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ORIGINAL ARTICLE

Autologous mesenchymal stem cells in chronic spinal cord injury

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Abstract
Spinal cord injury (SCI) occurs in the most productive part of life. Treatment options for treatment of chronic SCI are few and have limited impact on clinical outcome. Central nervous system (CNS) has limited intrinsic regeneration capability. The study included patients with chronic complete SCI. Previously harvested autologous mesenchymal stem cells were administered at the site of injury after a laminectomy. Follow-up was done by a neutral examiner not involved in the surgery every 3 months. One patient had improvement in motor power. Two patients had a patchy improvement in pin prick sensation below the level of injury. Three different, progressively increasing doses did not result in improvement in the clinical outcome. Though the administration of allogenic human mesenchymal stem cells is safe in patients with SCI, it may not be efficacious; especially in patients with chronic SCI.

Key words: *Stem cells, autologous mesenchymal stem cells, autologous stem cells, spinal cord injury, chronic spinal cord injury.*

Introduction

Despite major progress in pharmacological and surgical procedures, spinal cord injury (SCI) still remains a complex medical and psychological challenge, both for the patients as well as medical practitioners. The enormous impact of this problem can be gauged from the fact that there are ~11,000 new cases of SCI annually, and more than 253,000 people are living with devastating disabilities resulting from SCI according to the Spinal Cord Injury Information Network (in 2006). Currently, the average age of a patient at the time of injury is 38.0 years. Thus, SCI affects individuals in the most productive part of their lives, causing detrimental effects on their quality of life, and a considerable financial burden on society.[1] Also, the socio-economic burden of the disease is extremely high due to that fact the people affected are mostly young males (33 years; 4:1, male:female ratio).[2] Currently, methylprednisolone administration at high doses immediately after injury is the only therapy with recognised benefit; however its clinical effect is relatively minor.[3] Thus, it is imperative to look for alternate therapies for patients suffering from SCI.

Neurons and oligodendrocytes are exquisitely vulnerable to secondary cell death after SCI. Neurons have a high rate of oxidative metabolism which makes them susceptible to injury by reactive oxygen species following ischemia causing degeneration of the gray matter and disruption of the local spinal cord circuits within the injury center.[1]

It is believed that intrinsic repair is restricted after SCI.[1] The pathophysiological mechanism of SCI is more than a simple mechanical disruption or contusion of certain nerve tracts. It is a multistep process in which the primary lesion progressively expands by massive inflammatory reaction and secondary injury, leading to further disruption of nerve tracts.[4] The problem is further aggravated by lack of intrinsic regeneration ability. The failure of axons to regenerate after SCI has been attributed to growth-inhibitory molecules.[5,6] lack of appropriate trophic support,[7,8] proliferation of fibroblasts, astrocytes, microglia and endothelial cells at the site of injury forming a physical and/or chemical barrier[9] and reactions of the immune system.[10] The stem cells in the central nervous system after the injury are stimulated to produce scar tissue by the inflammatory cascade.

Many experimental studies have shown that transplantation of bone marrow cells, neural progenitor cells or olfactory ensheathing cells can promote functional improvements after SCI.[11–14]

Mesenchymal stem cells have been shown to have the capacity of differentiating into multiple mesodermal lines including bone,[15] cartilage,[16,17] fat,[15]

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muscle,[18] liver[19–21] and cardiac cells.[22,23] Additionally, they have been shown to have phenotypic plasticity wherein they can differentiate into cells expressing neural and glial lineage markers.[24,25] Thus, a lot of interest has been shown in the use of mesenchymal stem cells for SCI.

