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Treatment of Traumatic Brain Injury in Mice with Bone Marrow Stromal Cell-Impregnated Collagen Scaffolds

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Abstract

Object—This study was designed to investigate new ways of delivering human marrow stromal cells (hMSCs) into the injured brain by impregnating them into collagen scaffolds to treat traumatic brain injury (TBI).

Methods—C57BL/6J mice were injured with controlled cortical impact ($n = 8$) and treated with 0.3×10^6 hMSCs impregnated into three-dimensional porous collagen scaffolds transplanted into the lesion cavity. Additional experimental groups ($n = 8$ mice/group) were treated with scaffolds implanted alone into the lesion cavity, and hMSCs administered alone intracerebrally or intravenously or saline injected into the lesion core. All treatments were performed 7 days after TBI. Spatial learning was measured using a modified Morris water maze test and brain tissue was processed for histopathological analysis.

Results—The results showed that hMSCs when delivered with scaffolds were more effective than hMSCs administered alone (intravenously or intracerebrally) in improving spatial learning, reducing lesion volume, and increasing vascular density after TBI.

Conclusion—Collagen scaffolds populated with hMSCs may be a new way to reconstruct injured brain and improve neurological function after TBI.

Keywords

scaffolds; human marrow stromal cells; lesion volume; traumatic brain injury; collagen

INTRODUCTION

The use of marrow stromal cells (MSCs) as a means to restore neurological function has generated great interest in the neuroscience community.^{15–18,20,22,23,25–30} We have employed rodent as well as human marrow stromal cells (hMSCs) to treat traumatic brain injury (TBI) and promising results have been achieved^{17,18, 20,22,23,25–30} with intracranial as well as intravenous modes of administration to deliver hMSCs.^{20,26,27,30} The intraarterial route of administration was abandoned after preliminary studies demonstrated that it caused embolic

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stroke.²² Both intracranial as well as intravenous modes of administration have been efficacious in improving functional outcome after TBI but there are limitations to their use.^{20,25–28,30} Intravenous injection of hMSCs results in body-wide distribution of hMSCs^{20,25,28,30}; whereas after intracerebral transplantation only a small percentage of hMSCs are able to survive^{26,27} since the lesion core rapidly changes to a cystic cavity - a hostile environment to the hMSCs. In addition, though both intravenous as well as intracerebral modes of administration reduce functional deficits after TBI, the lesion volume of cortex is unaffected.^{17,18,20,22,23,25–30} Therefore, in an effort to optimize hMSC therapy of TBI, and in particular to reduce lesion volume we investigated the use of hMSC-impregnated collagen scaffolds as hMSC delivery vehicles. The engineered scaffolds have been used for delivery of neural stem cells after cerebral ischemia,³⁷ spinal cord injury,² and for treatment of TBI in rats.²¹ However, to date, this approach has not been utilized for the delivery of donor cells within TBI sites in mice. Therefore, to further investigate the utility of hMSC-impregnated collagen scaffolds in the treatment of TBI and to expand their application to another species, mice were treated with scaffolds impregnated with hMSCs implanted into the lesion core after TBI. The reason mice were selected is that mechanistic studies are much easier to perform in mice and if functional benefit is observed with scaffold/hMSC therapy, future studies can be designed to understand the molecular and biochemical basis of this benefit. Our data show significant improvement in spatial learning, reduction in lesion volume and enhanced vascular density in mice treated with hMSC-impregnated scaffolds compared with control populations.

MATERIALS AND METHODS

All experimental procedures were approved by Henry Ford Hospital's Institutional Animal Care and Use Committee.

