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Bone Marrow-derived Mesenchymal Stem Cell Transplant Survival in the Injured Rodent Spinal Cord

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Abstract

Transplantation of bone marrow-derived mesenchymal stem cells (MSCs) is a promising therapy for spinal cord repair. Its potential, however, is limited by poor survival of the cells in the damaged nervous tissue. A number of studies have tried to improve MSC transplant survival, yet often with limited or short-term effects. Survival enhancing strategies include optimizing timing of transplantation, suppressing the immune response, transplantation within a scaffold to limit anoikis, reducing reactive oxygen species and/or macrophages, genetically modifying MSCs, and electrical stimulation of the spinal cord. This review provides an overview of studies that have investigated MSC survival after transplantation into animal models of spinal cord injury.

Keywords: Transplantation; survival; Neurotrophic factors; Bone Marrow-derived MSC

Introduction

Traumatic spinal cord injury results in immediate functional impairments below the level of injury caused by the loss of neural cells and axons due to the initial impact. Following this primary injury, a secondary pathophysiological cascade causes progressive tissue loss for weeks to months after the insult, leading to the formation of fluid-filled cysts surrounded by scar tissue [1]. The endogenous response within the injured spinal cord fails to reorganize spinal cord tissue in a way that leads to functional repair. Currently no treatments exist that can effectively restore lost motor, sensory and autonomous function after spinal cord injury.

Mesenchymal stem cells (MSCs), derived mostly from bone marrow, but also from adipose tissue and umbilical cord, are being studied as a potential repair strategy for spinal cord injury. Typically, MSCs can be easily isolated, cultured and prepared for transplantation into a spinal cord lesion. MSCs secrete numerous molecules that are known to exert paracrine effects resulting in repair. After spinal cord injury, MSCs have the potential to decrease secondary tissue loss after spinal cord injury and this neuroprotective effect has been shown to be correlated with moderate functional improvements [2]. MSCs secrete neurotrophic factors, such as brain-derived neurotrophic factor, glial-derived growth factor and nerve growth factor that have the potential to decrease neuronal apoptosis and/or promote axonal regeneration [3]. In addition, MSCs secrete factors that have proliferative and stabilizing effects on blood vessels, including vascular endothelial growth factor and angiopoietin-1, respectively [4]. However, survival of MSCs in the injured spinal cord is poor, limiting the availability of these trophic factors to the nearby nervous tissue and thus their effects that lead to repair. Because it has been shown that improved survival of MSCs is associated with improved anatomical and/or functional repair [5-13], it is important to understand mechanisms of transplanted cell death and to develop strategies to improve MSC survival. This review provides an overview of studies that have investigated MSC survival in the injured spinal cord and summarizes current MSC survival promoting strategies, specifically focusing on bone marrow- derived MSCs.

MSC Survival Rates

MSCs can be tracked after transplantation by virally transducing the cells to express green-fluorescent protein (GFP) or isolating cells from a GFP-transgenic donor. Most reports on MSC transplantation after spinal cord injury provide only qualitative or semi-quantitative data on MSC transplant survival. Table 1 provides an overview of studies that have reported on bone marrow-derived MSC survival after transplantation into animal models of spinal cord injury. Studies that provide quantitative data on MSC survival after SCI report survival rates between 0 [14,15] and 52% [8] one week after transplantation and between 0 [6,16-20] and 8% [21] one month after transplantation without survival enhancing therapies. In some cases, presence of MSCs up to two months [2,13,22-25] and even three months [11,26,27] was reported after transplantation into models of spinal cord injury, but usually no or very few cells survive at these time points. The large variation in survival rates can in part be explained by the model system used. Interestingly, in spinal cord transection models, cells are usually reported to be present at the end point of the study, with reported survival rates up to 7% at eight weeks after transplantation [28]. In the transected spinal cord, a piece of gelfoam is often used to fill the injury gap and/or to provide a scaffold for the MSCs. Alternatively, cells are injected directly in the lesion penumbra, i.e., the nervous tissue adjacent to the actual transection. The environment in the lesion penumbra is different from the lesion epicenter in terms of immune cell presence, scar tissue and blood supply [29]. Together, this may explain why survival seems to be better in transection models than in contusion models in which the cells are mostly injected into the lesion environment. Because contusion models are clinically more relevant, in more than 70% of the cases a contusion is the mechanism of injury, it is imperative to understand the low and variable survival rates in contusion models of spinal cord injury.

