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# Olfactory ensheathing cell transplantation improves sympathetic skin responses in chronic spinal cord injury

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## Research Highlights

- (1) Recovery of autonomic nerve function is difficult in the treatment of spinal cord injury.
- (2) Olfactory ensheathing cell transplantation improves somatic nerve function and sympathetic skin responses in patients with chronic spinal cord injury.
- (3) Sympathetic skin response can objectively reflect the recovery of autonomic nerve function in patients with spinal cord injury after cell transplantation.

## Abstract

Forty-three patients with chronic spinal cord injury for over 6 months were transplanted with bryonic olfactory ensheathing cells,  $2-4 \times 10^6$ , into multiple sites in the injured area under the surgical microscope. The sympathetic skin response in patients was measured with an electromyography/evoked potential instrument 1 day before transplantation and 3–8 weeks after transition. Spinal nerve function of patients was assessed using the American Spinal Injury Association impairment scale. The sympathetic skin response was elicited in 32 cases before olfactory ensheathing cell transplantation, while it was observed in 34 cases after transplantation. tantly, sympathetic skin response latency decreased significantly and amplitude increased cantly after transplantation. Transplantation of olfactory ensheathing cells also improved American Spinal Injury Association scores for movement, pain and light touch. Our findings indicate that factory ensheathing cell transplantation improves motor, sensory and autonomic nerve functions in patients with chronic spinal cord injury.

## Key Words

neural regeneration; spinal cord injury; clinical practice; olfactory ensheathing cells; cell tation; olfactory ensheathing cell transplantation; sympathetic skin response; neurological function; autonomic nerve; paralysis; neuroregeneration

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## INTRODUCTION

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Paralysis and other dysfunctions caused by chronic spinal cord injury are major concerns for scientists and clinicians. In recent animal experiments and clinical trials, olfactory ensheathing cell transplantation has been shown to improve neurological functioning, including motor, sensory and autonomic nerve functions (such as skin sweating), after spinal cord injury<sup>[1-5]</sup>. Neurological functions following spinal cord injury have generally been assessed using the American Spinal Injury Association (ASIA) impairment scale<sup>[6]</sup>. However, the scale primarily evaluates motor function, and little attention has been given to autonomic nerve functions, such as defecation, sweating or skin response.

The sympathetic skin response test is a simple, safe, noninvasive electrophysiological detection method, and can objectively assess autonomic nerve functions in patients with spinal cord injury<sup>[7-8]</sup>.

In this study, we examined changes in sympathetic skin response and ASIA scores in spinal cord injury patients before and after olfactory ensheathing cell transplantation. Our aim was to provide insight into the effects of this therapeutic approach on neurological function in spinal cord injury patients.

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## RESULTS

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### Quantitative analysis of participants

Forty-three patients with chronic spinal cord injury were involved in the analysis.

### Baseline data on participants

The baseline data for the 43 patients with chronic spinal cord injury are shown in Table 1.

### Olfactory ensheathing cell transplantation increased somatic nerve function in patients with chronic spinal cord injury

Somatic nerve function in 43 patients with spinal cord injury was evaluated using the ASIA scale at 3–8 weeks after olfactory ensheathing cell transplantation. After trans-

plantation, the ASIA scores for pain, touch and motor functions increased, and patients showed improvements in sensory ability and motor power. Statistical analysis showed that, after transplantation, the pain scores in these patients increased significantly ( $P < 0.01$ ), while the touch and motor scores did not increase significantly ( $P > 0.05$ ; Table 2), compared with before transplantation.

### Olfactory ensheathing cell transplantation improved sympathetic skin response in patients with chronic spinal cord injury

After transplantation of olfactory ensheathing cells, the paraplegic patients exhibited increased skin sweating, a brighter skin tone, reduced desquamation, smoother stool, increased urine volume, and a reduction in residual urine volume. The sympathetic skin response was measured with an electromyography/evoked potential instrument. Among the 17 subjects with complete spinal cord injury, 8 cases showed a sympathetic skin response before transplantation and 10 cases showed a response after transplantation. Among the 26 subjects with incomplete spinal cord injury, a sympathetic skin response was observed in 24, both before and after transplantation (the number of cases was unchanged). In total, 32 patients were included in the statistical analysis. After transplantation, the sympathetic skin response amplitude significantly increased, while the latency period significantly decreased (both  $P < 0.01$ ; Table 3).