Apart from this, there has been an enhanced interest in the use of mesenchymal stem cells in various diseases as they can easily be isolated,[26] they can be rapidly and extensively expanded in cell cultures,[24,27] there is no evidence of tumour production *in vivo*,[28,29] they have the capacity for tissue repair,[30,31] they secrete growth factors *in vivo* which could enhance regeneration and repair[32] and they are immuno-privileged: they can escape the immune mechanism of the body as they do not express co-stimulatory molecule B7-1, B7-2, CD40 and CD40 ligand and hence do not activate alloreactive T cells,[33,34] do not express Human Leucocyte Antigen (HLA) class II antigens of the cell surface and fail to elicit a proliferative response from allogeneic lymphocytes in many vital tissues. In addition, Mesenchymal Stem Cells (MSCs) differentiated into various mesenchymal lineages do not appear to alter their interaction with T cells.[35,36]

A large number of animal studies have been done using mesenchymal stem cells for treatment of SCI. Many studies done in rats using both autologous[37–43] and xenogenic[44–46] (human) mesenchymal stem cells have shown that these MSCs migrated to the site of injury and lead to functional improvement.[37–46] Even studies done on non-rodent species have shown that mesenchymal stem cells are efficacious in improving the functional status in them.

To our knowledge, one study has been reported previously using mesenchymal stem cells in humans.[47] In our study, we transplanted human mesenchymal stem cells using different routes of delivery, in patients suffering from SCI to study the safety and efficacy of this treatment and also to find out the best dose.

Materials and methods

Patient selection

This pilot study was approved by the hospital ethics committee. All procedures were done after obtaining written informed consent. Only patients with complete SCI [American Spinal Injury Association (ASIA) impairment scale (AIS) grade A] between 18–60 years of age, having a chronic (>8 weeks) SCIs were included in this study. Exclusion criteria included ventilator assisted breathing, coma, serious pre-existing medical diseases, patients having low haemoglobin (Hb < 10 g%, high creatinine (>2 mg%) and bilirubin (total bilirubin >2 mg%) levels. Pregnant, nursing and child bearing women not using contraception were excluded as were patients already taking part in other trials. Baseline electrophysiological studies like somatosensory evoked potential, motor evoked potentials and nerve conduction study were recorded. The patient demographics are summarised in Table I.

Technique

After appropriate consent, the first step was aspiration of bone marrow from the iliac crest which was the source of stem mesenchymal stem cells for our study.

Bone marrow aspiration. Sixty millilitres of bone marrow was aspirated from the posterior iliac crest under local anaesthesia/short general anaesthesia and collected in blood bags containing citrate phosphate dextrose ascorbic acid (CPDA).

Isolation and culture of mesenchymal stem cells. Mesenchymal stem cells were isolated and cultured according to the standard protocol inside a class 100 biosafety hood in class 10,000 cGMP facility. In brief, bone marrow aspirate was passed through the cell strainer (100 μm) to remove bone spicules and cell aggregates. The aspirated bone marrow was combined with Knockout Dulbecco's Modified Eagle's Medium (KO-DMEM) (Invitrogen, Carlsbad, CA) in 1:1 ratio and centrifuged at 1200 rpm for 10 min at room temperature. The pellet was re-suspended with culture medium and the mononuclear cells which contain MSCs were isolated by gently layering onto a lymphoprep density gradient (Axis-Shield PoC AS) in 1:2 ratio. The mononuclear cells are washed again with the culture medium and centrifuged. The complete culture medium was prepared by adding 10% FBS (Hyclone), 200 mM Glutamax (Invitrogen), Pen-Strep (Invitrogen) and basic fibroblast growth factor (bFGF; 2 ηg/ml) to DMEM-KO (Dulbecco's modified Eagle Medium-Knock Out) medium (Invitrogen, Carlsbad, CA). The cells were again re-suspended in culture medium and transferred to T-75 cm^2 flasks (BD Biosciences, San Jose, CA). The culture was maintained at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. After 48 h the non-adherent cells were removed and fresh medium supplemented. Once the cells became confluent, they were dissociated with 0.25% trypsin/0.53 mM EDTA (Invitrogen) and trypsinized cells were re-seeded at a density of 5000 cells per cm^2 in one cell stack (Corning). Thus the MSCs were further subcultured and expanded in order to procure the adequate number of cells depending on the weight of the patient.

