

● PERSPECTIVE

## The future of bone marrow stromal cell transplantation for the treatment of spinal cord injury

Bone marrow stromal cell (BMSC) transplantation therapy is a promising approach for treating spinal cord injury (SCI), based on a number of experimental and clinical reports (Wright et al., 2011). BMSCs are a source of neuroregenerative somatic stem cells that are without the potential for tumorigenicity. Although clinical studies of autologous BMSC transplantation have been reported in Asia (Jiang et al., 2013; Yoon et al., 2007), in Japan, it is currently an uncommon procedure and highly controversial as well. This perspective paper provides an overview of the clinical effectiveness of BMSC transplantation and a proposal to enhance its use as a viable therapy.

Based on findings of experimental SCI animal models, there are two key characteristics of BMSCs that make them ideal for clinical use for the improvement of function following SCI. One is that BMSCs protect the injured CNS from further cellular damage (Vaquero and Zurita, 2011). The other is that BMSCs support nerve fiber regeneration (Hofstetter et al., 2002; Ukegawa et al., 2014). BMSCs produce in abundance various trophic factors, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF), which participate in either neural protection or regeneration. Six weeks after SCI, neurotrophin receptors such as tyrosine kinase B (trkB) or trkC are still expressed in neurons and glial cells (Widenfalk et al., 2001). Thus, neurotrophic factors, such as BDNF, released by transplanted BMSCs may be able to prevent or attenuate the extent of neuronal cell death since neurotrophin receptors are still expressed long after SCI. GDNF appears to be a key trophic factor as the number of papers has confirmed its role as a key trophic factor in the protection of damaged neural tissue and axon regeneration, particularly after SCI. In fact, BMSCs, compared to neural progenitor cells or Schwann cells, show higher mRNA expression of GDNF *in vitro* and GDNF receptors are still expressed 6 weeks after SCI (Enomoto et al., unpublished data 2013). In case of direct delivery of these trophic factors, without the use of cells, beneficial results have yet to be demonstrated since these proteins do not cross the blood-brain barrier and accumulate in extremely low quantities in the brain and spinal cord. A method to deliver trophic factors into the CNS should consider safety as well as high efficiency. Compared to directly protein delivery, cell therapy, *via* a paracrine effect, would appear to be a safer and more efficient method to deliver trophic factors to the site of injury.

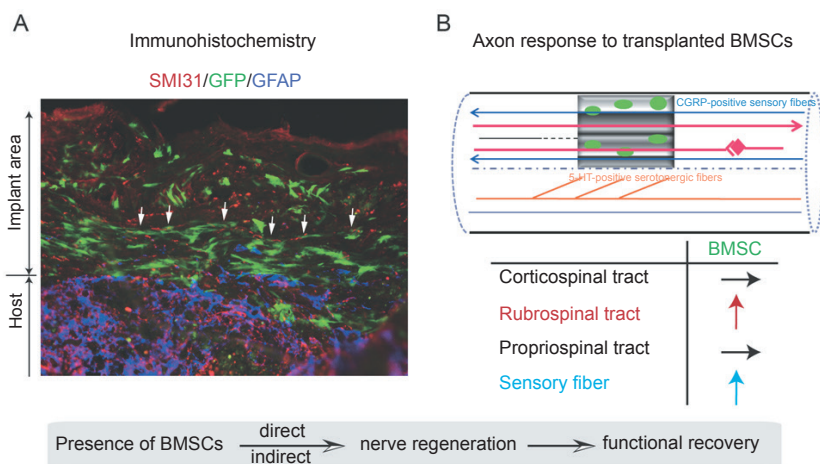
BMSCs could also have a neuroprotective effect *via* an anti-inflammatory mechanism, either alone or when co-transplanted with mesenchymal stromal cells. Bone marrow mononuclear cells are activated by a number of inflammatory substances including interleukin-6 (IL-6), PGE2, galectins, IL-10 and IL-12 (Bernardo and Fibbe, 2013). Thus, such substances in the region of the injury could activate BMSCs to produce and release trophic factors. However, BMSCs do not survive long within the injury site after transplantation when transplanted in the subacute phase, 1 to 2 weeks following SCI, in rats (Ide et al., 2010). This is a serious limitation that needs to be resolved for BMSC transplantation to be a useful clinical treatment.