Experimental Groups

Forty male wild type C57BL/6J mice were randomly divided into five groups with 8 mice per group: 1) TBI + saline, 2) TBI + scaffold, 3) TBI + hMSCs ic, 4) TBI + hMSCs iv, and 5) TBI + scaffold/hMSCs. Mice in the TBI + saline group were subjected to TBI and saline was injected into the lesion core. Mice in the TBI + scaffold group were subjected to TBI, and scaffolds without cells were transplanted directly into the lesion core. Mice in the TBI + hMSCs group received 0.30×10^6 hMSCs injected intracerebrally (ic) directly into the lesion core or intravenously (iv) into the tail vein after TBI. Mice in the TBI + scaffold/hMSCs group were transplanted with scaffolds seeded with 0.30×10^6 hMSCs into the lesion core. All treatments were performed 7 days after TBI. All mice were tested on the Morris water maze (MWM) test during the last five days before sacrifice and then sacrificed on Day 35 after TBI.

Animal Models

We employed a controlled cortical impact model of TBI.^{39,40} Forty male wild type C57BL/6J mice weighing 21 to 26 g were anesthetized with chloral hydrate 400 mg/kg body weight administered intraperitoneally. Rectal temperature was controlled at 37 °C with a feedbackregulated water-heating pad. A controlled cortical impact device was used to induce the injury. Mice were placed in a stereotactic frame. One 4-mm-diameter craniotomy was performed adjacent to the central suture, midway between the lambda and bregma. The dura was kept intact over the cortex.

Injury was induced by impacting the left cortex (ipsilateral cortex) with a pneumatic piston containing a 2.5-mm-diameter tip at the rate of 4 m/s and 0.8 mm of compression. Velocity was measured with a linear velocity displacement transducer.

Cell Culture

hMSCs were isolated and expanded, as has been previously described.¹ Briefly, human bone marrow aspirates obtained from an 18-year-old, consenting, non-smoking donor (Clonetics-Poietics, Walkersville, MD) were resuspended in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mM nonessential amino acids, 100 U/ml penicillin and streptomycin, and 1 ng/ml basic fibroblast growth factor (bFGF). They were then plated at 10 μ l aspirate/cm² in tissue culture flasks, and maintained at 37°C in an atmosphere of 95% air and 5% carbon dioxide. All tissue culture components were obtained from Life Technologies (Rockville, MD). hMSCs were selected based on their ability to adhere to the tissue culture plastic after approximately 10 days in culture; non-adherent hematopoietic cells were removed during medium replacement. Medium was changed twice per week thereafter. hMSCs were subsequently detached using 0.25% trypsin/1 mM EDTA, replanted at 5×10^3 cells/cm², and cultured to 90% confluence to passage-one cells. hMSCs were subsequently frozen in 10% fetal calf serum (FCS) and 8% dimethyl sulfoxide (DMSO) in DMEM. hMSCs were then thawed and ex vivo expanded to passage-three cells as described above and subsequently utilized for scaffold seeding.

Scaffold Preparation and Cell Seeding

Ultrafoam scaffolds, collagen type I (Fig 1), were obtained from commercial sources (Daval, RI, USA). Scaffolds, cylindrical in shape, 3 mm diameter and 3 mm thick, were placed in 24 well-plates (one scaffold/well) and were pre-wetted for 2 hours in culture medium consisting of DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml streptomycin, 0.1 mM nonessential amino acids, and 1 ng/ml of basic fibroblast growth factor (bFGF) (Life Technologies, Rockville, MD). The medium was then aspirated from the scaffolds in preparation for cell seeding. The hMSCs used from passage three, prepared as described above, were trypsinized (0.25% trypsin/1 mM EDTA) and then resuspended in culture medium (same as described above) at a density of $2.5\text{--}5 \times 10^7$ cells/mL. Scaffolds were seeded with 10–20 μ L of this cell suspension to yield 0.3×10^6 cells/scaffold. The seeding volume was divided in two and applied in 2 successive applications to both sides of the body of the cylindrical scaffolds. The scaffolds with loaded cells were incubated for 30–60 minutes in a humidified incubator at 37°C to facilitate the primary cell seeding in terms of cell interactions with the scaffold and distribution in the scaffolds. One hour after seeding, 3 mL of culture medium was added to each well and the well plates with scaffolds were returned to the humidified incubator at 37°C and kept until scaffold implantation.