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	Donor/ recipient	Timing (days psci)	Dose (# MSCs)	Sci model	Delivery method	Survival enhancing therapy	Cell survival (times point post-injection)
Ukegawa et al. 2014 [46]	Fischer rMSC/ Fischer rat	0	3×10^4	hemi	il	(honeycomb collagen sponge scaffold)	4wk: +
Ding et al. 2014 [23]	Wistar rMSC/ Wistar rat	9	5×10^5	trans	il	-	2wk: many 8wk: very few
Torres-Espin et al. 2014 [16]	SD MSC / SD rat	0 vs. 7	4.5×10^5	cont	il	acute >7d-delayed	14d: + 21d: 0
Chen et al. 2014 [47]	SD rMSC/ SD rat	0	5×10^5	hemi	il	(acellular spinal cord scaffold)	8wk: +
Ritfeld et al. 2014 [6]	SD rMSC/ SD rat	3	5×10^5	cont	il	poly-urethane based biogel: ↑	1wk: 20-70% 4 wk: 0
Tan et al. 2013 [12]	C57BL6 mMSC/ C57BL6 mouse	3	1×10^5	cont	il	IL-6/IL-6R blokkade: ↑	28d: 1.2% - 17.8 %
Nakano et al. 2013 [15]	SD rMSC/ SD rat	7 vs. 14 vs. 28	5×10^6	cont	it	7d > 14d = 28d-delayed	2d: 0 - a few 7d: 0
Edalat et al. 2013 [10]	SD rMSC/ SD rat	7	5×10^5	cont	il	P75-siRNA MSC: ↑	3wk: +
Mitsuhara et al. 2013 [9]	Fischer rMSC/ Fischer rat	0	3×10^5	cont	iv	microgravity culture conditions: ↑	21d: +
Aizawa et al. 2013 [48]	Wistar rMSC/ Wistar rat	9	3×10^5	trans	il	(genetic neural induction)	7wk: 5.9%
Quertainmont et al. 2012 [14]	Wistar rMSC/ Wistar rat	7	1×10^6	cont	il	(medium-induced neural induction)	7d: 0
Hodgetts et al. 2013 [17]	hMSC/ CBH-rnuArc (nude) rat	7	5×10^5	cont	il	CsA: ↑	2wk: + 4wk: 0 - a few
Ding et al. 2013 [41]	SD rMSC/ SD rat	0	1×10^5	trans	il	(gelfoam, TrkC/Lacz-overexpressing MSC)	10wk: +
Boido et al. 2014 [49]	C57BL6J mMSC/ C57BL6J mouse	0	1×10^5	cont	il		26d: <1%
Kang et al. 2012 [33]	SD rMSC/ SD rat	1	1×10^6	cont	iv vs. il	il > iv (CsA)	6wk: +
Ritfeld et al. 2012 [2]	SD rMSC/ SD rat	3	1×10^6	cont	il		8wk: <1%
Yazdani et al. 2012 [50]	Wistar rMSC/ Wistar rat	7	1×10^6	cont	il	(medium –induced neural induction)	5wk: +
Kang et al. 2012 [28]	hMSC/ Fischer rat	0	1×10^5 vs. 2×10^4 vs. 4×10^3	trans	il	dose: no effect (PLGA scaffold)	4wk: 8% 8wk: 7%
Liu et al. 2012 [40]	SD rMSC/ SD rat	7	7.5×10^3	cont	il	electroacupuncture: ↑	7wk: +
Zhilai et al. 2011 [22]	SD rMSC/ SD rat	7	2×10^5	cont	il	NOGO-66R antagonist (neural induction)	9wk: 0.09 - 0.24 %
Zeng et al. 2011 [51]	SD rMSC/ SD rat	0	1×10^5	trans	il	(gelatin sponge scaffold)	1wk : + 8wk: ?
Wu et al. 2011 [42]	SD rMSC/ SD rat	7	5×10^5	cont	il	electrical stimulation: ↑	7wk: +
Alexanian et al. 2010 [44]	mMSC/ SD rats	7	1×10^5	cont	il	neural induction: no effect	1wk: 37% 2wk: 2% 3wk: 0.5% 4wk: < 0.5%
Fang et al. 2010 [52]	hMSC/ SD rats	7	2×10^5	cont	il		2wks: a few
Xu et al. 2011 [45]	C57B6Kr15mMSC/ C57B6Kr15 mouse	7	3×10^4	cont	il	coculture with Schwann cells pre-injection: no effect	2wk: 3-4 % 6wk: 1%
Cizkova et al. 2011 [31]	Wistar rMSC/ Wistar rat	3 vs. 7 vs. 3,4,5 vs. 7,8,9	5×10^5	cont	it	repeated 7d-delayed injections: ↑	28d: 0 - 5%
Zhang et al. 2010 [53]	SD rMSC/ SD rat	0	5×10^5	trans	il	(pretreatment with retinoic acid, gelfoam)	67d: +
Ritfeld et al. 2010 [35]	SD rMSC/ SD rat	3	1×10^6	cont	il	MC vs. CsA vs. MP: no effect	7d: 21-33%
Luo et al. 2009 [13]	SD rMSC/ SD rat	0	3×10^6	trans	il	G-CSF ↑	8wk: +
Ding et al. 2009 [54]	SD rMSC/ SD rat	0	5×10^5	trans	il	electroacupuncture: ↑ (gelfoam)	8wk: +
Itosaka et al. 2009 [39]	mMSC/ SD rat	0	3×10^5	hemi	il	fibrin matrix: ↑ (CsA)	4wk: +
Samdani et al. 2009 [55]	hMSC/ SD rat	0	1.5×10^5	cont	il	(CsA)	3wk: 1.3%