As mentioned earlier, a key characteristic of BMSCs is that they support nerve fiber regeneration. We have shown that BMSCs in a honeycomb collagen sponge (HC) scaffold can directly influence nerve fiber regeneration (Ukegawa et al., 2014). Honeycomb collagen utilizes “artificial extracellular matrix geometry”, which highlights the importance of a three-dimensional structure of the scaffold *via* a

serial tunnel structure of 400  $\mu$ m pores, the end result being a highly advantageous scaffolding for guiding sprouting neurites (Fukushima et al., 2008). The geometry of the scaffold significantly influences cellular differentiation and nerve growth. BMSCs in HC facilitate the regeneration of nerve fibers (arrows in Figure 1A), such as rubrospinal axons and primary afferent sensory fibers, in injured spinal cord. As a result, in the rodent models, BMSC survive for longer periods of time in the injured environment, support regenerating axons and promote functional recovery without the need to genetically modify BMSC (*e.g.*, exogenous insertion of the neurotrophin gene into BMSCs). However, in terms of scaffolds, current difficulties include finding the best-fitting scaffold biomaterial as a bridging structure or hydrogel. Other issues related to scaffolds include the size and nature of the injury and the duration of the SCI, whether implantation will occur subacutely or long after SCI. These problems need to be resolved in order to successfully reconstruct injured spinal cord, whether acutely or long after SCI.

Recently, a clinical trial of an autologous BMSC transplant was performed in China (Jiang et al., 2013). The authors reported that BMSC transplantation was effective for the treatment of SCI and was without notable adverse side effects. From a larger sample, eight ASIA impairment scale (AIS) A patients (1 patient with a cervical-level SCI, 6 with a thoracic-level SCI and 1 patient with a lumbar-level SCI) were given BMSCs *via* either lumbar puncture or by CT-guided injection. Improvements from A to B or C in the AIS were observed in four patients 30 days after treatment. In contrast, according to data from the Spinal Injuries Center, Fukuoka, Japan, 12.1% of Frankel A patients with either a thoracic or lumbar SCI improved to B–D (17 out of 141 patients) with reduction and stabilization surgery after a 6 month follow-up period. Thus, compared to conventional treatment, a surprising 50% of AIS A patients treated with BMSC showed sensory and motor functional recovery in a short period of time. Furthermore, the duration of SCI in a study from Jiang et al. ranged from 3 months to 10 years. It is unclear, as the authors pointed out, if the effect of BMSC transplantation on recovery was related to age, gender, cause or duration of injury since the sample size was small and that there was a lack of a parallel control that corresponded to each SCI case. A Japanese group recently reported that ten SCI patients underwent BMSC transplantation *via* lumbar puncture (Suzuki et al., 2014). Patients were treated 80 days to 1 year after SCI. Out of five AIS A patients (2 cervical-level and 3 thoracic-level SCI), one thoracic SCI patient recovered to AIS B (20%) 6 months after BMSC transplantation. The authors suggested that increasing cell numbers may increase efficacy and further recommended aggregating SCI patients in a multicenter study to overcome the recurring issue of small sample size. With respect to efficacy, it should be noted that, in Europe, spontaneous recovery occurs in 30% of AIS A patients, from A to AIS B–E (42 out of 139 patients) and 68% of AIS B patients, from B to C–E (27 out of 40 patients) 12 months post-SCI (Spiess et al., 2012). With this in mind, spontaneous recovery is likely to underlie the apparent efficacy observed in both the Chinese and Japanese studies, though improvements in function obtained in the Chinese study cannot be attributed solely to spontaneous recovery. Methodology could greatly influence efficacy, such as BMSC delivery *via* lumbar puncture or injection of BMSC into the site of injury—Jiang et al. could have listed their method of BMSC delivery for each patient. At the acute or subacute phase following SCI, direct injection of BMSCs into or around the injury site would probably lead to good functional recovery whereas long after the SCI, or the chronic phase, BMSCs on a scaffold should be considered since some kind of structural continuity is necessary to bridge the injury gap. In any case, each potential use of BMSC transplantation for the treatment of SCI should be thoroughly discussed with a neurologist, spinal surgeon and neurosurgeon beforehand to maximize the potential for success. With such careful consideration, it is hoped that BMSC transplantation could be utilized to promote functional recovery after SCI.

In summary, it is proposed that the mechanisms of BMSC which promote recovery following SCI are: 1) providing protection by the



**Figure 1 Honeycomb collagen sponge (HC) supports the survival of bone marrow stromal cells (BMSCs) in the lesion area as BMSCs stimulate and guide nerve fiber regeneration.**

Representative image after BMSC transplantation into hemisectioned rat spinal cord (A) and scheme of axons in response to transplanted BMSCs (B). (A) Immunofluorescent photomicrograph showing green fluorescent protein (GFP)-positive cells (green) within the implantation site of a HC seeded with BMSCs. Regenerating nerve fibers are shown as SMI31-positive fibers (red) in a GFAP (blue)-negative area. Arrows point to a subset of GFP-positive cells located along regenerating nerve fibers. (Reproduced from Ukegawa et al., 2014.) (B) The axonal response to surviving BMSCs in the HC. Ukegawa et al. showed increased calcitonin gene-related peptide (CGRP)-positive sensory fibers found at the site of implantation and 5-HT-positive serotonergic fibers contralateral to the implantation site in spinal cords implanted with BMSCs. Retrograde tracing showed increased rubrospinal neuron projections distal to the HC implant containing BMSCs. The application of BMSCs in a HC scaffold in the injured spinal cord directly promoted nerve regeneration, thus resulting in functional recovery.

