic analyses. No immune rejection was found, and ^{18}F-FDG uptake was significantly higher in areas transplanted with MSCs and in the adjacent cortex. Neurologic deficit scores were significantly lower in the MSC-treated groups indicating the better recovery of animals in this group. Pathologic analyses revealed higher

blood vessel densities surrounding the MSC-injected brain tissue (Feng et al., 2011). Li et al. transplanted human-derived MSCs to treat a model of brain ischemia in *M. fascicularis* monkeys and reported no serious adverse reactions. They showed that transplantation of MSCs into ischemic brain tissue improved neurological function and increased IL-10 expression. IL-10 is well-known as an anti-inflammatory cytokine with neuroprotective properties (Li et al., 2010). Lu et al. (2006) reported that human umbilical cord-derived MSCs expressed low levels of human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I but did not express HLA MHC class II and costimulatory molecules (CD40, CD80, and CD86). The production of tolerogenic TGF-β and IL-10 was significantly higher in MSCs, MSCs showed a significantly higher proliferation activity, more robust in vitro activation of allogeneic lymphocytes and delayed rejection in vivo (Deuse et al., 2011). Carrade et al. used allogeneic umbilical cord-derived MSCs to treat acute equine lesions using intradermal injections. They did not note any acute graft rejection or a delayed-type of hypersensitivity response (Carrade et al., 2011). Wang et al. administered human umbilical cord-derived MSCs via intravenous injection in cynomolgus monkeys. Toxicity was evaluated using clinical observations, pathology, immunologic consequences and anatomic pathology. All animals survived until a scheduled euthanasia timepoint, and no stem cell transplantation-related toxicity was reported (Wang et al., 2011).

To explore the mechanism that underlies stem cell-induced restoration, MSCs were transplanted into a mouse model of TBI. They were found to migrate to the sites of injury and to differentiate into neurons, glial cells, and vascular endothelial cells. These differentiated cells secreted glial cell line-derived neural factor (GDNF), brain-derived neurotrophic factor (BDNF) and stromal-derived factor 1 (SDF-1), which promote neural regeneration, neovascularization and blood supply at the site of the lesion (Mitchell et al., 2003; Zanier et al., 2011).

In this study, 20 patients with sequelae of TBI were treated by administering UCMSCs via transplanted into the subarachnoid space using lumbar punctures. The efficacy of the transplantations was assessed using both FMA and FIM scores for extremity motor function, sensation, balance and the ability to live independently (Connell and Tyson, 2012; Nichol et al., 2011). The two groups of patients showed equivalent baseline scores indicating that the two groups were comparable. Based on FMA evaluations at the 6-month follow-up, the upper extremity motor sub-score, lower extremity motor sub-score, sensation sub-score and balance sub-score of the control group was not significantly improved, and there were no changes in lower extremity motor sub-score or sensation sub-score. In contrast, in the stem cell transplantation group, the upper extremity motor sub-score, lower extremity motor sub-score, sensation sub-score and balance sub-score improved markedly at 6 months after transplantation ($P<0.05$). There were highly significant differences between baseline and the 6-months timepoint after transplantation in the upper extremity motor sub-score and the balance sub-score ($P<0.001$) in the stem cell transplantation group. The FIM evaluations showed that after 6 months of observation, the self-care sub-score, sphincter control sub-score, mobility sub-score, locomotion sub-score, communication sub-score, and social cognition sub-score were not significantly improved in the control group ($P>0.05$). In contrast, in the stem cell transplantation group, the self-care sub-score, mobility sub-score, locomotion sub-score and communication sub-score were significantly improved at 6 months after treatment ($P<0.05$). The sphincter control sub-score and social cognition sub-score in the stem cell transplant group were also improved after 6 months (FIM scores increased by 0.58 ± 1.16 and 0.42 ± 1.00, respectively) but these differences were not statistically significant ($P>0.05$). There are 2 possible explanations for this lack of significance: (1) the sample size was too small, and the scales used to functionally evaluate sphincter control and social cognition are not very sensitive thereby making it difficult to detect improvements; and (2) the effects of the stem cell treatment on sphincter control and social cognition are not as robust as the effects noted on other functions. A larger sample size should be used in future studies, and some specific examinations (e.g., neuroelectrophysiology and urodynamics) should be performed in further analyses.