Transplantation

Scaffolds were seeded with 0.3×10^6 hMSCs and transplanted into the lesion cavity (one scaffold/animal) days after TBI. Under aseptic conditions and general anesthesia with ketamine (40 mg/kg) and xylazine (8 mg/kg) the original scalp incision was reopened and the lesion cavity induced by TBI was exposed in the left hemisphere. A scaffold seeded with hMSCs was placed directly into the cavity without removal of additional brain tissue, and subsequently covered by surgical foam (polyurethane foam), and the incision was closed with 4-0 absorbable gut surgical suture.

Tissue preparation

Thirty-five days after TBI, the animals were euthanized with an overdose of chloral hydrate (450 mg/kg body weight) administered intraperitoneally. All animals were perfused with intracardiac heparinized saline followed by 10% buffered formalin. The brains were removed and stored in 10% buffered formalin for two days at room temperature. Standard 1-mm-thick blocks were cut by the use of rodent brain matrix and processed for paraffin sectioning. A series of adjacent 6- μ m thick sections were cut, and a section of each block was stained with

hematoxylin and eosin (H&E). Standard H&E staining was employed to calculate lesion volume.

Immunofluorescence Studies

Single staining was performed for identification of hMSCs using a primary mouse anti-human nuclei monoclonal antibody (MAB1281), specific for all human cell types, and a secondary Cy5-conjugated F(ab')₂ Fragment rabbit anti-mouse IgG. Briefly, 6- μ m-thick sections from the TBI + hMSC ic, TBI + hMSC iv and TBI + scaffolds/hMSC groups were deparaffinized and the sections were put in boiling citrate buffer (pH = 6) in a microwave oven for 10 minutes for identification of neurons. After cooling at room temperature, the sections were incubated with a primary mouse anti-human nuclei monoclonal antibody (a human cellular nuclei antigen, MAB1281, dilution, 1: 200) and a Cy5-conjugated F(ab')₂ fragment mouse anti-human IgG (dilution, 1: 20). The slides were analyzed using a fluorescent microscope (Nikon ECLIPSE 80i). Negative control sections from each animal received identical staining preparation, except that the primary or secondary antibodies were omitted.

To identify the vascular structure, deparaffinized brain tissue sections were incubated in 1% Bovine serum albumin/PBS at room temperature for 1 hour and subsequently treated with mouse anti-vWF antibody (Dako Cytomation, Carpinteria, CA) diluted to 1:200 in PBS at 4 ° C overnight. vWF is a marker of endothelial cells and is routinely used to identify vessels in tissue sections.⁴¹ Following sequential incubation with biotin-conjugated anti-mouse immunoglobulin G (dilution 1:100; Dakopatts, Carpinteria, CA), the sections were treated with an avidin-biotin-peroxidase system (ABC kit; Vector Laboratories, Inc., CA). Diaminobenzidine was then used as sensitive chromogen for light microscopy. Negative control sections from each animal received identical staining preparation except that secondary anti-mouse immunoglobulin G was omitted.

Cell Counting

For measurement of MAB 1281 reactive cells, an average number of five equally spaced slides were obtained from brain block E, and MAB 1281 reactive cells were counted within the boundary zone. To reduce biases introduced by sampling parameters, all sections for MAB 1281 identification were stained simultaneously. The criteria for MAB 1281 positive cells were defined before the cells were counted by observers blinded to the individual treatment.

Volumetric analysis

To estimate the lesion volume of the cortex, tissue sections were stained with H&E and analyzed using a 10x objective and a computer imaging analysis system.⁴² The lesion volume was calculated by measuring the area of the lesion, including the lesion cavity and boundary zone, from each section and multiplying the lesion area by the section thickness and the sampling intervals.