Harvesting of cells for transplantation. Confluent cell stacks were washed twice with Dulbecco's phosphate buffered saline (DPBS). Cells were separated by adding 0.25% trypsin-EDTA. Trypsin was neutralised by adding culture medium. The cell suspension was centrifuged and the pellet washed five times with

TABLE I. Stem cell doses, duration from time of injury and results

Patient no.	Age (years)	Sex	Site of injury	Duration of injury (in months)	Total Dose of stem cells given (in million cells/kg body weight)	Dose of stem cells given directly at site of injury (million/kg body wt.)	Dose of stem cell given via 1st LP (million cells/kg body wt.)	Dose of stem cell given via 2nd LP (million cells/kg body wt.)	Follow-up time (months)	Results
1	44	F	T11	18	3	1	1	1	38	Improved by one ASIA grade
2	18	M	C5	30	3	1	1	1	21	No improvement
3	35	M	C5	3	3	1	1	1	17	No improvement
4	30	M	C6	48	3	1	1	1	12	Patchy improvement in sensations below the injured level
5	28	M	T4-T6	24	3	1	1	1	12	No improvement
6	28	M	T4	24	3	1	1	1	12	No improvement
7	27	M	C5	7	3	1	1	1	13	No improvement
8	27	M	T7	10	3	1	1	1	12	Patchy improvement in sensations below the injured level
9	52	F	T7-T8	7	3	1	1	1	11	No improvement
10	28	F	T3	10	6	2	2	2	10	Patient subjectively felt improved sense of bladder filling
11	28	M	T5	48	6	2	2	2	6	No improvement
12	43	M	T10-11	4	8	4	2	2	6	No improvement
13	31	M	C5	132	8	4	2	2	6	No improvement

DPBS and finally once with normal saline. Finally, the cell pellet is resuspended in 1 ml normal saline for transplantation.

Quality control of MSCs. Cell surface markers analysis using CD45, CD73 and CD90 (BD, San Diego) was done for the MSCs by flow cytometry. The multilineage differential potential of the MSCs were determined by differentiating them into adipogenic and osteogenic lineages. Karyotyping was performed by visualising chromosomes by standard G-banding procedure and reported according to the International System for Human Cytogenetic Nomenclature. Endotoxin level was tested using LAL test and mycoplasma testing using PCR-ELISA was performed. Cell viability was measured by flow cytometry using 7AAD (7-amino actinomycin D). Any sample testing positive for endotoxin or mycoplasma or having an abnormal karyotype were discarded appropriately. The cells were released for transplantation when the sample showed more than 90% cells positive for CD 73 and CD 90, less than 10% cells positive for CD 45 and more than 90% cells were viable. Before administration, the cells were trypsinised, washed with DPBS and suspended in 1 ml normal saline.

Dosage and administration of mesenchymal stem cells. Patients were operated by the principal author and his team at another hospital. Patients were admitted a day prior to the first injection of the stem cells. All surgeries were done under general anaesthesia. The protocol used included three injections: the first dose was given directly at the site of injury followed by two doses given via lumbar puncture within a span of 21 days of the first injection under local anaesthesia. The first of the three injections was done locally via a posterior laminectomy approach at or below the clinical motor level. This level was chosen for two reasons. First, this acted as a guide to the area where the injection was required which corresponded to the area maximally damaged by the injury needing a possible 'repair' by the stem cells. Second, this approach prevented any further motor deficits which were especially important in the patients with mid cervical injury who would be maximally dependent on the residual motor function. All patients have undergone MRI scans. No patient was found to have complete cord trans-section, injury at more than one level. We explain our choice of clinical level as the determining factor with the following example: a patient has radiological injury at C5–6. He has grade 2/5 motor power at shoulder and elbow level, grade 0/5 below. Correcting for plus-one shift between vertebral and cord levels in the cervical spine, intraparenchymal injection at or above C5 could produce a second lesion jeopardising motor function at this level. Injection would be carried out instead at C7-T1, at or below which level there is no discernable motor power.