Nandoe Tewarie et al. 2009 [8]	SD rMSC/ SD rat	0 vs. 3 vs. 7 vs. 21	1 ×10^6	cont	il	0 or 3d-delayed > 7d- or 21d-delayed)	7d: 9 - 52% 28d: 0 - 2%
Hollis et al. 2009 [56]	Fischer rMSC/ Fischer rat	0	2 × 10^5	dcl	il		4wk: +
Parr et al. 2008 [26]	Wistar rMSC/ SD rat	0	2 × 10^5	cont	il	(CsA)	12wk: +
Parr et al. 2008 [21]	Wistar rMSC/ SD rat	9	1.25 ×10^5	cont	il	CsA high dose > CsA low dose = no CsA	28d: 8-11%
Sheth et al. 2008 [57]	hMSC/ nude SD rat	7	6 × 10^5	cont	il	(nude rats)	6wk: a few
Bi et al. 2008 [43]	SD rMSC/ SD rat	7	1 × 10^6	cont	il	Salvianolic acid B: ↑	28d: +
Yano et al. 2006 [30]	SD rMSC/ SD rat	7	7.5 × 10^4	cont	8 mm rostral to injury		4wk: +
Yoshihara et al. 2006 [27]	Fischer rMSC/ Fischer rat	9	1 × 10^6	cont	il	(Vitrogen matrix, CsA)	3month: +
Cizkova et al. 2006 [34]	Wistar hMSC/ Wistar rat	7	1 (or 2?)* × 10^6	cont	iv		3wk: 4%
Shi et al. 2006 [32]	rabbit MSC/ rabbit	-2	1 × 10^8	ischemia	it		14d: +
Himes et al. 2006 [58]	hMSC/ SD rat	7	0.5-1 × 10^6	cont	il	(CsA)	11wk: a few
Bakshi et al. 2006 [7]	Fischer rMSC/ SD rat	<14d vs. >14d#	2 × 10^6 vs. 1 × 10^6 vs. 4 × 10^6	cont	it	[<14d] > [>14d]# dose: no effect (CsA)	6wk: +
Yano et al. 2005 [59]	mMSC/ Wistar rat	7	7 × 10^4	cont	il	(CsA)	4wk: +
Lu et al. 2005 [11]	Fischer rMSC/ Fischer rat	0	2 × 10^5	dcl	il	BDNF-overexpressing MSC ↑	1month: many 3month: many
Ankeny et al. 2004 [25]	Wistar rMSC/ Wistar rat	2	3 × 10^5	cont	il		8wk: +
Satake et al. 2004 [19]	Lewis rMSC/ Lewis rat	3, 5, 7	1 × 10^6	cont	it	(repeated injections)	14d: + 28d: 0
Ohta et al. 2004 [20]	SD rMSC/ SD vs. Wistar rat	0	5 × 10^6	cont	it	inbred = outbred no CsA = CsA	2wk: + 3wk: 0
Lee et al. 2003 [60]	C57BL6 mMSC/ C57BL6 mouse	7	3 × 10^3	cont	il + penumbra (2mm)		4wk: +
Hofstetter et al. 2002 [5]	Lewis rMSC/ Lewis rat	0 vs. 7	3 × 10^5	cont	il + penumbra (2mm)	7d-delayed > acute	4wk: 1% (delayed) 5wk: 0.2% (acute)
Chopp et al. 2000 [61]	Wistar rMSC/ Wistar rat	7	2.5 × 10^5	cont	il		4wk: +

psci, post-spinal cord injury; MSC, mesenchymal stem cell; sci, spinal cord injury; rMSC, rat mesenchymal stem cell; SD, Sprague-Dawley; mMSC, mouse mesenchymal stem cell; hMSC, human mesenchymal stem cell; hemi, hemisection; trans, transection; cont, contusion; dcl, dorsal column lesion; il, intralesionally; iv, intravenously; it, intrathecally; CsA, Cyclosporine A; PLGA, poly(D,L-lactide-co-glycolide); MC, minocycline; MP, methylprednisolone; G-CSF, granulocyte-colony stimulating factor; ↑, increased MSC survival compared to control; +, MSCs are present, but report lacks absolute numbers or percentages; #, <14d includes transplantation 4d-, 9d-, or 13d-delayed and >14d includes transplantations 20d- or 27d-delayed; *, report inconsistent. When a range of numbers of surviving cells is given, the higher number represents the observed survival with the survival enhancing strategy and the lower rate represents the observed survival without the survival enhancing strategy. Therapies in parenthesis represent treatments/factors that likely have had an effect on MSC survival, but that were not compared to a control group to study its effect on MSC survival.

Table 1: Overview of studies that have investigated bone marrow-derived MSC survival after transplantation into the injured spinal cord.