Measurement of vascular density

Five sections with 50- μ m intervals through the dorsal dentate gyrus were stained for vWF and the images were digitized with a light microscope at magnification of x 200 (at the interaural 1.98-mm levels). The vWF-positive vessels were counted in the boundary zone of the lesion and the CA3 region of the hippocampus, using the Meta Imaging Series system (Nikon, Inc., Melville NY). The boundary zone was defined as the area surrounding the lesion cavity, which morphologically differs from surrounding intact brain. The vascular density in the boundary zone and CA3 region of the hippocampus was determined by dividing the number of the immunoreactive vessels by the corresponding area.⁴²

Morris Water Maze Test

The testing procedure is a modification of the Morris water maze test (MWM) as described previously.^{6,7,19} The experimental apparatus consisted of a circular water tank (140 cm in diameter and 45 cm high). An invisible platform (15 cm in diameter and 35 cm high) was placed 1.5 cm below the surface of the water, with a temperature of 30 °C. The pool was located in a large test room where there were many clues external to the maze (for example, pictures, lamps and so forth); these clues were visible from the pool and presumably used by the mice for spatial orientation. The position of the clues remained unchanged throughout the test. Data collection was automated using the HVS Image 2020 Plus Tracking System (US HVS Image, San Diego, CA). For descriptive data collection, the pool was subdivided into four equal quadrants formed by imaging lines. Spatial learning was tested towards the end of the study (days 31–35) since the neurorestorative processes require certain time periods to be clinically evident.¹⁸ Each animal was subjected to five trials. One trial was performed each day from Days 31 to 35 after TBI. At the start of a trial, the mouse was placed randomly at one of four fixed starting points, randomly facing either toward the wall or inwardly (designated North, South, East and West) and allowed to swim for 90 seconds or until it found the platform. The platform was located in a randomly changing position within the NE (correct) quadrant throughout the test period (e.g., sometimes equidistant from the center and edge of the pool, against the wall, near the center of the pool, and at the edges of the NE quadrant). If the animal found the platform, it was allowed to remain there for 15 seconds before being returned to its cage. If the animal was unable to find the platform within 90 seconds, the experiment was terminated and a maximum score of 90 seconds was assigned. The percentage of time traveled within the NE quadrant was calculated relative to the total amount of time spent swimming before reaching the platform.

Statistical analysis

Repeated measure analysis of variance (ANOVA) was used to study the effect of hMSC treatment on functional recovery. Analysis began with testing for the treatment by time interaction, followed by testing the effects of treatment at each time point, if interaction or main effect of time or treatment was detected at the 0.05 level. The same analytic approach was used to test the effects of MSCs on vascular density in the lesion boundary zone and hippocampus. The mean and standard deviation (SD) by time are presented as data illustration.

RESULTS

Spatial Learning Improvement

The mean percentage of time spent in the correct quadrant in the MWM test was significantly higher in all treatment groups compared to control ($p < 0.05$) with improvement seen at Days 32–35 after TBI (Fig. 2). The scaffolds alone or hMSCs-alone treatment (ic, or iv) induces improvement in spatial learning compared to saline control ($p < 0.05$). However, the TBI + scaffold/hMSC group performed significantly better than all other treatment groups.

Scaffold + hMSC treatment reduces lesion volume

Fig. 3 shows that scaffold + hMSC treatment reduces tissue loss in the injured cortex. There were no significant differences in the lesion volume among the hMSCs or scaffolds-alone treated groups compared with control (Fig. 4). However, treatment with scaffold + hMSCs significantly reduced lesion volume ($p < 0.05$).

Scaffolds increase hMSC migration into lesion boundary zone after TBI

No MAB1281 positive cells (hMSCs) were detected in the brains of the TBI + saline or TBI + scaffold groups. MAB1281 positive cells were seen in the brains of TBI + hMSCs delivered

ic, TBI + hMSCs delivered iv and TBI + scaffold/hMSCs (Fig. 5). These cells were mainly seen in the lesion boundary zone though some scattered cells were also seen in other areas of the ipsilateral hemisphere. The number of MAB1281 positive cells was significantly increased in the TBI + scaffold/hMSC groups compared with TBI + hMSCs delivered ic or TBI + hMSCs delivered iv groups (Fig. 6), indicating that scaffolds enhance the presence of hMSCs in the lesion boundary zone.