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## DISCUSSION

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Effective treatment of chronic spinal cord injury remains a medical challenge. Transplantation of olfactory ensheathing cells may promote neurological recovery in patients with chronic spinal cord injury<sup>[1-4]</sup>; however, the degree of recovery is limited<sup>[5]</sup>. While a great deal of attention has been given to the recovery of somatic nerve (sensory and motor) functions, autonomic nerve function has been neglected. Although the ASIA scale<sup>[6]</sup> and the Frankel grading system<sup>[9]</sup> are considered the most effective evaluation

methods for spinal cord function and spinal cord injury, respectively, they do not include or reflect autonomic function. In addition, there is no effective method for the evaluation of spinal autonomic nerve function<sup>[10]</sup>. Furthermore, there is a biased and narrow perception of clinical effectiveness. For example, while no therapeutic effect may be indicated by the outcome index “standing and walking, bowel controlling”, it ignores other aspects of improved neurological functioning. The neurological functions of the spinal cord are complex, and functional

abnormalities in the autonomic system manifest as the following<sup>[11]</sup>: (1) skin color, temperature and nutrition: skin cyanosis, chilling, drying, thinning, subcutaneous tissue slightly swelling, brittle nails, hair loss, trophic ulcer; (2) sphincter functional impairment: urinary retention or incontinence, constipation or incontinence; (3) circulation disorder: increased or lowered blood pressure; (4) peripheral vasomotor disorders: spastic and expanded; (5) sexual dysfunction; (6) hypohidrosis: no or reduced sweat after spinal cord injury.

