

# A 37-year-old spinal cord-injured female patient, transplanted of multipotent stem cells from human UC blood, with improved sensory perception and mobility, both functionally and morphologically: a case study

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*HLA-matched UC blood-derived multipotent stem cells were directly transplanted into the injured spinal cord site of a 37-year-old female patient suffering from spinal cord injury (SCI). In this case, human cord blood (UCB)-derived multipotent stem cells improved sensory perception and movement in the SCI patient's hips and thighs within 41 days of cell transplantation. CT and MRI results also showed*

*regeneration of the spinal cord at the injured site and some of the cauda equina below it. Therefore, it is suggested that UCB multipotent stem cell transplantation could be a good treatment method for SCI patients.*

## Keywords

*clinical trial, multipotent stem cells, spinal cord injury, UC blood.*

## Introduction

Spinal cord injury (SCI) is a major medical problem worldwide. Great efforts have been made to improve the condition of SCI patients, not only regarding sensory perception but also functional ability [1]. There are some recent reports related to animal model experiments that indicate some hope for SCI patients [2,3].

The blood remaining in the UC following birth contains hematopoietic precursors, which represent an important alternative source for transplantation for hematopoietic diseases [4–6]. However, controversy exists as to whether such blood also contains multipotent stem cells (MSC) that are capable of differentiating into cells of different connective tissue lineages, such as bone, cartilage and adipose tissues. Stem cells are the best candidates for tissue engineering of musculoskeletal tissues [7–9]. To date, the most common source of MSC has been BM, but aspirating BM from a patient is an invasive procedure. In addition, it has been demonstrated that the number and differentiating

potential of BM-derived MSC decrease with age [10]. Therefore, the search for alternative sources of MSC is of significant value. So far, little success has been reported regarding the isolation, characterization and differentiation of MSC from umbilical cord blood (UCB). Erices *et al.* [11] reported that UCB-derived mononuclear cells gave rise to two adherent cell types, one of which expressed MSC related to surface Ag. Mareschi *et al.* [12] reported that, in given conditions, it was possible to isolate MSC from BM but not from UCB. However, Goodwin *et al.* and our laboratory [13,14] have recently reported cells that have multilineage differentiation activity, isolated from UCB, and express bone, fat and neural markers. Kakinuma *et al.* [15] reported that UCB-derived MSC could differentiate into hepatic progenitor cells. However, none of these reports provided sufficient evidence to fulfill the qualifying criteria for MSC, because relatively heterogeneous cells were reported by the groups. It has been reported that MSC from BM can improve SCI functional models in the

laboratory [3]. However, there has been no report of cord blood MSC related to SPI. This study is the first report regarding a clinical trial for a chronic SPI patient using MSC derived from UCB. In this study, we report that MSC from UCB can show functional and morphologic improvement in a chronic SPI patient.