Based on this study, UCMSC transplantation can significantly improve numerous neurological functions. Moreover, although the mechanisms that underlie the observed improvements remain unclear, we initially suggest the following possibilities: (1) cell replacement by proliferation and differentiation of the transplanted stem cells into the phenotype of the damaged and lost cells, (2) trophic support, (3) manipulation of the environment to stimulate endogenous neural repair and regeneration.

4. Conclusion

This study confirmed the efficacy and safety of the treatment with UCMSCs in patients with TBI sequelae. In future, we will use UCMSCs to treat spinal cord injuries, stroke sequelae, cerebral palsy and other neurological diseases. Our next step is to prepare collaborations with imaging centers and neuroelectrophysiological and urodynamics centers to perform large sample size clinical trials to define UCMSCs as a promising candidate for the treatment of diseases of the central nervous system.

5. Experimental procedures

5.1. *Patient recruitment*

A randomized, single-blind controlled clinical study was conducted. The treatment regimens have been registered in the Chinese Clinical Trials Registry Platform of the World Health Organization (WHO; Registration no. ChiCTR-TNRC-11001528). This study also obtained approval of the Medical Ethics Committee of the General Hospital of the Chinese People's Armed Police Forces.

Inclusion criteria: (1) patients were diagnosed as having sequelae of TBI based on clinical manifestations, head CTs and MR examinations; (2) patients suffered from central nervous system dysfunction at the time of recruitment; (3)

Table 3 – Patients' demographics, cause of injury and injury severity.

Variable	Stem cell transplantation group (N=20)	Range	Control group (N=20)	Range
Age, years	27.50±9.43	5–48	28.64±10.13	7–57
Time from injury to assessment (years)	4.80±2.69	1–10	5.86±4.54	2–11
GCS score	6.60±1.60	6–10	6.92±1.38	5–11
	N	%	N	%
Gender				
Male	17	85.00	15	75.00
Female	3	15.00	5	25.00
Type of injury				
Motor vehicle accident	13	65.00	13	65.00
Falling	4	20.00	6	30.00
Assault	3	15.00	1	5.00
CT, MRI scans				
Normal	2	10.00	3	15.00
Atrophy	2	10.00	3	15.00
Focal abnormality	13	65.00	12	60.00
Atrophy & focal abnormality	3	15.00	2	10.00
Clinical examination				
Dyskinesia	20	100.00	19	95.00
Sensory disability	16	80.00	17	85.00
Allolalia	13	65.00	11	65.00

patients did not undergo any surgery or medical treatment that could have interfered with the functional assessment within 3 months of the baseline and during the study period; (4) patients were willing to be recruited and signed a written, informed consent form.

Exclusion criteria: (1) skull defects excluding cranioplasty; (2) intracranial infection or hydrocephalus; (3) inherited metabolic diseases of the CNS; (4) intractable epilepsy; (5) allergies or autoimmune diseases; (6) A history of tumors and/or blood disease; (7) a positive result upon serologic testing for AIDS, hepatitis or syphilis; (8) patient who showed dysfunction or exhaustion of the heart, liver, kidneys or other vital organs.

Forty patients with sequelae of TBI were randomly assigned to the stem cell transplantation group or the control group at the Department of Cell Transplantation, the General Hospital of the Chinese People's Armed Police Forces between May 2011 and May 2012. Forty patients were recruited to the trial and were randomly divided into the stem cell treatment group and the control group. Twenty patients in the stem cell treatment group underwent four stem cell transplantations via lumbar puncture and received a 6-months follow-up. Twenty patients were included in the control group. At the endpoint, 40 patients finished the entire trial. Fifteen of the 20 patients who were assigned to the stem cell transplantation group had previously undergone cranial bone flap decompression and intracranial hematoma clearance surgery, and the other 5 patients had received conservative medical treatment after the injury. For the 20 patients in the control group, 13 had been previously treated with cranial bone flap decompression and intracranial hematoma clearance, and the other 7 patients had been treated only with conservative medical therapy after the TBI. All patients who underwent cranial bone flap surgery had also undergone cranioplasty before being recruited into this study. Baseline demographics, cause of injury and injury severity (GCS, CT, MRI and clinical examination) are described in Table 3.

Differences in average patient age, gender, time from injury to assessment, cause of injury and injury severity between the 2 groups were not statistically significant ($P>0.05$).