Table 1 Baseline information on patients with chronic spinal cord injury

| Case No. | Gender | Age (years) | Course of disease     | Injury area        | Injury cause      | ASIA grading | Number of transplanted cells ( $\times 10^6$ ) | Follow-up time (day) | Sympathetic skin response before transplantation |
|----------|--------|-------------|-----------------------|--------------------|-------------------|--------------|--|----------------------|--|
| 1        | Male   | 46          | 1 year and 6 months   | C <sub>3-4</sub>   | Traffic accident  | B            | 3.4  | 246                  | Yes  |
| 2        | Male   | 24          | 4 years and 6 months  | C <sub>3-4</sub>   | Falling injury    | B            | 3.1  | 36                   | Yes  |
| 3        | Male   | 43          | 1 year and 6 months   | C <sub>3-4</sub>   | Traffic accident  | D            | 2.8  | 58                   | Yes  |
| 4        | Male   | 12          | 8 years               | C <sub>5</sub>     | Traffic accident  | C            | 3.2  | 26                   | Yes  |
| 5        | Male   | 30          | 2 years               | C <sub>5</sub>     | Knife injury      | B            | 2.4  | 35                   | Yes  |
| 6        | Male   | 22          | 2 years               | C <sub>5</sub>     | Traffic accident  | A            | 3.4  | 24                   | Yes  |
| 7        | Male   | 36          | 2 years and 6 months  | C <sub>5-6</sub>   | Traffic accident  | B            | 3.4  | 28                   | Yes  |
| 8        | Male   | 43          | 1 year and 8 months   | C <sub>5-6</sub>   | Falling injury    | B            | 3.0  | 30                   | Yes  |
| 9        | Male   | 47          | 2 years               | C <sub>6-7</sub>   | Traffic accident  | B            | 2.1  | 44                   | Yes  |
| 10       | Male   | 22          | 9 months              | C <sub>6-7</sub>   | Traffic accident  | A            | 2.6  | 27                   | No   |
| 11       | Male   | 24          | 1 year and 10 months  | C <sub>6-7</sub>   | Traffic accident  | A            | 2.8  | 30                   | Yes  |
| 12       | Male   | 35          | 1 year and 6 months   | C <sub>6-7</sub>   | Traffic accident  | A            | 2.4  | 31                   | No   |
| 13       | Male   | 41          | 8 months              | C <sub>7</sub>     | Falling injury    | C            | 3.0  | 26                   | Yes  |
| 14       | Male   | 28          | 2 years               | T <sub>1</sub>     | Traffic accident  | D            | 3.1  | 42                   | Yes  |
| 15       | Male   | 42          | 2 years               | T <sub>10</sub>    | Traffic accident  | A            | 3.1  | 32                   | No   |
| 16       | Female | 33          | 1 year and 6 months   | T <sub>3-4</sub>   | Traffic accident  | C            | 3.4  | 27                   | Yes  |
| 17       | Male   | 29          | 1 year                | T <sub>3-4</sub>   | Crash injury      | A            | 2.8  | 30                   | Yes  |
| 18       | Male   | 26          | 9 months              | T <sub>4-6</sub>   | Traffic accident  | A            | 3.8  | 32                   | No   |
| 19       | Male   | 35          | 2 years               | T <sub>5-6</sub>   | Traffic accident  | B            | 3.4  | 44                   | No   |
| 20       | Male   | 27          | 25 days               | T <sub>5-6</sub>   | Traffic accident  | D            | 3.5  | 29                   | Yes  |
| 21       | Male   | 39          | 1 year and 5 months   | T <sub>6</sub>     | Falling injury    | A            | 3.2  | 29                   | No   |
| 22       | Male   | 46          | 1 year                | T <sub>6</sub>     | Traffic accident  | A            | 3.0  | 34                   | No   |
| 23       | Male   | 19          | 7.5 months            | T <sub>7</sub>     | Traffic accident  | B            | 3.0  | 22                   | Yes  |
| 24       | Male   | 39          | 1 year and 5 months   | T <sub>8</sub>     | Traffic accident  | A            | 3.0  | 25                   | No   |
| 25       | Male   | 32          | 10 months             | T <sub>8-9</sub>   | Crash injury      | B            | 3.0  | 26                   | Yes  |
| 26       | Female | 43          | 1 year and 6 months   | T <sub>9</sub>     | Falling injury    | A            | 2.5  | 29                   | Yes  |
| 27       | Male   | 32          | 2 years and 6 months  | T <sub>9-10</sub>  | Crash injury      | B            | 3.0  | 26                   | No   |
| 28       | Female | 30          | 18 years              | T <sub>11</sub>    | Traffic accident  | D            | 4.0  | 34                   | Yes  |
| 29       | Male   | 30          | 2 years and 10 months | L <sub>2</sub>     | Traffic accident  | B            | 3.0  | 30                   | Yes  |
| 30       | Male   | 31          | 2 years and 2 months  | L <sub>3</sub>     | Traffic accident  | B            | 3.5  | 23                   | Yes  |
| 31       | Male   | 33          | 5 years and 7 months  | L <sub>3-4</sub>   | Traffic accident  | C            | 3.6  | 29                   | Yes  |
| 32       | Male   | 39          | 3 years               | T <sub>11-12</sub> | Iatrogenic injury | B            | 2.0  | 23                   | Yes  |
| 33       | Male   | 32          | 1 year and 6 months   | T <sub>11-12</sub> | Traffic accident  | B            | 3.0  | 29                   | Yes  |
| 34       | Male   | 37          | 11 months             | T <sub>11-12</sub> | Traffic accident  | B            | 3.4  | 27                   | Yes  |
| 35       | Male   | 37          | 7 months              | T <sub>11-12</sub> | Crash injury      | A            | 3.5  | 31                   | Yes  |
| 36       | Male   | 30          | 7 years and 6 months  | T <sub>11-12</sub> | Falling injury    | A            | 3.0  | 36                   | No   |
| 37       | Male   | 35          | 9 years and 6 months  | T <sub>11-12</sub> | Traffic accident  | A            | 2.5  | 24                   | No   |
| 38       | Female | 25          | 8 months              | T <sub>11-12</sub> | Crash injury      | A            | 3.6  | 26                   | Yes  |
| 39       | Male   | 54          | 7.5 months            | T <sub>11-12</sub> | Iatrogenic injury | D            | 3.0  | 30                   | Yes  |
| 40       | Male   | 36          | 8 months              | T <sub>12</sub>    | Traffic accident  | C            | 3.5  | 26                   | Yes  |
| 41       | Male   | 24          | 2 years               | T <sub>12-L1</sub> | Traffic accident  | B            | 4.0  | 22                   | Yes  |
| 42       | Male   | 42          | 11 years and 6 months | T <sub>12-L1</sub> | Traffic accident  | C            | 3.0  | 22                   | Yes  |
| 43       | Male   | 31          | 1 year and 6 months   | T <sub>12-L1</sub> | Traffic accident  | A            | 3.5  | 41                   | Yes  |