Scaffold + hMSC treatment increases the vascular density in the lesion boundary zone and hippocampus after TBI

Figure 7 shows vWF positive vessels in the lesion boundary zone of TBI + hMSCs ic, TBI + hMSCs iv and TBI + scaffold/hMSC groups. The vascular density in the lesion boundary zone and CA3 region of hippocampus was significantly increased in the TBI + scaffold/hMSC group compared to all other groups (Fig. 8). The vascular density was also increased in the lesion boundary zone alone in the TBI + hMSCs delivered iv group compared to hMSCs delivered ic, scaffold-alone and saline-treated groups. However, this increase was significantly less than the TBI + scaffold/hMSCs group.

DISCUSSION

Our data demonstrate that scaffolds suffused with hMSCs improve functional outcome and reduce lesion volume after TBI compared to saline-scaffold and hMSC-alone treated mice.

Tissue engineering approaches have become a focus in regenerative medicine³³ in particular in the context of critically sized defects. These biomedical interventions have mainly focused on treating skeletal defects such as disorders of bones and cartilage.^{14,33} Though there are several reports of scaffolds used in the treatment of spinal cord injury² and cerebral ischemia,³⁷ they have not been used extensively for the treatment of TBI. Many non-biological and biological biomaterials for scaffolds have been studied covering a range of mechanical properties, including ceramics,¹³ metals,³ synthetic polymers³¹ and natural polymers.^{34,35} Scaffolds utilized for tissue repair should mimic the structure and biological function of the extracellular matrix (ECM), and collagen seems to be an appropriate biomaterial for this purpose.³⁸ The scaffolds should provide mechanical support, deliver inductive molecules or cells to repair the site and provide cues to control the structure and function of newly formed tissue.¹¹ Many techniques have been developed to fabricate three-dimensional porous architecture to fill this role such as particle backing,²⁴ phase separation,³⁶ self assembly,⁹ and salt porogen.¹² We have used the technique of salt porogen¹² in generating these scaffolds and we believe it is an optimum engineering approach since the structures generated by it resemble the topographic features of ECM. In our study, collagen scaffolds were well accepted by the host brain with minimal mononuclear cell infiltration and astroglial scarring. Bakshi et al implanted hydrogel scaffolds into the spinal cord after cervical hemisection² and also found minimal gliosis. This indicates that scaffolds do not induce a toxic inflammatory reaction.

Our research represents a marriage of cell therapy and tissue engineering aimed at restoring neurological function and reducing biostructural damage following TBI. In our study, administration of hMSCs after impregnating them into collagen scaffolds increased their survival and concentration in the lesion boundary zone and the functional outcome of these animals was significantly better than all other groups. hMSCs within the scaffolds may provide neurotrophic support for the surrounding brain tissues and hMSCs which migrate into the lesion boundary zone may promote remodeling of the injured tissue.¹⁸ The synthetic extracellular matrix (ECM), as represented by the scaffold, defines a potential space for tissue development and can serve as a permissive substrate for cell growth. This is the likely reason that administration of hMSCs along with scaffolds reduced the lesion volume whereas administration of scaffolds-alone or hMSCs-alone (intracerebrally and intravenously

delivered) had no effect on lesion volume. The scaffolds can provide spaces to allow for vascularization of new tissues which in turn can also produce angiogenic factors. In our study, vascular density in the lesion boundary zone as well as in the CA3 region of hippocampus was significantly enhanced in the mice treated with scaffolds + hMSCs compared with mice treated with scaffolds or hMSCs alone.

We have previously studied the treatment of TBI in rats with hMSCs impregnated-collagen scaffolds.²¹ However, that was a pilot study and we only compared the treatment with hMSCs + scaffolds with hMSCs injected intracerebrally. The present study was more comprehensive and treatment with hMSCs + scaffolds was compared with hMSCs injected intracerebrally as well as intravenously. However, the previous study also showed that treatment with hMSCs + scaffold was more efficacious in improving functional outcome and reducing lesion volume in rats compared to hMSCs injected-alone intracerebrally. Therefore, this technique of hMSC delivery (i.e., impregnating them into collagen scaffolds) has been shown to be very effective in two species and this extends its in vivo application.