Effect of Timing, Dose and Delivery Method on MSC Transplant Survival

One important factor that should be accounted for is the timing of cell transplantation. Table 2 gives an overview of studies that have studied the effect of timing of transplantation on MSC survival in spinal cord injury models. Of the four studies that so far looked at survival after intralesional transplantation, three report better or similar survival after acute or 3-day-delayed transplantation than after 7-, 14- or 21-days-delayed transplantation [8,12,16]. The fourth study described better survival after 7-day-delayed transplantation than after acute transplantation. However, here the survival rate was reported at 28 days after the injury, implicating that the transplant was in fact quantified after 21 days, which could account for the discrepancy. MSCs seem to be able to migrate to the injury site and survive to some extent both when injected in the spinal cord away from the injury site [30], intrathecally [7,15,19,20,31,32] and intravenously [9,33,34]. A direct comparison of cell survival after an intralesional injection or intravenous injection of MSCs revealed that the former approach resulted in better survival [33]. Studies of direct comparisons between the other delivery modes are absent. After intrathecal delivery up to 5% of MSCs can survive 28 days after transplantation after repeated weekly injections starting 7 days post-injury [31]. With intrathecal delivery, three-day-delayed [19,31] or late injection (≥ 14-day-delayed) [7,15] seems less beneficial for cell survival than 7-day-delayed injection. However, a 5-day-delayed injection was shown to lead to better survival than a 7-day-delayed injection [19]. Given the current data, it seems reasonable to conclude that the optimal time point for intralesional transplantation is three days post-lesion. Although less data is available about the beneficial effects of intrathecal MSC injections and differences in methodology between

	Time point of transplantation (days psci)	Outcome	Delivery	Remarks
Torres-Espin et al. 2014 [16]	0 vs. 7	7d pi: 0 > 7 14d pi: 7 > 0 28d pi: 0 = 7 (few cells)	il	
Tan et al. 2013 [12]	1 vs. 3 vs. 7 vs. 14	3,7,14d pi: 3 > 1 > 7 > 14 28d pi: 1 = 3 = 7 = 14 (few cells)	il	
Nandoe Tewarie et al. 2009 [8]	0 vs. 3 vs. 7 vs. 2	7d pi: 3 > 0 > 7 = 21 28d pi: 0 = 3 = 7 = 21 (few cells)	il	
Hofstetter et al. 2002 [5]	0 vs. 7	5wk psci: 7 > 0	il + penumbra	Quantification 5w pi for acute group, 4w pi for 7d-delayed group
Cizkova et al. 2011 [31]	3 vs. 7 vs. 3,4,5 vs. 7,8,9 (repeated injections)	28d psci: 3 = 7 (no cells) 28d psci: 7,8,9 > 3,4,5	it	Quantification 21d pi for 7d-delayed group, 25d pi for 3d-delayed group
Nakano et al. 2013 [15]	7 vs. 14. vs. 28	2dpi: 7 > 14 = 28 7dpi: 7=1 =28 (no cells)	it	
Bakshi et al. 2006 [7]	<14 (4, 9 or 13) vs. >14 (20 or 27)	14d pi: [<14d] > [>14d]	it	
Satake et al. 2004 [19]	3 vs. 5 vs. 7	7d pi: 5 > 3 = 7 14d pi: 5 > 3 = 7	it	

psci, post-spinal cord injury; pi, post-injection; >, better survival than; = similar survival as; il, intralesional; it, intrathecal

Table 2: Overview of studies that have investigated the effect of timing of MSC transplantation on survival of MSCs in animal models of spinal cord injury.

studies make direct comparisons difficult, current data seem to suggest the use of intrathecal injections as a delivery method for MSC therapy for spinal cord injury [7,15,31]. For intrathecal injection the optimal time point seems to be five to seven days after injection. These time points are also favorable for clinical translation. Repeated deliveries (three deliveries at weekly intervals) seem to have beneficial effects on cell survival and associated anatomical and functional recovery after intrathecal delivery of MSCs [7,31]. A repeated injection regimen, however, seems problematic for intralesional injections where multiple surgeries will be necessary. Studies using single MSC injections did not find a dose-effect on MSC survival or associated recovery [7,28].