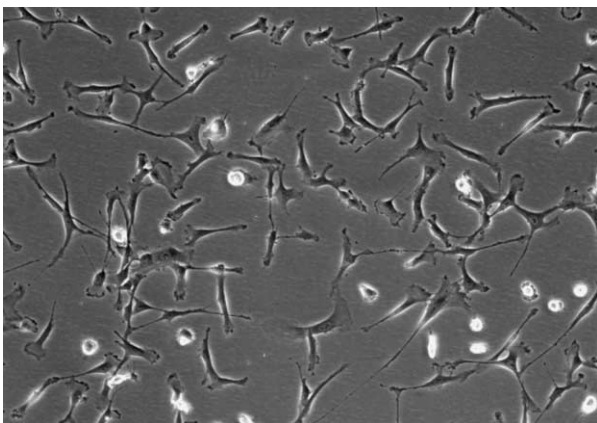
## Methods

### Human UCB harvest and preparation of MSC

Human UCB samples were harvested (Seoul Cord Blood Bank, Seoul, Korea) from term and pre-term deliveries at the time of birth with the mothers' consent. Blood samples were processed within 24 h of collection. The mononuclear cells were separated from UCB using Ficoll-Paque™ PLUS (Amersham Bioscience, Uppsala, Sweden) and were suspended in culture medium (DEME; Gibco, Grand Island, NY, USA) containing 15% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate. The cells were then seeded at a density of  $1 \times 10^6$  cells/cm<sup>2</sup> in culture flasks. After 7 days of culture, suspended cells were removed and adherent cells were additionally cultured (Figure 1). Cells were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide, with a change of culture medium every 3–4 days. Approximately 50–60% of confluent cells was detached with 0.1% trypsin-EDTA and replated at a density of  $2 \times 10^3$  cells/cm<sup>2</sup> in culture flasks.

### Patients and cell transplantation

A 37-year-old female who fell into a ditch in July 1985 underwent an emergency operation, approved by the Korean Food and Drug Administration, to repair the SPI



**Figure 1.** The morphology of multipotent stem cells from human UCB. The morphology is fibroblastic and spindle-shaped.

derived from a compression fracture of the 12th thoracic vertebra and fracture dislocation of the 11th–12th thoracic vertebrae. She eventually became paraplegic and was dependent on the use of an electromotive wheel chair for her movement for 19 years and 6 months before treatment with MSC.

The patient was admitted to Chosun University Hospital, Kwang-ju, Korea, for UCB-derived MSC therapy via the Department of Neurosurgery in 2004. Her vital signs were within the normal range, her motor power of upper extremities was 4/5 and of lower extremities 0/5 on a manual muscle test. The range of motion of joint was absent and grade I spasticity was noticed on lower extremities using the Ashworth's scale. On sensory–neural examination, the dermatome of the 9th thoracic vertebra was normal in range but one of the 10th–12th thoracic vertebrae was decreased on the left side. The dermatome up to the 11th thoracic vertebra was normal in range, one of the 12th thoracic vertebra was decreased, and one below the 1st lumbar vertebra was absent in right side. Deep tendon reflex of both knees and ankles was decreased. Perianal sensitivity and voluntary anal sphincter contraction were not observed. The extent of neural injury according to the standard of the ASIA (American Spinal Injury Association) was complete paraplegia of the 10th thoracic vertebra.

An open procedure (posterior approach with laminectomy) was performed under general anesthesia to ensure proper stem cell administration. After observation of the injured dura of the spinal cord at the level of the 10th–12th thoracic vertebrae, 1 mL ( $1 \times 10^6$  cells) of cord blood multipotent stem cells was injected into the subarachnoid space of the most distal part of the normal spinal cord. Then an additional 2 mL (one million) stem cells was injected diffusely into the intradural and extradural space of the injured spinal cord. Hemovac was not inserted for the purpose of implanted stem cell protection. Before and after treatment, no immunosuppressive drugs were used.

### Neural differentiation of multipotent stem cells *in vitro*

Cells were plated at 1000–2000 cells/cm<sup>2</sup> in complete medium with the addition of 10 ng/mL basic fibroblast growth factor (bFGF; Roche, Indianapolis, IN, USA Switzerland), 10 ng/mL human epidermal growth factor (hEGF; Roche) and 10 ng/mL human neural growth factor (hNGF; Invitrogen, Grand Island, NY, USA) for 14 days.