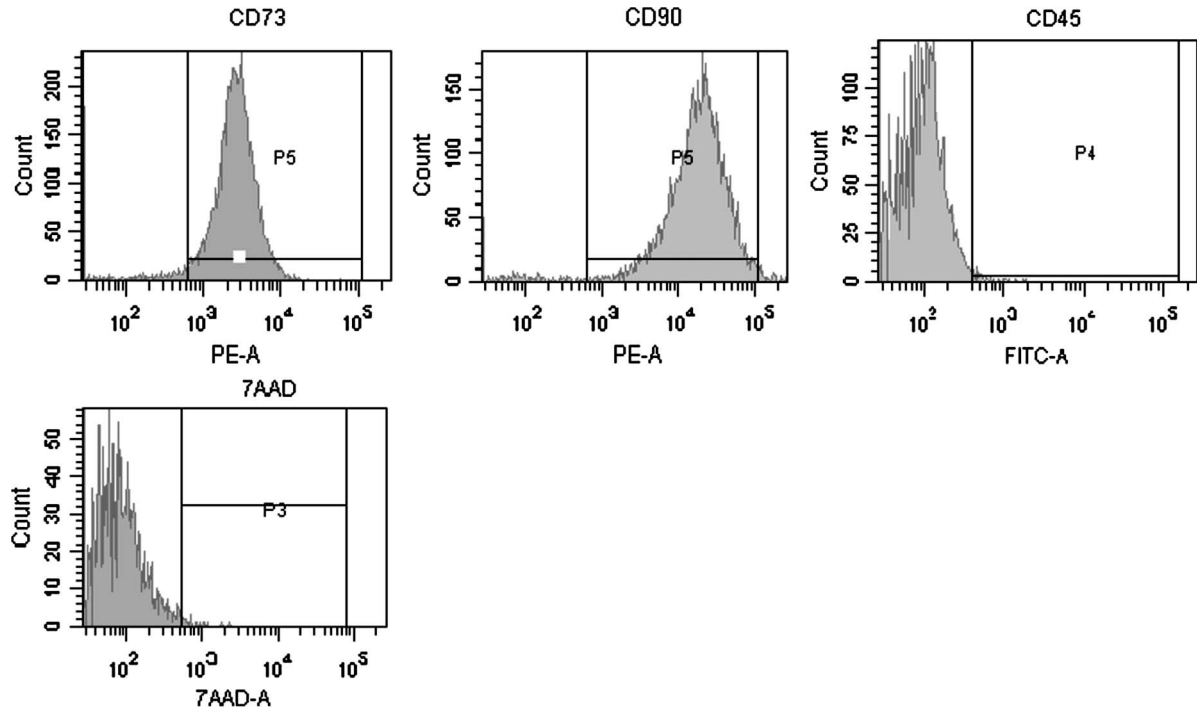

FIG. 1. Flow cytometric analysis of MSCs

The first injection was done locally at the site of injury because all the patients included in our study had a chronic SCI injury and we believed that a chronic injury does not produce chemokine signals strong enough to attract the stem cells towards the site of injury if they are simply injected via a lumbar puncture.

For the first injection, a standard laminectomy was done at or below the motor level as determined by clinical examination. Mesenchymal stem cells were injected in the cord substance at the predetermined level after a midline durotomy. Cells were supplied to us suspended in 1 cc of normal saline solution. We used a 27-gauge spinal needle to inject the cells.

In three patients where the scattered residual motor activity was present below the level of injury or there was a partially preserved bladder function, it was felt that injection of stem cells into the cord substance could jeopardise this activity. In such cases, a small midline myelotomy was done to create a tiny area of injury at the posterior midline. The stem cells were then poured directly over the myelotomy site and into the adjacent subarachnoid space. We assumed that the myelotomy would work in two ways. It would open up a physical pial barrier to the cells, and lead to the generation of chemokine signals for the stem cells. In all other patients the cells were injected into the cord substance.

The second and the third injections were given via a lumbar puncture into the lumbar intrathecal space 1 and 2 weeks, respectively, after the first injection considering that the cells will be attracted towards the chemokine signals emanating from the site of the previous surgery. Patients were discharged 1 day after the third dose was given after suture removal.

Patients were given a total dose varying between 3 and 8 million cells/kg body weight. The first dose (administered directly at the site of injury) varied from 1 to 4 million cells/kg body weight and the next two doses (via lumbar puncture) varied between 1 and 2 million cells/kg body weight each. Lesser dosage (1 million cells/kg body weight) was tried in the initial cases. The dose was increased to 4 million cells/kg body weight as no side effects were seen and good results were not obtained with the lower dosage (Table I).