The ASIA classification system categorizes the injury into four grades: A, complete spinal cord injury; B–D, incomplete spinal cord injury. Yes: Autonomic nerve function present; No: loss of autonomic nerve function.

Table 2 Changes in pain, touch and motor scores using the American Spinal Injury Association scale in patients with spinal cord injury before and after olfactory ensheathing cell transplantation

| Time                   | Pain      | Touch     | Motor     |
|------------------------|-----------|-----------|-----------|
| Before transplantation | 60.1±18.3 | 63.6±16.8 | 52.9±15.7 |
| After transplantation  | 69.8±17.9 | 67.9±17.8 | 56.0±15.7 |
| <i>t</i>               | -3.164    | -1.551    | -1.322    |
| <i>P</i>               | 0.002     | 0.128     | 0.193     |

Data are expressed as mean ± SD of 43 patients. Paired *t*-test shows that the scores for pain, touch and motor function were increased (especially the pain score) after cell transplantation. The higher scores indicate better neurological function in patients.

Table 3 Change in sympathetic skin response amplitude (mV) and latency (second) in spinal cord injury patients before and after olfactory ensheathing cell transplantation

| Time                   | Amplitude | Latency   |
|------------------------|-----------|-----------|
| Before transplantation | 1.35±0.56 | 2.83±0.33 |
| After transplantation  | 1.77±0.64 | 2.41±0.27 |
| <i>t</i>               | -6.330    | 11.841    |
| <i>P</i>               | 0.000     | 0.000     |

Data are expressed as mean ± SD. A total of 32 patients were evaluated for the sympathetic skin response. Paired *t*-test shows that the sympathetic skin response amplitude was significantly increased, while the latency period was significantly shortened, after cell transplantation. This indicates recovery of autonomic nerve function.

Therefore, improvements in autonomic nerve functions can alleviate or prevent complications following spinal cord injury, and can greatly impact the patient's health and quality of life.

The sympathetic skin response is a skin sudomotor reflex involving the brain and spinal cord, and is recorded as an epidermal voltage change, which is related to sweat gland secretion<sup>[12-14]</sup>. It is a sympathetic polysynaptic reflex induced by exogenous and endogenous stimuli (including non-noxious)<sup>[15-16]</sup>. These stimuli recruit thick myelinated sensory nerve fibers or sympathetic sudomotor efferent fibers after integration in the acoustic nerve afferent center, which induces synchronous activity in the sweat glands. The electrical activity potential on the skin surface is recorded. This activity reflects the body-sympathetic nerve reflex, which involves the efferent nerve center composed of the medulla oblongata and the spinal cord. This pathway also involves the sympathetic postganglionic unmyelinated C fibers. Although the efferent central pathways in the human body are not entirely clear, the peripheral pathways include the sympathetic ganglia, preganglionic fibers, postganglionic fibers and skin sweat glands<sup>[17]</sup>. The degree of autonomic

nerve functional impairment in spinal cord injury patients is associated with the severity of injury<sup>[7]</sup>. The measurement of the sympathetic skin response allows for the evaluation of sympathetic preganglionic fiber integrity, exclusive of supraspinal center abnormalities or sympathetic postganglionic fiber abnormalities<sup>[18]</sup>. It also shows high specificity and sensitivity in the detection of autonomic nerve function<sup>[19-20]</sup>.