We have reported previously that our MSC have been seen to differentiate into neuronal cells, *in vitro*, by immunofluorescent assay, RT-PCR and Western blot analysis [14]. In this study, to confirm the neural differentiation ability again, the expression of neural related Ag was examined. Rabbit polyclonal Ab were used against NSE (Chemicon, Temecula, CA, USA) and GFAP (Chemicon). For immunocytochemical NSE and GFAP labeling, cells were rinsed with PBS and fixed with 3.7% formaldehyde in PBS for 10 min at room temperature. They were then treated with ice-cold 100% methanol for 10 min, 100% acetone for 5 min and 0.4% Triton X-100 in PBS for 10 min, with triple PBS rinses between treatments. Samples were treated with 2% horse serum (Gibco-BRL, Gaithersburg, MD, USA) and 2% goat serum (Zymed Laboratories Inc., San Francisco, CA, USA) in PBS containing 4% BSA (PBS/BSA) for 100 min at 37°C to block non-specific binding of primary Ab. Ab were diluted in PBS/BSA plus 2% horse and 2% goat sera at 1:200 for NSE and 1:200 for GFAP. The primary Ab were incubated on cells for 1 h at 37°C. Samples were rinsed three times with PBS. Fluorescent secondary Ab were added concurrently: FITC, TRITC anti-rabbit (Zymed Laboratories Inc.) diluted 1:200 in PBS/BSA plus 2% horse and 2% goat sera, for 45 min at 37°C. Slides were rinsed with PBS and mounted in Gelvatol (Lab Vision, Fremont, CA, USA). Fluorescence was visualized using a fluorescent microscope.

### Flow cytometric analysis of cord-derived multipotent stem cells

To detect surface Ag, cells were detached and washed with PBS (Jeil Biotechserveices Inc., Daegu, Korea) and incubated at 4°C for 30 min with the following cell-specific Ab conjugated with FITC or PE (Becton Dickinson, San Jose, CA, USA): SH2 (CD105, endoflin), SH3 (CD73), CD13, CD29 ( $\beta_1$  intergrin), CD44, CD49e ( $\alpha_5$  intergrin), CD54 (ICAM-1), CD90 (Thy-1), CD14, CD34, CD45, CD31, CD49d ( $\alpha_4$  intergrin), CD106 (VCAM-1), HLA-ABC and HLA-DR.

### Results

In order to assess their ability to differentiate into neuronal cells, cord blood-derived MSC were cultured in neurogenic medium. When exposed to hEGF/hFGF/hNGF for 2 weeks, UCB-derived MSC could express neural-specific Ag and showed some morphologic features of neural cells, such as long multi-polar extensions and branching ends.

After neuronal differentiation, the UCB-derived MSC expressed NSE and GFAP, which are cytoskeletal proteins in neurons and astrocytes, respectively. Therefore, MSC in human UCB can expand *in vitro* and differentiate into non-mesenchymal cells, as we previously reported [14].

Before using the isolated MSC, we confirmed cell viability by trypan blue exclusion and counted cells from primary cultured MSC using a hemocytometer. We used only populations with >95% viable cells (the total viable cell number,  $1 \times 10^6$ ) for the transplantation. The isolated MSC are shown in Figure 1. The morphology was fibroblastoid and spindle-shaped. Flow cytometric analysis revealed that the cells were negative for CD24 (B cells), CD62L (B cells, T cells, monocytes, NK cells), CD62P (platelets, megakaryocytes) and CD20 (B cells). UCB-derived cells were found to be positive for CD29 (leukocytes), CD44 (hyaluronidase receptor), CD14 (myelomonocytic cells), CD49b ( $\alpha_2$  intergrin) and CD135 (multipotent precursors).

Before the operation, electrodiagnostic results had shown that the somatosensory and motor-evoked potentials of both tibial nerves revealed no response. A nerve conduction study of the common peroneal and tibial nerves also showed no response. The dermatomal somatosensory-evoked potential showed no response below the right 12th thoracic and left 11th thoracic vertebra (Table 1), and no somatosensory and motor-evoked potential below the right 10th and left 9th thoracic vertebra (Table 2). For radiologic findings, thoracolumbar plain films, myelogram and MRI revealed the old compression fracture of the 12th thoracic vertebra and posterior dislocation of the 11th vertebra, and the resultant SPI by compression at the 10th–12th thoracic vertebrae level (Figure 2e).