Follow-up

Follow-up was done by an independent examiner not involved in the surgery. Patients were regularly followed up after every 3 months and at each follow-up, urodynamic study, complete neurological assessment and AIS (using AIS, a five-scale subdivision was used: (A) a complete loss of motor and sensory function; (B) sensory but not motor function is preserved below the neurological level and includes the sacral segments S4–S5; (C) motor function is preserved below the level; (D) motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more; (E) motor and sensory function are normal) scale assessment were done. At 6th and 12th month of follow-up, electrophysiological studies including SSEP, MEP and NCV were also done.

Results

Patient profile and bone marrow aspiration

A total of 67 patients of chronic SCI were screened during the study between May 2006 and June 2009. Twenty-five were eligible for stem cell therapy according to the protocol. Of the 25 eligible patients, 13 patients (10 males and 3 females) agreed to participate in this pilot study with the mean age being 32.2 years (range 18–52 years). All of them were patients with chronic SCI with an average duration of injury of 28 months (range 3–132 months). One patient had SCI secondary to tuberculosis of bony elements of spine at D-4 level. All other patients had traumatic SCI. Out of the total number of cases recruited, 38.5% (five patients) had injury in the cervical level and 61.5% (8 patients) had injury in the thoracic spine. All patients underwent autologous bone marrow aspiration. There were no complications related to bone marrow aspiration. Detailed patient profile, dosage received and outcomes are listed in Table I.

Stem cell transplantation and complications

Of the 13 patients included in the study, 69.2% received a total dose of 3 million cells/kg body weight (with each dose being 1 million cells/kg body weight) and 15.4% received 6 million cells/kg body weight (with each dose being 2 million cells/kg body weight and 8 million cells/kg body wt each (with first dose being 4 million cells/kg body weight and the next two being 2 million cells/kg body weight) (Table I).

The most frequent post-operative complication seen in 50% of the patients was a transient increase in spasticity in the lower limbs which returned to the pre-procedural levels within 2 weeks.

Two patients had post-operative fever. None of the patients had wound infection or meningitis. Three patients had episodes of vomiting, three patients also complained of general body ache. Apart from these three patients who were injected cells directly into the spinal cord complained of tingling/burning girdle sensation which lasted for 2–3 days and then subsided. All of these symptoms responded to symptomatic treatment. There were no serious adverse events like respiratory distress, angiodema and infection at the site of surgery or sepsis.

Follow-up and results

Average follow-up period was 13.5 months (range 6–38 months). During the follow-up, only one (11.1%) patient showed neurological improvement from AIS A to B. This patient was able to walk with support with the assistance from spasticity in the lower limbs. Two patients developed patchy improvement in pinprick sensation below the pre-operative sensory level. One patient subjectively felt that she had developed sensation of fullness of bladder. No improvement was seen in other patients (Table I).

Discussion

Patients with chronic SCI receiving autologous mesenchymal stem cells were used in this pilot study. The present study has demonstrated that administration of autologous mesenchymal stem cells (in three doses and at different dose concentrations) was safe in the short- and intermediate-term (Table I). However, none of the patients, except one, with chronic SCI who had undergone at least 6 months of follow-up improved after the transplantation. The neurological improvement in this single patient may be a part of the natural history of the disease. Higher doses of 8 million cells/kg (body weight) used in the later part of our study failed to improve clinical outcome when evaluated at 6 months after the surgery (Table I).

Though many studies have been done in animal models of SCI;[36–46,48,49] most of them have come to conclusion that stem cells work best when they are administered within a therapeutic window period of 7–21 days after SCI.[50]

This study excluded acute injuries as the isolation, culture, administration and upscaling of autologous stem cells takes about 4 weeks from the day that marrow blood is drawn. Allogenic stem cells were not cleared for use in trials. Another reason for selecting autologous MSCs was that most of the patients were young (mean age 33.2 years), and the growth of the MSCs isolated from such patients was robust.[51]

In addition, scientists also found that animal models of SCI are not representative of human cases as even subtle physiological differences between humans and animals can manifest as profound differences in disease physiology and treatment effectiveness and safety.[52] Numerous differences in spinal cord physiology and reaction to injury exist between species and even strains within a species.[53–55] These differences likely contribute to the repeated failure of spinal cord treatments that have tested safe and effective in animals to translate into human benefit.