5.2. *Preparation of UCMSCs*

With the written consent of the parents, a fresh human umbilical cord was collected. After the cord was disinfected in 75% ethanol for 30 s, the blood vessels were removed. The cord was cut into cubes of approximately 0.5 cm^3 and centrifuged at 250*g* for 5 min. Following removal of the supernatant, these cubes were washed with serum-free Dulbecco's modified Eagle's medium (DMEM; Gibco) and centrifuged at 250*g* for 5 min. After aspiration of the supernatant, the cubes were placed into a 6-well plate, cultured in DMEM supplemented with 10% FBS and incubated at 37 °C in a humidified tissue culture incubator in 5% CO_2 and 95% air. After 10 days in culture, the adherent cells from individual explanted cord tissue sections were observed. The cord tubes were removed from the cultures, and the adherent cells were cultured to 80% confluence. UCMSCs at between passages 6 and 8 were used for transplantation. Prior to clinical applications, multiple tests were performed on the UCMSCs to ensure the quality of cells: (1) cell morphology: cells grew vigorously and showed the typical phenotype of mesenchymal cells under phase contrast microscopy (Fig. 1); (2) cell surface markers: fluorocytometry was performed to check the cell surface markers; the percentage of cells that were positive for CD44, CD73, CD90 and CD105 was greater than 95%, and the expression rates of CD19 (2.73%), CD45 (0.01%), CD11b (0.11%), CD34 (0.54%) and HLA-DR was not higher than 5% (Fig. 2); (3) sterility tests: bacterial and mycoplasma contaminations were excluded; (4) endotoxin tests were negative.

5.3. *Transplantation methods*

After a routine examination to exclude surgical contraindications and administration of local anesthesia, a lumbar puncture was performed in the lumbar 3–4 or lumbar 4–5 intervertebral space. After the puncture needle had been confirmed to penetrate into the subarachnoid space, 2 ml of stem cell suspension (containing 1×10^7 stem cells) was slowly injected into the subarachnoid space. This transplantation procedure was performed 4 times over an interval of 5–7 days. After transplantation, patient body temperatures, heart rates, blood pressures, oxygen saturations and respiratory rates were monitored for 6 h using a multi-functional monitor.

5.4. *Evaluation of the treatment effect*

The original report of the Fugl-Meyer Assessment (FMA; Fugl-Meyer et al., 1975), which was published in 1975, outlined the purpose and target population for this scale, its content, and instructions for scoring. It is a multi-item Likert-type scale that was developed as a measure to evaluate recovery from hemiplegic stroke. The FMA is a 226-point multi-item scale that assesses motor function, sensory function, balance, joint range of motion, and joint pain. Each domain comprises multiple items, each of which is scored on a 3-point ordinal scale (0=cannot perform, 1=performs partially, 2=performs fully). The motor score ranges from 0 (hemiplegia) to a maximum of 100 points (normal motor performance) and is divided into 66 points for the upper extremity and 34 points for the lower extremity. Similarly, there is a maximum of 24 points for sensation, 14 points for sitting and standing balance, 44 points for joint range of motion, and 44 points for joint pain (Gladstone et al., 2002). In China, the responsiveness and validity of the Fugl-Meyer Assessment has been completed. The responsiveness of the FMA was significantly greater than that of the ARAT and the WMFT-TIME but not the WMFT functional ability scores. With respect to construct validity, correlations between the FMA and other measures were relatively high (P=0.42–0.76). The FMA and the WMFT performance time scores at pre-treatment showed moderate predictive validity with the FIM scores at post-treatment (P=0.42–0.47). These results support the fact that FMA is suitable for detecting changes over time in patients after stroke rehabilitation. While simultaneously considering the validity and responsiveness attributes, the FMA may be a relatively sound measure of motor function for stroke patients based on our results (Hsieh et al., 2009).

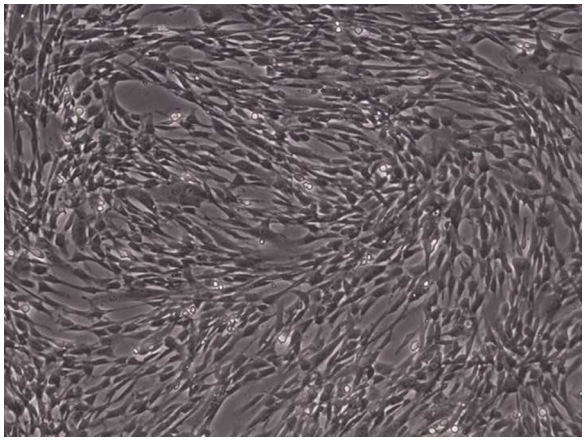

Fig. 1 – UCMSCs' morphology.