The Role of the Immune System in MSC Transplant Survival

There are a number of plausible causes of death of MSCs after transplantation into the injured spinal cord. The overwhelming presence of neutrophils in the first days after injury, followed by activation of resident microglia and a massive influx of macrophages may cause MSC death by direct phagocytosis. However, simply reducing the presence of macrophages does not increase MSC survival [35]. Neutrophils and macrophages may also cause MSC death by the formation of reactive oxygen species that cause membrane damage leading to death. Indeed, transplanting the cells within a polyurethane-based biogel with anti-oxidative properties increases short term (one week) survival of MSCs [6]. Longer term (4 week) survival however, is unaffected by this gel, probably due to biodegradation of the gel. The adaptive immune response may also play a role in MSC death after transplantation, the extent of which, however, is debated. MSCs have low expression of MHC class I molecules and absence of co-stimulatory molecules and have been reported to suppress the function of T-cells, B-cells, natural killer cells and dendritic cells [36]. The effect of immunosuppressants on MSC survival has been studied by different groups with conflicting results. In syngeneic transplantation, where cells are taken from an inbred strain and transplanted into a genetically similar individual from the same inbred strain, immunosuppressants are deemed unnecessary because the contribution of the immune system to MSC death is thought to be low [24]. In allogeneic transplantation, i.e. cells from an outbred strains and transplanted into individuals from another strain, immunosuppressants were shown to be effective by some investigators [21,24] but not by others [20,35]. Cyclosporine A (CsA) dose may partly explain these differences. Indeed, Parr et al. [21] studied the effect of CsA dose on MSC survival after 28 days and showed no effect of low dose CsA treatment (10 mg/kg/day) but a small statistically significant effect of high dose CsA treatment (20 mg/kg/day) on MSC survival 28 days after injection (8% vs 11%) in an allogeneic model system. Similarly, Swanger et al. [24] showed increased graft volumes, which were interpreted as larger cell numbers, with high dose CsA treatment (30 mg/kg/day for three days prior to transplantation, followed by 15 mg/kg/day for the duration of the study), compared to low dose (10 mg/kg/day) CsA treatment at 4 and 8 weeks post-transplantation. This difference was seen in an allogeneic model using MSCs from transgenic Fischer (inbred) rats transplanted into Sprague-Dawley (SD; outbred) rats without a spinal injury. In another study, in which MSCs from SD rats were transplanted into SD rats, this same dose regimen did not result in improved MSC transplant survival [35]. Aside from the strain difference, the fact that the latter study used spinal cord injured rats whereas the former study used uninjured rats likely explains the difference, since MSC death mechanisms in the injured spinal cord are different from those in the uninjured spinal cord. Xenotransplantation of MSCs, in which cells from one species (typically humans) are transplanted into another species (typically rats), usually results in poor survival even when using nude rats or CsA after injection into a spinal cord contusion [17]. This kind of rejection, however, is less relevant from a translational point of view, since a projected MSC therapy for human spinal cord injury would optimally be autologous and at the least allogeneic.

Deprivation from Oxygen, Nutrients and Growth Substrates

After spinal cord injury, there is a shortage of oxygen and nutrients resulting from rupturing of blood vessels and death of endothelial cells caused by the initial impact and subsequent inflammatory processes.

The ischemic environment likely contributes to MSC death after transplantation, although there is some evidence that MSCs are relatively resistant to hypoxia [37]. An increasing body of research is focusing on strategies to restore vascularization after spinal cord injury, the effect of which on MSC survival has yet to be determined. Another mechanism of MSC death in an injury site may be anoikis, which is defined as apoptosis induced by the lack of a substrate for attachment. Indeed, MSCs express integrin receptors, providing ligands for attachment to extracellular matrix molecules including collagen, fibronectin and laminin [38]. MSCs are anchorage-dependent cells and lack of a substrate to adhere to may induce apoptotic pathways. Transplantation of MSCs within scaffolds, in addition to possibly providing protection against macrophages, may prevent anoikis. Indeed, transplantation within a fibrin matrix was shown to increase MSC survival compared to controls [39].

Alternative Survival Enhancing Therapies

In addition to the survival enhancing strategies described above, including optimizing timing, immunosuppression and transplantation within a scaffold for protection against anoikis, reactive oxygen species and macrophages, a number of other treatments have been shown to be successful at increasing MSC survival. Table 1 summarizes these studies. Therapies include blockade of the IL-6/IL-6receptor [12], silencing p75 receptors in MSCs [10], culturing MSCs under microgravity conditions [9], electroacupunture [40,41], electrical stimulation [42], co-treatment with granulocyte-colony stimulating factor [13], Salvianolic acid B [43] or a NOGO-66R antagonist [22] and BDNF-overexpression in MSCs [11]. Strategies that failed to improve MSC survival include neural induction of MSCs [44] and co-culture with Schwann cells prior to transplantation [45]. Most of these studies however, did not provide quantitative data from which percentages of surviving cells could be derived. Instead, increases in MSC staining intensity, cell number per unit area, graft volume or only qualitative data are reported, making these studies very difficult to interpret and to compare.

From Bench to Bedside

There have been some early clinical trials assessing the safety and primary efficacy of MSC transplantation into spinal cord injured patients, concluding that MSC transplantation is feasible and safe [62,63]. Larger randomized, controlled, blinded clinical trials are needed to assess efficacy of MSCs in humans. Moreover, to our knowledge, survival rates of MSCs in humans have not been studied, but it is plausible that MSC survival enhancing strategies developed in rodents will benefit survival rates and efficacy of MSCs in humans.

Conclusion

MSC survival after transplantation in the injured spinal cord is poor, especially in the clinically relevant contusion models, which limits their therapeutic efficacy. Optimal timing, route of delivery, pretreatment of MSCs and co-treatment strategies may enhance survival to an extent, but quantitative data is scarce and when provided shows only small or short-term improvements in survival. It is clear from the current literature that more research is needed to elucidate mechanisms of MSC transplant death, so that rational survival enhancing strategies may be developed that can further develop MSC transplantation as a clinically relevant spinal cord injury therapeutic.