A few human studies have also been done till date in which haematopoietic stem cells and olfactory stem cells have been used in the treatment of SCI.[47] In a few studies done in Korea, it was seen that though patients having acute or sub-acute SCI did show some improvement after autologous haematopoietic stem cell transplantation and administration of granulocyte-macrophage colony stimulating factors, patients with chronic SCI did not show any improvement.[56–58] Another observational study done from the largest human experiment in chronic SCI done in China using olfactory ensheathing cells revealed that no clinically useful sensorimotor, disability or autonomic improvements were found

in patients treated with these cells.[59] A similar data were found in a pilot study done in Portugal using olfactory ensheathing cells.[60] In a small study using autologous hematopoietic stem cells, Deda et al. have reported encouraging outcomes.[47]

It must also be recognised that the demonstration of transformation of transplanted MSCs into native or host tissue cells may be unrealistically extrapolated into an implicit assumption that normal functional recovery will follow. The un-answered question here is: what is the 'quality' of the transformed MSCs vis-à-vis normal host tissue? Further, in the case of the central nervous system, functional clinical recovery requires that the tracts proximal and distal to the injury connect up across the area of injury restoring normal signal traffic. This has to occur not only across simple axonal tracts, but in relation to more complex things such as synapses. The transformation of MSCs into identifiable neuronal tissue does not guarantee that this will occur.

In other words, the steps beginning with successful MSCs harvest and replicate, progressing to a usable 'product', and ending in successful local growth and metamorphosis does appear to be achievable. The gap between this stage and the stage of clinical recovery in the patient will be bridged only by further developments around the events that occur after successful implantation.

Conclusions

Direct injection of autologous MSCs at the site of injury and into the lumbar subarachnoid space appears to be safe in the short- and intermediate-term in patients with chronic SCI. The quality and extent of clinical recovery, however, has not been very encouraging, when evaluated at the end of 6 months and 1 year. An increase in the dosage to 8 million cells/kg body weight has not resulted in better clinical outcome when evaluated at the end of 6 months.

Thus, though the administration of allogenic human mesenchymal stem cells is safe in patients with SCI, it may not be efficacious; especially in patients with chronic SCI. Other avenues treatment for patients with chronic SCI may require further research.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Eftekharpour E, Karimi-Abdolrezaee S, Fehlings MG. Current status of experimental cell replacement approaches to spinal cord injury. *Neurosurg Focus* 2008;24 (3&4):e18.
2 Wyndaele M, WyndaeleJJ. Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey? *Spinal Cord* 2006;44:523–9.