The initial full version of the Functional Independence Measure (FIM; Granger and Gresham., 1984) was developed during the 1980s to assist rehabilitation clinicians to reliably document and report a patient's level of disability. It is widely used in the United States and has been implemented in a limited manner in England. The FIM is an 18-item rating scale

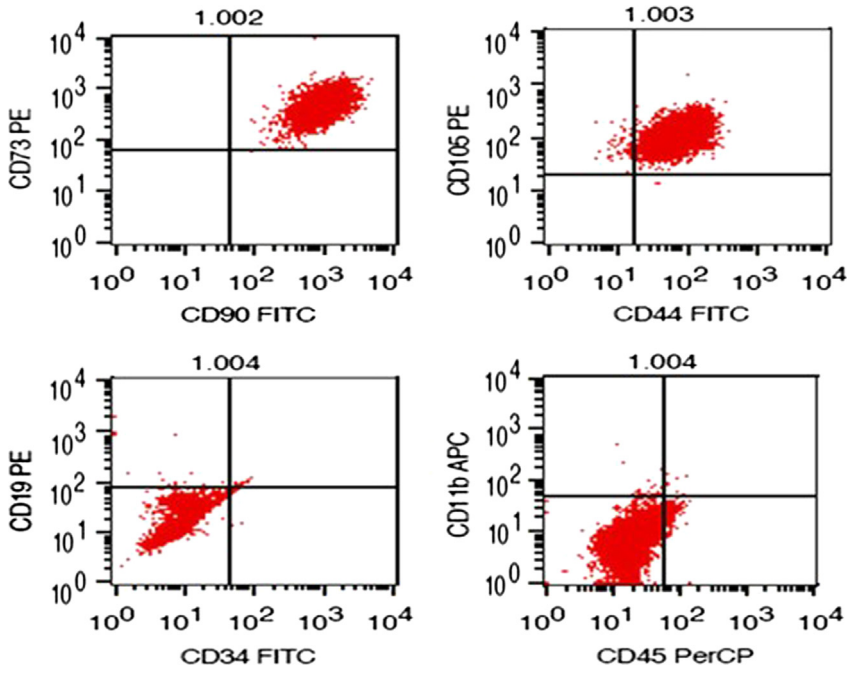

Fig. 2 – The result of UCMSCs surface markers checked by fluorocytometry.

that assesses self-care, bowel and bladder management, mobility, communication, cognition, and psychosocial adjustment. The total score range from 18 to 126 and comprises subscale score ranges of 42 for self-care, 14 for bowel and bladder management, 21 for mobility, 14 for locomotion, 14 for communication, and 21 for cognition and psychosocial adjustment. A total FIM score of <108 indicates a limitation in activities and a requirement for assistance from another person, whereas scores of 109–126 indicate functional independence. In China, the reliability and validity of the Functional Independence Measure has been completed, and the FIM shows good intra-rater and inter-rater reliability ($r>0199$, $P<0.01$) and good overall internal consistency (admission FIM, $\alpha=0.83$; discharge FIM, $\alpha=0.82$). With regard to FIM validity, FIM demonstrated some responsiveness (45% FIM score improvement, $P<0.05$) and showed a positive correlation with BI and MMSE ($r=0.9174$, $P<0.001$; $r=0.3162$, $P<0.05$). The FIM motor sub-score was positively correlated with BI ($r=0.9546$, $P<0.001$). The FIM cognitive subscore was also positively correlated with MMSE ($r=0.8567$, $P<0.001$). These results indicate that the FIM and FMA show good retest reliability, good internal consistency and good validity in the functional evaluation of inpatients with commonly encountered disabilities (Qiu et al., 1998).

Patients in the stem cell transplantation group underwent face-to-face testing with the FMA and the FIM tools prior to stem cell transplantation and at 6 months post-transplantation. Patients in the control group underwent FMA and FIM testing at their first visit and at 6 months later. All assessments for each case were completed by the same qualified rehabilitator. Single-blinding was used for each evaluation (the rehabilitator did not know the group each patient was in). To eliminate other factors that could interfere with nerve function assessment, the two groups of patients were not permitted to take any drugs that would affect the assessment or to undergo any surgery during the 6-month period between the assessments.

5.5. *Statistical methods*

SPSS 16.0 software was used for statistical analyses. The clinical data are presented as the average plus/minus the standard deviation ($\bar{x}\pm s$). Data from the 2 timepoints within the same group were compared using the paired *t*-test. $P<0.05$ was considered to be statistically significant.

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