However, there is one important question; do scaffolds provide mostly mechanical support or do they in some way modify the genetic structure of hMSCs? Mauney et al³² reported that culturing hMSCs on a denatured collagen matrix versus tissue culture polystyrene significantly reduced cellular aging, increased the proliferation capacity, reduced the rate of morphological changes and resulted in dramatic increase in the retention of osteogenic-specific functions. hMSCs have been shown to primarily utilize a large family of transmembrane heterodimeric receptors, i.e. integrins, to facilitate their adhesion to a variety of ECM components such as collagen Type I.⁸ Interactions between integrins and their ligands have been linked to many cellular processes including proliferation, differentiation, survival and apoptosis.⁴⁻⁸ Moreover, the ability of the intracellular portion of integrins to bind to various tyrosine kinases and adapter proteins in response to ECM adhesion has implicated them in cellular signaling pathways.^{5,10} We are conducting mechanistic studies to investigate if culturing hMSCs with collagen scaffolds alters their genetic and molecular structure.

We are using recent advances in tissue engineering as means of enhancing the neurorestorative functions of hMSCs. This interface between two scientific disciplines can increase the clinical relevance of cell therapy for treatment of TBI and also serve as a prototype for multidisciplinary strategies against other complex problems in the nervous system.

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References

1. Altman GH, Horan RL, Lu HH, Moreau J, Martin I, Richmond JC, et al. Matrix for tissue engineered anterior cruciate ligaments. *Biomaterials* 2002;23:4131–4141. [PubMed: 12182315]
2. Bakshi A, Fisher O, Dagci T, Himes BT, Fischer I, Lowman A. Mechanically engineered hydrogel scaffolds for axonal growth and angiogenesis after transplantation in spinal cord injury. *J Neurosurg (Spine1)* 2004;3:322–329.
3. Bancroft GN, Sikavitsas VI, van den Dolder J, Sheffield TL, Ambrose CG, Jansen JA, et al. Fluid flow increases mineralized matrix deposition in 3D perfusion culture of marrow stromal osteoblasts in a dose-dependent manner. *Proc Natl Acad Sci USA* 2002;99:12600–5. [PubMed: 12242339]
4. Boudreau NJ, Jones PL. Extracellular matrix and integrin signaling: the shape of things to come. *Biochem J* 1999;339:481–488. [PubMed: 10215583]
5. Chen Q, Kinch MS, Lin TH, Burridge K, Juliano RL. Integrin-mediated activation of mitogen-activated protein kinases. *J Biol Chem* 1994;269:26602–26609. [PubMed: 7929388]