3 Bracken MB, Shepard MJ, Holford TR, *et al.* Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury: results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial – National Acute Spinal Cord Injury Study. *JAMA* 1997;277:1597–604.
4 Ronsyn MW, Berneman ZN, Van Tendeloo VFI, *et al.* Can cell therapy heal a spinal cord injury? *Spinal Cord* 2008;46:532–9.
5 Hofsetter XCP, Hess SD, Widenfalk J, *et al.* Marrow stromal cells form guiding strands in injured spinal cord and promote recovery. *Proc Natl Acad Sci USA* 2002;99(4):2199–204.
6 Fawcett JW, Asher RA. The glial scar and central nervous system repair. *Brain Res Bull* 1999;49:377–91.
7 Skene JH. Axonal growth-associated proteins. *Ann Rev Neurosci* 1989;12:127–56.
8 Widenfalk J, Lundstromer K, Jubran M, *et al.* Neurotrophic factors and receptors in the immature and adult spinal cord after mechanical injury or Kainic acid. *J Neurosci* 2001;21:3457–75.
9 Fu-jiang C, Shi-qing F. Human umbilical cord mesenchymal stem cells and the treatment of spinal cord injury. *Chin Med J* 2009;122(2):225–31.
10 Schwartz M. Autoimmune involvement in CNS trauma is beneficial if well controlled. *Prog Brain Res* 2000;128:259–63.
11 Ramer LM, Au E, Richter MW, *et al.* Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. *J Comp Neurol* 2004;473:1–15.
12 Pearse DD, Marcillo AE, Oudega M, *et al.* Transplantation of Schwann cells and olfactory ensheathing glia after spinal cord injury: does pretreatment with methylprednisolone and interleukin-10 enhance recovery? *J Neurotrauma* 2004;21: 1223–39.
13 Ogawa Y, Sawamoto K, Miyata T, *et al.* Transplantation of *in vitro* expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. *J Neurosci Res* 2002;69:925–33.
14 Koshizuka S, Okada S, Okawa A, *et al.* Transplanted hematopoietic stem cells from bone marrow differentiate into neural lineage cells and promote functional recovery after spinal cord injury in mice. *J Neuropathol Exp Neurol* 2004;63:64–72.
15 Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–7.
16 Lennon DP, Haynesworth SE, Young RG, *et al.* A chemically defined medium supports in vitro proliferation and maintains the osteochondral potential of rat marrow-derived mesenchymal stem cells. *Exp Cell Res* 1995;219:211–22.
17 Richardson SM, Curran JM, Chen R, *et al.* The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on poly-L-lactic acid (PLLA) scaffolds. *Biomaterials* 2006;27(22):4069–78.
18 Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve* 1995;18:1417–26.
19 Kang XQ, Zang WJ, Song TS, *et al.* Rat bone marrow mesenchymal stem cells differentiate into hepatocytes *in vitro*. *World J Gastroenterol* 2005;11(22):3479–84.
20 Lee KD, Kuo TKC, Whang-Peng J, *et al.* *In vitro* hepatic differentiation of human mesenchymal stem cells. *Hepatology* 2004;40(6):1275–84.
21 Ong S, Dai H, Leong KW. Hepatic differentiation potential of commercially available human mesenchymal stem cells. *Tissue Eng* 2006;12:3477–85.
22 Xu W, Zhang X, Qian H, *et al.* Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype *in vitro*. *Exp Bio Med* 2004;229:623–31.
23 Makino S, Fukuda K, Miyoshi S, *et al.* Cardiomyocytes can be generated from marrow stromal cells *in vitro*. *J Clin Invest* 1999;103:697–705.
24 Brazelton TR, Rossi FM, Keshet GI, *et al.* From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 2000;290:1775–9.