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References

1. Hagg T, Oudega M (2006) Degenerative and spontaneous regenerative processes after spinal cord injury. J Neurotrauma 23: 264-280.
2. Ritfeld GJ, Nandoe Tewarie RD, Vajn K, Rahiem ST, Hurtado A, et al.(2012) Bone marrow stromal cell-mediated tissue sparing enhances functional repair after spinal cord contusion in adult rats. Cell Transplant 21: 1561-1575.
3. Chen X, Katakowski M, Li Y, Lu D, Wang L, et al. (2002) Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. J Neurosci Res 69: 687-691.
4. Nakano N, Nakai Y, Seo TB, Yamada Y, Ohno T, et al. (2010) Characterization of conditioned medium of cultured bone marrow stromal cells. Neurosci Lett 483: 57-61
5. Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, et al. (2002) Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc Natl Acad Sci 99: 2199-2204.
6. Ritfeld GJ, Rauck BM, Novosat TL, Park D, Patel P, et al. (2014) The effect of a polyurethane-based reverse thermal gel on bone marrow stromal cell transplant survival and spinal cord repair. Biomaterials 35: 1924-1931.
7. Bakshi A, Barshinger AL, Swanger SA, Madhavani V, Shumsky JS, et al. (2006) Lumbar puncture delivery of bone marrow stromal cells in spinal cord contusion: a novel method for minimally invasive cell transplantation. J Neurotrauma 23: 55-65.
8. Nandoe Tewarie RD, Hurtado A, Ritfeld GJ, Rahiem ST, Wendell DF, et al. (2009) Bone marrow stromal cells elicit tissue sparing after acute but not delayed transplantation into the contused adult rat thoracic spinal cord. J Neurotrauma 26: 2313-2322.
9. Mitsuhara T, Takeda M, Yamaguchi S, Manabe T, Matsumoto M, et al. (2013) Simulated microgravity facilitates cell migration and neuroprotection after bone marrow stromal cell transplantation in spinal cord injury. Stem Cell Res Ther 4:35.
10. Edalat H, Hajebrahimi Z, Pirhajati V, Movahedin M, Tavallaei M, et al. (2013) Transplanting p75-suppressed bone marrow stromal cells promotes functional behavior in a rat model of spinal cord injury. Iran Biomed J 17: 140-145.
11. Lu P, Jones LL, Tuszynski MH (2005) BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. Exp Neurol 191: 344-360.
12. Tan Y, Uchida K, Nakajima H, Guerrero AR, Watanabe S, et al. (2013) Blockade of interleukin 6 signaling improves the survival rate of transplanted bone marrow stromal cells and increases locomotor function in mice with spinal cord injury. J Neuropathol Exp Neurol 72: 980-993.
13. Luo J, Zhang HT, Jiang XD, Xue S, Ke YQ (2009) Combination of bone marrow stromal cell transplantation with mobilization by granulocyte-colony stimulating factor promotes functional recovery after spinal cord transection. Acta Neurochir (Wien) 151: 1483-1492.
14. Quertainmont R, Cantinieaux D, Botman O, Sid S, Schoenen J, et al. (2012) Mesenchymal stem cell graft improves recovery after spinal cord injury in adult rats through neurotrophic and pro-angiogenic actions. PLoS One 7: e39500.
15. Nakano N, Nakai Y, Seo TB, Homma T, Yamada Y, et al. (2013) Effects of bone marrow stromal cell transplantation through CSF on the subacute and chronic spinal cord injury in rats. PLoS One 8: e73494.
16. Torres-Espin A, Redondo-Castro E, Hernandez J, Navarro X (2014) Bone marrow mesenchymal stromal cells and olfactory ensheathing cells transplantation after spinal cord injury--a morphological and functional comparison in rats. Eur J Neurosci 39: 1704-1717.
17. Hodgetts SI, Simmons PJ, Plant GW (2013) Human mesenchymal precursor cells (Stro-1(+)) from spinal cord injury patients improve functional recovery and tissue sparing in an acute spinal cord injury rat model. Cell Transplant 22: 393-412.
18. Ronsyn MW, Daans J, Spaepen G, Chatterjee S, Vermeulen K, et al. (2007) Plasmid-based genetic modification of human bone marrow-derived stromal cells: analysis of cell survival and transgene expression after transplantation in rat spinal cord. BMC Biotechnol 7:90.
19. Satake K, Lou J, Lenke LG (2004) Migration of mesenchymal stem cells through cerebrospinal fluid into injured spinal cord tissue. Spine (Phila Pa 1976) 29: 1971-1979.

20. Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, et al. (2004) Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. Exp Neurol 187: 266-278.

21. Parr AM, Kulbatski I, Wang XH, Keating A, Tator CH (2008) Fate of transplanted adult neural stem/progenitor cells and bone marrow-derived mesenchymal stromal cells in the injured adult rat spinal cord and impact on functional recovery. Surg Neurol 70: 600-707.

22. Zhilai Z, Hui Z, Yinhai C, Zhong C, Shaoxiong M, et al. (2011) Combination of NEP 1-40 infusion and bone marrow-derived neurospheres transplantation inhibit glial scar formation and promote functional recovery after rat spinal cord injury. Neurol India 59: 579-585.