6. Day LB, Schallert T. Anticholinergic effects on acquisition of place learning in the Morris water task: spatial mapping deficit or inability to inhibit nonplace strategies? *Behav Neurosci* 1996;110:998–1005. [PubMed: 8919002]
7. Day LB, Weisend M, Sutherland RJ, Schallert T. The hippocampus is not necessary for a place response but may be necessary for pliancy. *Behav Neurosci* 1999;113:914–924. [PubMed: 10571475]
8. Gronthos S, Simmons PJ, Graves SE, Robey PG. Integrin-mediated interactions between human marrow stromal precursor cells and the extracellular matrix. *Bone* 2001;28:74–81.
9. Hartgerink JD, Beniash E, Stupp SI. Peptide-amphiphile nanofibers: a versatile scaffold for the preparation of self-assembling materials. *Proc Natl Acad Sci USA* 2002;99:5133–5138. [PubMed: 11929981]
10. Heino J. The collagen receptor integrins have distinct ligand recognition and signaling functions. *Matrix Biol* 2000;19:319–323. [PubMed: 10963992]
11. Hirano Y, Mooney DJ. Peptide and protein presenting materials for tissue engineering. *Adv Matter (Weinheim, Fed Repub Ger)* 2004;16:17–25.
12. Kim UJ, Park J, Kim HJ, Wada M, Kaplan D. Three-dimensional aqueous-derived biomaterial scaffolds from silk fibroin. *Biomaterials* 2005;26:2775–2785. [PubMed: 15585282]
13. Kon E, Muraglia A, Corsi A, Bianco P, Marcacci M, Martin I, et al. Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones. *J Biomed Mater Res* 2000;49:328–337. [PubMed: 10602065]
14. Li C, Vepari C, Jin HJ, Kim HJ, Kaplan DL. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. *Biomaterials* 2006;27:3115–3124. [PubMed: 16458961]
15. Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, et al. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology* 2002;59:514–523. [PubMed: 12196642]
16. Liu Z, Li Y, Qu R, Shen L, Gao Q, Zhang X, et al. Axonal sprouting into the denervated spinal cord and synaptic and postsynaptic protein expression in the spinal cord after transplantation of bone marrow stromal cell in stroke rats. *Brain Res* 2007;1149:172–180. [PubMed: 17362881]
17. Lu D, Li Y, Mahmood A, Wang L, Rafiq T, Chopp M. Neural and marrow-derived stromal cell sphere transplantation in a rat model of traumatic brain injury. *J Neurosurg* 2002;97:935–940. [PubMed: 12405384]
18. Lu D, Mahmood A, Chopp M. Biological transplantation and neurotrophic induced neuroplasticity after traumatic brain injury. *J Head Trauma Rehabil* 2003;18:357–376. [PubMed: 16222130]
19. Lu D, Mahmood A, Goussev A, Schallert T, Qu C, Zhang ZG, et al. Atorvastatin reduction of intravascular thrombosis, increase in cerebral microvascular patency and integrity, and enhancement of spatial learning in rats subjected to traumatic brain injury. *J Neurosurg* 2004;101:813–821. [PubMed: 15540920]
20. Lu D, Mahmood A, Li Y, Chopp M. Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. *Neuroreport* 2001;12:559–563. [PubMed: 11234763]
21. Lu D, Mahmood A, Qu C, Chopp M. Collagen scaffolds populated with human marrow stromal cells reduce lesion volume and improve functional outcome after traumatic brain injury. *Neurosurgery* 2007;61:596–602. [PubMed: 17881974]
22. Lu D, Wang L, Chen J, Mahmood A, Chopp M. Intraarterial administration of marrow stromal cells in a rat model of traumatic brain injury. *J Neurotrauma* 2001;18:813–819. [PubMed: 11526987]
23. Lu M, Chen J, Lu D, Yi L, Mahmood A, Chopp M. Global test statistics for treatment effect of stroke and traumatic brain injury in rats with administration of bone marrow stromal cells. *J Neurosci Res* 2003;128:183–190.
24. Ma PX, Coi JW. Biodegradable polymer scaffolds with well-defined interconnected spherical pore network. *Tissue Eng* 2001;7:23–33. [PubMed: 11224921]
25. Mahmood A, Lu D, Chopp M. Treatment of traumatic brain injury in adult rats with human bone marrow stromal cells. *Neurosurgery* 2003;53:697–700. [PubMed: 12943585]
26. Mahmood A, Lu D, Li Y, Chen JL, Chopp M. Intracranial bone marrow transplantation after traumatic brain injury improves functional outcome in adult rats. *J Neurosurg* 2001;94:589–595. [PubMed: 11302657]