25 Corti S, Locatelli F, Donadoni C, *et al*. Neuroectodermal and microglial differentiation of bone marrow cells in the mouse spinal cord and sensory ganglia. *J Neurosci Res* 2002;70:721–33.
26 Kitchel SH, Wang MY, Lauryssen CL. Techniques for aspirating bone marrow for use in spinal surgery. *Neurosurgery* 2005;57(4 Suppl):286–9.
27 Azzizi SA, Stokes D, Augelli BJ, *et al*. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats – similarities to astrocyte grafts. *Proc Natl Acad Sci USA* 1998;95:3908–13.
28 Abdallah BM, Haack-Sorensen M, Burns JS, *et al*. Maintenance of differentiation potential of human bone marrow mesenchymal stem cells immortalized by human telomerase reverse transcriptase gene despite extensive proliferation. *Biochem Biophys Res Commun* 2005;326:527–38.
29 Du Y, Jenkins NA, Copeland NG. Insertional mutagenesis identifies genes that promote the immortalization of primary bone marrow progenitor cells. *Blood* 2005;106:3932–9.
30 Akiyama Y, Radtke C, Kocsis JD. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. *J Neurosci* 2002;22:6623–30.
31 Lu D, Mahmood A, Wang L, *et al*. Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. *Neuroreport* 2001;12:559–63.
32 Chen X, Li Y, Wang L, *et al*. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. *Neuropathology* 2002;22:275–9.
33 Di Nicola M, Carlo-Stella C, Magni M, *et al*. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002;99:3838–43.
34 Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005;105: 1815–22.
35 Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol* 2000;28:875–84.
36 Le Blanc K, Tammik C, Rosendahl K, *et al*. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003;31:890–6.
37 Koda M, Okada S, Nakayama T, *et al*. Hematopoietic stem cell and marrow stromal cell for spinal cord injury in mice. *Neuroreport* 2005;16:1763–7.
38 Sykova E, Jendelova P. Magnetic resonance tracking of implanted adult and embryonic stem cells in injured brain and spinal cord. *Ann N Y Acad Sci* 2005;1049:146–60.
39 Chopp M, Zhang XH, Li Y, *et al*. Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. *Neuroreport* 2000;11:3001–05.
40 Wu S, Suzuki Y, Ejiri Y, *et al*. Bone marrow stromal cells enhance differentiation of co-culture neurosphere cells and promote regeneration of injured spinal cord. *J Neurosci Res* 2003;72:343–51.
41 Hofstetter CP, Schwarz EJ, Hess D, *et al*. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci USA* 2002;99:2199–304.
42 Zurita M, Vaquero J. Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation. *Neurosci Lett* 2006; 402:51–6.
43 Ohta M, Suzuki Y, Noda T, *et al*. Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. *Exp Neurol* 2004;187:266–78.
44 Cizkova D, Rosocha J, Vanicky I, *et al*. Transplants of human mesenchymal stem cells improve functional recovery after spinal cord injury in the rat. *Cell Mol Neurobiol* 2006;26(7–8):1165–1178.
45 Hee Lee K, Suh-Kim H, Choi JS, *et al*. Human mesenchymal stem cell transplantation promotes functional recovery following acute spinal cord injury in rats. *Acta Neurobiol Exp* 2007;67:13–22.
46 Sheth RN, Manzano G, Li X, *et al*. Transplanatation of human bone marrow-derived stromal cells in contused spinal cord of nude rats. *J Neurosurg Spine* 2008;8:153–62.
47 Deda H, Inci MC, Kürekçi AE, *et al*. Treatment of chronic spinal cord injured patients with autologous bone marrow-derived hematopoietic stem cell transplantation: 1-year follow-up. *Cytotherapy* 2008;10(6):565–74.
48 Urdzí ková L, Jendelová P, Glogarová K, *et al*. Transplantation of bone marrow stem cells as well as mobilization by granulocyte-colony stimulating factor promote recovery after spinal cord injury in rat. *J Neurotrauma* 2006;23:1379–91.
49 Sykova E, Urdzikova L, Jendelova P, *et al*. Bone marrow cells – a tool for spinal cord injury repair. *Exp Neurol* 2005;193: 261–2.
50 Lim HJ, Byeon YE, Ryu HH, *et al*. Transplantation of canine umbilical cord blood-derived mesenchymal stem cells in experimentally induced spinal cord injury in dogs. *J Vet Sci* 2007;8(3):275–82.
51 Mendes SC, Tibbe JM, Veenhof M, *et al*. Bone tissue-engineered implants using human bone marrow stromal cells: effect of culture conditions and donor age. *Tissue Eng* 2002;8(6):911–20.
52 Sykova E, Homola A, Mazanec R, *et al*. Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. *Cell Transplant* 2006;15:1–13.
53 Hackam DG. Translating animal research into clinical benefit. *BMJ* 2007;334:163–4.
54 Ma M, Wei P, Wei T, *et al*. Enhanced axonal growth into a spinal cord contusion injury site in a strain of mouse (129X1/ SvJ) with a diminished inflammatory response. *J Comp Neurol* 2004;474:469–86.
55 Popovich PG, Wei P, Stokes BT. Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. *J Comp Neurol* 1997;377:443–64.
56 Ha Y, Yoon SH, Park SR, *et al*. Treatment of complete spinal cord injury patients receiving autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor – report of three cases. *J Korean Neurosurg Soc* 2004;35:459–64.
57 Park HC, Shim YS, Ha Y, *et al*. Treatment of complete spinal cord injury patients by autologous bone marrow cell transplantation and administration of granulocyte macrophage-colony stimulating factor. *Tissue Eng* 2005;11(5/6): 913–22.
58 Yoon SH, Shim YS, Park YH, *et al*. Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage- colony stimulating factor: phase I/II clinical trial. *Stem Cells* 2007;25:2066–73.
59 Dobkin BH, Curt A, Guest J. Cellular transplants in China: observational study from the largest human experiment in chronic spinal cord injury. *Neurorehabil Neural Repair* 2006;20(1):5–13.
60 Lima C, Pratas-Vital J, Escada P, *et al*. Olfactory mucosa autografts in human spinal cord injury: a pilot study. *J Spinal Cord Med* 2006;29(3):191–203.