The sympathetic skin response can be used for the evaluation of autonomic nerve function in patients with spinal cord injury<sup>[21]</sup>, especially to assess spinal cord core integrity<sup>[22]</sup>. The sympathetic skin response is not elicited in all patients with spinal cord injury; it is dependent on the degree and the position of the spinal cord injury, and it is affected by the site of electrical stimulation<sup>[18]</sup>. Complete transection of the spinal cord results in an absent sympathetic skin response<sup>[8, 23]</sup>. Thus, the presence of an evoked skin response indicates intact sympathetic spinal cord functioning. Consequently, the ASIA grading system is not perfect because of its inability to fully assess autonomic nerve impairment. The sympathetic skin response is a supplementary tool for the detection of complete spinal cord injury and for assessing prognosis.

In this study, 17 patients with complete spinal cord injury were included. Before transplantation, 8 of these patients showed an evoked sympathetic skin response, while after transplantation, 10 patients showed a response. This suggests that spinal cord center functions were improved in two patients. A total of 32 subjects showed an improved sympathetic skin response after transplantation, indicating that olfactory ensheathing cell transplantation can improve the functioning of sympathetic spinal centers in patients with spinal cord injury.

Transplantation of olfactory ensheathing cells may promote the recovery of neurological function following spinal cord injury<sup>[24-26]</sup>. After transplantation of olfactory ensheathing cells, ASIA scores for movement, touch sensation and pain were significantly increased<sup>[27]</sup>. A total of 43 patients with spinal cord injury were included in the present study. After olfactory ensheathing cell transplantation, the patients showed improvements in sensory ability and motor force. While the ASIA scores for pain, touch sensation and motor function were increased, only the pain scores were significantly higher than before transplantation. This lack of statistical significance may be associated with the small sample size.

From the results of the somatic nerve function (sensory and motor) and autonomic nerve function assessment,

we can conclude that olfactory ensheathing cell transplantation can improve neurological functioning in patients with chronic spinal cord injury. The sympathetic skin response can reflect changes in autonomic nerve function, and can be used to assess autonomic nerve functional recovery after transplantation of olfactory ensheathing cells. Consequently, it can be used as a supplement to ASIA scale evaluation.

## SUBJECTS AND METHODS

### Design

A self-controlled clinical case study.

### Time and setting

Experiments were performed in the Department of Orthopedics, Taian Central Hospital, and the Department of Spine and Spinal Cord, Taian Rongjun Hospital, China between July 2009 and July 2010.

### Subjects

Spinal cord injury patients who were candidates for olfactory ensheathing cell transplantation were selected from the Department of Orthopedics, Taian Central Hospital, and the Department of Spine and Spinal Cord, Taian Rongjun Hospital, China.

### Inclusion criteria

(1) Clinical data are complete, the duration of spinal cord injury is more than 0.5 years, with no recovery of neurological functions after operation, medical treatment or acupuncture and moxibustion therapy (after understanding the potential of olfactory ensheathing cell transplantation, all patients volunteered for the study); (2) The patients can tolerate the cell transplantation operation, with no contraindications; (3) Spinal cord signals display anatomic continuity under MRI.

### Exclusion criteria

(1) Clinical or electrophysiological evidence of peripheral nerve injury, other central nerve injury, diabetes or depression; (2) Receiving oral administration of anticholinesterase drugs and adrenaline to affect autonomic nerve activity.

A total of 43 patients with spinal cord injury were included in this study; 33 males and 10 females. No improvement was observed after operation, medication or acupuncture treatment. The spinal cord function was graded according to the ASIA classification system<sup>[7]</sup>: complete spinal cord injury (grade A) in 16 cases, incomplete spinal injury

in 26 cases (grade B in 16 cases, grade C in 6 cases, grade D in 5 cases). The mean age of patients was  $30.7 \pm 10.3$  years, and the average course of disease was  $3.7 \pm 3$  years. The spinal cord injury was localized to the cervical segment in 16 cases, to the thoracic segment in 19 cases, and to the lumbar segment in eight cases. The cause of injury was traffic injury in 29 cases, falling injury in six cases, crush injury in five cases, iatrogenic injury in two cases, and wounds in 1 case. The patients and their relatives signed the informed consent, and the experiments met the *Helsinki Declaration* ethics requirements.