The patient could move her hips and feet her hip skin on day 15 after transplantation. On day 25 after transplantation, her feet responded to stimulation. On post-operative day (POD) 7, motor activity was noticed and improved gradually in her lumbar paravertebral and hip muscles. She could maintain an upright position by herself on POD 13. From POD 15 she began to elevate both lower legs about 1 cm, and hip flexor muscle activity gradually improved until POD 41. The posterior tibial nerve conduction, which was not observed pre-operatively, showed 100 mV amplitude, 55.3 m/s conduction velocity on POD 20. This conduction improved up to 300 mV amplitude, 56.3 m/s on POD 27.

**Table 1.** Changes of dermatomal somatosensory-evoked potential after transplantation

Date	10/11		10/12		10/26		11/1		11/8		11/15	
	R	L	R	L	R	L	R	L	R	L	R	L
T10	31.4	42.2	27.8	36.6	32.0	29.8	42.0	36.8	30.4	36.8	31.2	33.2
T11	34.2	46.8	34.6	37.4	40.2	39.6	44.6	40.8	48.8	39.6	43.8	47.0
T12	50.4	NE	51.2	44.6	49.2	51.6	55.6	53.8	51.6	45.4	64.2	56.8
L1	NE	NE	51.4	NE	NE	55.8	56.8	64.4	66.0	58.4	72.0	76.8
L2	NE	NE	NE	NE	NE	NE	NE	NE	74.8	72.0	NE	NE

NE, no effect; R, right leg; L, left leg.

Examinations were carried out for somatosensory and motor-evoked potentials. The potentials were activated down to L1 in the right leg and to T12 in the left leg, compared with T10 of the right leg and T9 of the left leg before transplantation. The time-course of these potentials was also examined. It became clear that the somatosensory and motor-evoked potentials had gone down to L2 in both legs (Table 2). Regarding the dermatomal somatosensory-evoked potential, we also examined the patient in a time-course manner. These results also showed that the dermatomal somatosensory-evoked potential was recovered to L2 in both legs on day 41 after operation compared with pre-operation (T12 in the right leg, T11 in the left leg; Table 1). These results suggested that the sensory and motor nerves of the patient might have been continuously improved and regenerated.

To prove regeneration of the spinal cord, CT and MRI scans were performed of the thoracolumbar spine. These results revealed that there was a compression fracture in T12 with retropulsion of the fragment into the spinal canal. Anterior subluxation of T11 on T12 caused a compression effect of the spinal cord before transplantation. The atrophied spinal cord was expanded after stem cell administration, with total laminectomy, and the low-

ermost portion of the atrophied spinal cord was enlarged, along with thinning and interruption of the calcified pia mater at the T12–L1 level on pre-contrast axial CT films (Figure 2b, d). Sagittal T2 weighted SE MRI revealed regenerating spinal cord at the injured level (Figure 2f, arrow) and some of the cauda equina below it (Figure 2f, arrow heads).