23. Ding P, Yang Z, Wang W, Wang J, Xue L (2014) Transplantation of bone marrow stromal cells enhances infiltration and survival of CNP and Schwann cells to promote axonal sprouting following complete transection of spinal cord in adult rats. Am J Transl Res 6: 224-235.

24. Swanger SA, Neuhuber B, Himes BT, Bakshi A, Fischer I (2005) Analysis of allogeneic and syngeneic bone marrow stromal cell graft survival in the spinal cord. Cell Transplant 14: 775-786.

25. Ankeny DP, McTigue DM, Jakeman LB (2004) Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. Exp Neurol 190: 17-31.

26. Parr AM, Kulbatski I, Zahir T, Wang X, Yue C, et al. (2008) Transplanted adult spinal cord-derived neural stem/progenitor cells promote early functional recovery after rat spinal cord injury. Neuroscience 155: 760-770.

27. Yoshihara H, Shumsky JS, Neuhuber B, Otsuka T, Fischer I,et al. (2006) Combining motor training with transplantation of rat bone marrow stromal cells does not improve repair or recovery in rats with thoracic contusion injuries. Brain Res 1119: 65-75.

28. Kang KN, Kim da Y, Yoon SM, Lee JY, Lee BN, et al. (2012) Tissue engineered regeneration of completely transected spinal cord using human mesenchymal stem cells. Biomaterials 33: 4828-4835.

29. Cregg JM, DePaul MA, Filous AR, Lang BT, Tran A,et al. (2014) Functional regeneration beyond the glial scar. Exp Neurol 253: 197-207.

30. Yano S, Kuroda S, Shichinohe H, Seki T, Ohnishi T, et al. (2006) Bone marrow stromal cell transplantation preserves gammaaminobutyric acid receptor function in the injured spinal cord. J Neurotrauma 23: 1682-1692.

31. Cizkova D, Novotna I, Slovinska L, Vanicky I, Jergova S, et al. (2011) Repetitive intrathecal catheter delivery of bone marrow mesenchymal stromal cells improves functional recovery in a rat model of contusive spinal cord injury. J Neurotrauma 28: 1951-1961.

32. Shi E, Kazui T, Jiang X, Washiyama N, Yamashita K, et al. (2006) Intrathecal injection of bone marrow stromal cells attenuates neurologic injury after spinal cord ischemia. Ann Thorac Surg 81: 2227-2233.

33. Kang ES, Ha KY, Kim YH (2012) Fate of transplanted bone marrow derived mesenchymal stem cells following spinal cord injury in rats by transplantation routes. J Korean Med Sci 27: 586-593.

34. Cizkova D, Rosocha J, Vanicky I, Jergova S, Cizek M (2006) Transplants of human mesenchymal stem cells improve functional recovery after spinal cord injury in the rat. Cell Mol Neurobiol 26: 1167-1180.

35. Ritfeld GJ, Nandoe Tewarie RD, Rahiem ST, Hurtado A, Roos RA, et al. (2010) Reducing macrophages to improve bone marrow stromal cell survival in the contused spinal cord. Neuroreport 21: 221-226.

36. Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, et al. (2005) T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. J Biomed Sci 12: 47-57.

37. Buravkova LB, Andreeva ER, Gogvadze V, Zhivotovsky B (2014) Mesenchymal stem cells and hypoxia: Where are we? Mitochondrion.

38. Prowse AB, Chong F, Gray PP, Munro TP (2011) Stem cell integrins: implications for ex-vivo culture and cellular therapies. Stem Cell Res.;6(1): 1-12.

39. Itosaka H, Kuroda S, Shichinohe H, Yasuda H, Yano S, et al. (2009) Fibrin matrix provides a suitable scaffold for bone marrow stromal cells transplanted into injured spinal cord: a novel material for CNS tissue engineering. Neuropathology 29: 248-257.

40. Liu H, Yang K, Xin T, Wu W, Chen Y (2012) Implanted electro-acupuncture electric stimulation improves outcome of stem cells' transplantation in spinal cord injury. Artif Cells Blood Substit Immobil Biotechnol 40: 331-337.

41. Ding Y, Yan Q, Ruan JW, Zhang YQ, Li WJ, et al. (2013) Electroacupuncture promotes the differentiation of transplanted bone marrow mesenchymal stem cells overexpressing TrkC into neuron-like cells in transected spinal cord of rats. Cell Transplant 22: 65-86.

42. Wu W, Zhao H, Xie B, Liu H, Chen Y, et al. (2011) Implanted spike wave electric stimulation promotes survival of the bone marrow mesenchymal stem cells and functional recovery in the spinal cord injured rats. Neurosci Lett 491: 73-78.

43. Bi XB, Deng YB, Gan DH, Wang YZ (2008) Salvianolic acid B promotes survival of transplanted mesenchymal stem cells in spinal cord-injured rats. Acta Pharmacol Sin 29: 169-176.