### Methods

#### Obtainment of olfactory bulb

The olfactory bulb was obtained from embryos at 4–6 months through unintended voluntary delivery in the Taian Central Hospital and the Maternal and Child Health Hospital of Taian, China. Maternal health examinations had excluded infectious and genetic diseases. Pregnant women gave informed consent and signed a voluntary donation consent.

#### Culture and identification of olfactory ensheathing cells

Olfactory bulbs were stripped and cut into pieces or small tissue blocks, which were then cultured in Dulbecco's modified Eagle's medium/F12 medium containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA)<sup>[1-2]</sup>. The cells were digested and prepared into a cell suspension at  $1 \times 10^{10}$  cells/L. Low-affinity neurotrophin receptor (p75) immunofluorescence staining showed that the cultured cells were olfactory ensheathing cells (Figure 1).

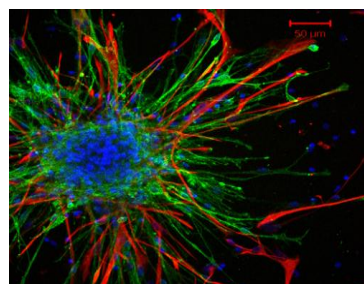


Figure 1 Olfactory ensheathing cells isolated from the olfactory bulb of embryos expressed low-affinity neurotrophin receptor (p75).

Red by immunofluorescence staining. Scale bar: 50  $\mu$ m.

#### Cell transplantation

Intramedullary transplantation of olfactory ensheathing cells was performed. In brief, patients under general anesthesia were fixed in the prone position, and using a posterior median approach, the dura mater was cut to

expose the injured spinal cord. The cultured olfactory ensheathing cell suspension was transplanted into the injured spinal cord under the surgical microscope (Möller, Nuremberg, Germany). There were 2–5 injection sites located at the ends of the injured area. A 50- $\mu$ L volume of cell suspension, containing  $2-4 \times 10^6$  cells, was injected at each site. After transplantation, the wound dressing was changed, and rehabilitation exercise was given. No immunosuppressive or neurotrophic drugs were given.

### **Evaluation of neurological function**

Patients were evaluated with the ASIA scale 1 day before transplantation and 3 weeks–2 months after transplantation<sup>[6, 27]</sup>. The scale is composed of three parts: movement, sensation and pain. The motor function was assessed as grade 1–5 according to the muscle force, for a total of 10 muscles and a total of 100 points. Sensation and pain were assessed as follows: 2 points for normal, 1 point for abnormal, and 0 point for no feeling. A total of 112 points were assessed: 56 pain points and 56 sensory points (including 28 key sensory points). Higher scores indicate better neurological function.

### **Determination of sympathetic skin response**

The sympathetic skin response was measured in a quiet and bright room at 24–26°C. The patients were lying in the supine position, with a palm and foot temperature > 31°C. Using the Keypoint-4 electromyography/evoked potential system (Medtronic; Minneapolis, Minnesota, USA), sympathetic skin responses were evoked by electrical stimulation (0.2 ms duration, 1 Hz frequency, 0.5 mV/D sensitivity). The stimulus intensity was initially 40 mA, and then increased to 60 mA. The left tibial nerve was stimulated using a saddle electrode in the left medial malleolus. All patients were given four stimuli until a stable waveform was observed. Each stimulation was given for 15 minutes, and the interval between stimuli was 1 minute. The recording electrode was a disposable surface electrode located on the right foot. The reference electrode was placed on the foot back, corresponding to the recording electrode, and the grounding electrode was a ring electrode located between the left heel and the left ankle. The sympathetic skin response latency and amplitude were recorded 1 day before transplantation and 3 weeks to 2 months after transplantation.

### **Statistical analysis**

Data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA) and were expressed as mean  $\pm$  SD. Differences before and after transplantation were compared with the paired *t*-test. A *P*-value less than 0.05 was considered significant.

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