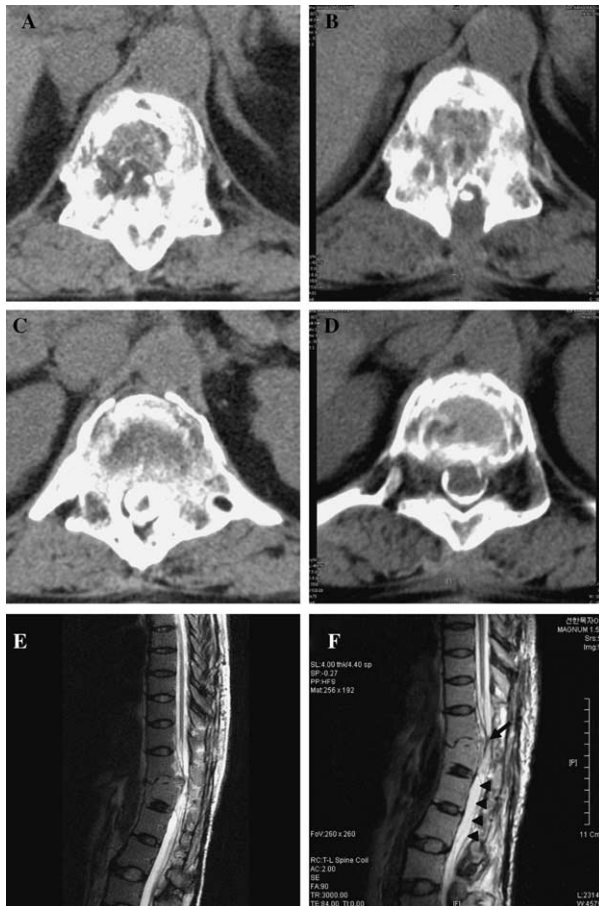
### Discussion

Although the presence of multipotent MSC/progenitor cells in cord blood is widely reported [9,11,16,17], these cells are known to proliferate poorly in the *in vitro* culture system. There has been skepticism regarding the presence of human multipotent stem/progenitor cells in UCB [12,18]. Despite the fact that BM represents the main available source of MSC, the use of BM-derived cell is not always acceptable because of the high degree of viral infection and significant drop in the number of the cells and proliferation/differentiation capacity with age. Houghton *et al.* [19] reported BM-derived stem cells as a potential source of gastric cancer. However, it has not yet been reported that UCB-derived cells can form tumor. Therefore, UCB-derived MSC have many advantages because of:

**Table 2.** Change of somatosensory and motor-evoked potentials after transplantation

Date	10/11		11/1		11/8		11/15	
	R	L	R	L	R	L	R	L
T9	14.3	14.5	13.9	13.7	14.0	13.7	14.2	14.5
T10	19.5	NE	17.6	18.8	15.8	16.3	16.5	16.1
T11	NE	NE	19.0	19.4	17.0	17.6	18.1	17.9
T12	NE	NE	19.8	20.0	17.6	18.0	18.3	18.7
L1	NE	NE	20.0	NE	18.5	18.8	19.5	19.0
L2	NE	NE	NE	NE	NE	NE	NE	NE

NE, no effect; R, right leg; L, left leg.



**Figure 2.** The atrophied spinal cord is expanded after stem cell administration with total laminectomy on pre-contrast axial CT films (b). The lowermost portion of the atrophied spinal cord is enlarged, along with thinning and interruption of the calcified pia mater at the T12–L1 level on pre-contrast axial CT films (d). Sagittal T2 weighted SE MRI reveal regenerating spinal cord at the injured level (arrow, f) and some of the cauda equina below it (arrow heads, f). CT images before cell transplantation (a, c) and MRI image before cell transplantation (e).

- the immaturity of newborn cells compared with adult cells
- the fact that immune reactions causing dysfunctional grafts can be avoided.

Therefore, we have established a new paradigm for stem cell therapy without immunosuppression, because cord blood-derived stem/progenitor cells are less likely to attack a recipient's body than BM-derived cells. In this case report, we have shown that UCB-derived MSC improved the condition, physically and morphologically, within 41 days after cell transplantation, of an SPI patient who had suffered for about 20 years. The specific

mechanisms and reasons regarding how the patient improved so quickly now remain to be explained fully. Further studies are urgently needed.

Possible explanations of the effects are:

- UCB-derived MSC are able to produce many cytokines and growth factors (unpublished data)
- MSC can directly reconstitute injured spinal cord (we have also demonstrated that cord blood-derived MSC are capable of differentiating into neural features *in vitro* [20]).

These results strongly support the clinical improvement of the patient. However, as well as these two possible explanations, we cannot exclude the act of laminectomy, which can release compressed areas of the spinal cord, because we have reported on only one case study.

Based on their large *ex vivo* expansion capacity as well as on their differentiation potential, cord blood-derived MSC can be visualized as an attractive source for cellular and gene transfer therapy for incurable and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Nieman-Pick disease, diabetes and so on. Furthermore, UCB provide no ethical problems for basic studies and clinical application compared with embryonic stem cells.

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