production of growth factors and anti-inflammatory molecules, 2) direct guidance of axonal regeneration. During the acute to sub-acute phase following SCI, BMSCs can have a crucial role in protecting injured tissue but their rate of survival is low within the site of injury. During the chronic phase, the protective effects of BMSC may not be as important as axonal regeneration but a combination of the best-fitting scaffolding with BMSC could lead to improved BMSC survival, increased axonal regeneration and thus functional recovery. In the future, to gain a more clear idea of the efficacy of BMSC on SCI and to better understand what issues need to be addressed so that BMSC transplantation can be utilized clinically as useful treatment for SCI, clinical studies should utilize similar methodological protocols and patient inclusion criteria-there should be serious consideration for an Asian multicenter study.

Given currently available somatic stem cells derived from autologous tissue, out of these, BMSC shows promise as a cell-based option that could be utilized for the treatment of SCI. Further efforts are needed to improve scaffold structure and to develop methods for promoting the long-term survival of BMSC and, thus, the regeneration of injured spinal cord tissue. It is entirely possible that given such improvements, BMSC could be utilized in other neurological disorders in which there are currently no cures.

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Enomoto M (2015) The future of bone marrow stromal cell transplantation for the treatment of spinal cord injury. *Neural Regen Res* 10(3):383-384.

## References

- Bernardo ME, Fibbe WE (2013) Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* 13:392-402.
- Fukushima K, Enomoto M, Tomizawa S, Takahashi M, Wakabayashi Y, Itoh S, Kuboki Y, Shinomiya K (2008) The axonal regeneration across a honeycomb collagen sponge applied to the transected spinal cord. *J Med Dent Sci* 55:71-79.
- Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ, Olson L (2002) Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci U S A* 99:2199-2204.
- Ide C, Nakai Y, Nakano N, Seo TB, Yamada Y, Endo K, Noda T, Saito F, Suzuki Y, Fukushima M, Nakatani T (2010) Bone marrow stromal cell transplantation for treatment of sub-acute spinal cord injury in the rat. *Brain Res* 1332:32-47.
- Jiang PC, Xiong WP, Wang G, Ma C, Yao WQ, Kendell SF, Mehling BM, Yuan XH, Wu DC (2013) A clinical trial report of autologous bone marrow-derived mesenchymal stem cell transplantation in patients with spinal cord injury. *Exp Ther Med* 6:140-146.
- Spieß MR, Müller RM, Rupp R, Schuld C, van Hedel HJ, Group E-SS (2009) Conversion in ASIA impairment scale during the first year after traumatic spinal cord injury. *J Neurotrauma* 26:2027-2036.
- Suzuki Y, Ishikawa N, Omae K, Hirai T, Ohnishi K, Nakano N, Nishida H, Nakatani T, Fukushima M, Ide C (2014) Bone marrow-derived mononuclear cell transplantation in spinal cord injury patients by lumbar puncture. *Restor Neurol Neurosci* 32:473-482.
- Ukegawa M, Bhatt K, Hirai T, Kaburagi H, Sotome S, Wakabayashi Y, Ichinose S, Shinomiya K, Okawa A, Enomoto M (2014) Bone marrow stromal cells combined with a honeycomb collagen sponge facilitate neurite elongation in vitro and neural restoration in the hemisectioned rat spinal cord. *Cell Transplant*. [Epub ahead of print]
- Vaquero J, Zurita M (2011) Functional recovery after severe CNS trauma: current perspectives for cell therapy with bone marrow stromal cells. *Prog Neurobiol* 93:341-349.
- Widenfalk J, Lundströmer K, Jubran M, Brene S, Olson L (2001) Neurotrophic factors and receptors in the immature and adult spinal cord after mechanical injury or kainic acid. *J Neurosci* 21:3457-3475.
- Wright KT, El Masri W, Osman A, Chowdhury J, Johnson WE (2011) Concise review: Bone marrow for the treatment of spinal cord injury: mechanisms and clinical applications. *Stem Cells* 29:169-178.
- Yoon SH, Shim YS, Park YH, Chung JK, Nam JH, Kim MO, Park HC, Park SR, Min BH, Kim EY, Choi BH, Park H, Ha Y (2007) 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* 25:2066-2073.