44. Alexanian AR, Kwok WM, Pravdic D, Maiman DJ, Fehlings MG (2010) Survival of neurally induced mesenchymal cells may determine degree of motor recovery in injured spinal cord rats. Restor Neurol Neurosci 28: 761-777.

45. Xu X, Geremia N, Bao F, Pniak A, Rossoni M, et al. (2011) Schwann cell coculture improves the therapeutic effect of bone marrow stromal cells on recovery in spinal cord-injured mice. Cell Transplant 20: 1065-1086.

46. Ukegawa M, Bhatt K, Hirai T, Kaburagi H, Sotome S, et al. (2014) Bone marrow stromal cells combined with a honeycomb collagen sponge facilitate neurite elongation in vitro and neural restoration in the hemisected rat spinal cord. Cell Transplant.

47. Chen J, Zhang Z, Liu J, Zhou R, Zheng X, et al. (2014) Acellular spinal cord scaffold seeded with bone marrow stromal cells protects tissue and promotes functional recovery in spinal cord-injured rats. J Neurosci Res 92: 307-317.

48. Aizawa-Kohama M, Endo T, Kitada M, Wakao S, Sumiyoshi A, et al. (2013) Transplantation of bone marrow stromal cell-derived neural precursor cells ameliorates deficits in a rat model of complete spinal cord transection. Cell Transplant 22: 1613-1625.

49. Boido M, Garbossa D, Fontanella M, Ducati A, Vercelli A (2014) Mesenchymal stem cell transplantation reduces glial cyst and improves functional outcome after spinal cord compression. World Neurosurg 81: 183-190.

50. Yazdani SO, Pedram M, Hafizi M, Kabiri M, Soleimani M, et al. (2012) A comparison between neurally induced bone marrow derived mesenchymal stem cells and olfactory ensheathing glial cells to repair spinal cord injuries in rat. Tissue Cell 44: 205-213.

51. Zeng X, Zeng YS, Ma YH, Lu LY, Du BL, et al. (2011) Bone marrow mesenchymal stem cells in a three-dimensional gelatin sponge scaffold attenuate inflammation, promote angiogenesis, and reduce cavity formation in experimental spinal cord injury. Cell Transplant 20: 1881-1899.

52. Fang KM, Chen JK, Hung SC, Chen MC, Wu YT, et al. (2010) Effects of combinatorial treatment with pituitary adenylate cyclase activating peptide and human mesenchymal stem cells on spinal cord tissue repair. PLoS One 5: e15299.

53. Zhang W, Yan Q, Zeng YS, Zhang XB, Xiong Y, et al. (2010) Implantation of adult bone marrow-derived mesenchymal stem cells transfected with the neurotrophin-3 gene and pretreated with retinoic acid in completely transected spinal cord. Brain Res 1359: 256-271.

54. Ding Y, Yan Q, Ruan JW, Zhang YQ, Li WJ, et al. (2009) Electro-acupuncture promotes survival, differentiation of the bone marrow mesenchymal stem cells as well as functional recovery in the spinal cord-transected rats. BMC Neurosci 10:35.

55. Samdani AF, Paul C, Betz RR, Fischer I, Neuhuber B (2009) Transplantation of human marrow stromal cells and mono-nuclear bone marrow cells into the injured spinal cord: a comparative study. Spine (Phila Pa 1976) 34: 2605-2612.

56. Hollis ER, 2nd, Lu P, Blesch A, Tuszynski MH (2009) IGF-I gene delivery promotes corticospinal neuronal survival but not regeneration after adult CNS injury. Exp Neurol 215: 53-59.

57. Sheth RN, Manzano G, Li X, Levi AD (2008) Transplantation of human bone marrow-derived stromal cells into the contused spinal cord of nude rats. J Neurosurg Spine 8: 153-162.

58. Himes BT, Neuhuber B, Coleman C, Kushner R, Swanger SA, et al. (2006) Recovery of function following grafting of human bone marrow-derived stromal cells into the injured spinal cord. Neurorehabil Neural Repair 20: 278-296.

59. Yano S, Kuroda S, Lee JB, Shichinohe H, Seki T, et al. (2005) In vivo fluorescence tracking of bone marrow stromal cells transplanted into a pneumatic injury model of rat spinal cord. J Neurotrauma 22: 907-918.

60. Lee J, Kuroda S, Shichinohe H, Ikeda J, Seki T, et al. (2003) Migration and differentiation of nuclear fluorescence-labeled bone marrow stromal cells after transplantation into cerebral infarct and spinal cord injury in mice. Neuropathology 23: 169-180.

61. Chopp M, Zhang XH, Li Y, Wang L, Chen J, et al. (2000) Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. Neuroreport 1: 3001-3005.

62. Karamouzian S, Nematollahi-Mahani SN, Nakhaee N, Eskandary H (2012) Clinical safety and primary efficacy of bone marrow mesenchymal cell transplantation in subacute spinal cord injured patients. Clin Neurol Neurosurg 114: 935-939.

63. Jiang PC, Xiong WP, Wang G, Ma C, Yao WQ, et al. (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.