27. Mahmood A, Lu D, Li Y, Chopp M. Intracerebral transplantation of marrow stromal cells (MSCs) improves outcome after traumatic brain injury. *J Neurotrauma* 2002;19:1609–1617. [PubMed: 12542861]
28. Mahmood A, Lu D, Qu C, Goussev A, Chopp M. Human marrow stromal cell treatment provides long lasting benefit after traumatic brain injury in rats. *Neurosurgery* 2005;57:1026–1031. [PubMed: 16284572]
29. Mahmood A, Lu D, Qu C, Goussev A, Chopp M. Long-term recovery after bone marrow stromal cell treatment of traumatic brain injury in rats. *J Neurosurg* 2006;104:272–277. [PubMed: 16509501]
30. Mahmood A, Lu D, Wang L, Chopp M. Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. *Neurosurgery* 2001;49:1196–1204. [PubMed: 11846913]
31. Martin I, Shastri VP, Padera RF, Yang J, Mackay AJ, Langer R, et al. Selective differentiation of mammalian bone marrow stromal cells cultured on three-dimensional polymer foams. *J Biomed Mater Res* 2001;55:229–35. [PubMed: 11255174]
32. Mauney JR, Kaplan DL, Volloch V. Matrix-mediated retention of osteogenic differentiation potential by human adult bone marrow stromal cells during ex vivo expansion. *Biomaterials* 2004;25:3233–3243. [PubMed: 14980418]
33. Meinel L, Fajardo R, Hofmann S, Langer R, Chen J, Snyder B, et al. Silk implants for the healing of critical size bone defects. *Bone* 2005;37:688–698. [PubMed: 16140599]
34. Meinel L, Hofmann S, Karageorgiou V, Zichner L, Langer R, Kaplan D, et al. Engineering cartilage-like tissue using human mesenchymal stem cells and silk protein scaffolds. *Biotechnol Bioeng* 2004;88:379–391. [PubMed: 15486944]
35. Meinel L, Karageorgiou V, Fajardo R, Snyder B, Shinde-Patil V, Zichner L, et al. Bone tissue engineering using human mesenchymal stem cells: effects of scaffold material and medium flow. *Ann Biomed Eng* 2004;32:112–22. [PubMed: 14964727]
36. Nam YS, Park TG. Biodegradable polymeric microcellular foams by modified thermally induced phase separation method. *Biomaterials* 1999;20:1783–1790. [PubMed: 10509188]
37. Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nature Biotechnol* 2002;20:1111–1117. [PubMed: 12379868]
38. Reddi AH. Morphogenetic messages are in the extracellular matrix: biotechnology from bench to bedside. *Biochem Soc Trans* 2000;28:345–349. [PubMed: 10961916]
39. Smith DH, Soares HD, Pierce JS, Perlman KG, Saatman KE, Meaney DF, et al. Model of parasagittal controlled cortical impact in the mouse: cognitive and histopathologic effects. *J Neurotrauma* 1995;12:169–178. [PubMed: 7629863]
40. Xiong Y, Shie FS, Zhang J, Lee CP, Ho Y. Prevention of mitochondrial dysfunction in post-traumatic mouse brain by superoxide dismutase. *J Neurochem* 2005;95:732–744. [PubMed: 16248885]
41. Zanetta L, Marcus SG, Vasile J, Dobryansky M, Cohen H, Eng K, et al. Expression of vonWillebrand factor, an endothelial cell marker is upregulated by angiogenesis factors: a potential method for objective assessment of tumor angiogenesis. *Int J Cancer* 2000;85:281–288. [PubMed: 10629090]
42. Zhang ZG, Zhang L, Jiang Q, Chopp M. Bone marrow derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in adult mouse. *Circ Res* 2002;90:284–288. [PubMed: 11861416]

Fig. 1. SEM images of collagen scaffolds: (A) disks used in implants, 3 mm in diameter scale bar = 2 mm, (B) higher magnification image, scale bar = 500 microns, (C) further magnification to show porous structure, scale bar = 200 microns.

Fig. 2.

Graph showing time spent in the correct quadrant in the TBI + saline, TBI + scaffold, TBI + hMSCs ic, TBI + hMSCs iv and TBI+ scaffold/hMSCs groups during the Morris water maze test. The percentage of time spent in the correct quadrant was significantly more in the scaffold/hMSCs-treated group than in the hMSCs ic, hMSCs iv, scaffolds-only and saline-treated groups from 32–35 days post TBI. * = $p < 0.05$. The saline-treated animals spent less time in the correct quadrant than animals from all other groups. # = $p < 0.05$.

Fig. 3.

Hematoxylin and eosin staining shows the representative sections from TBI + hMSC ic, TBI + hMSC iv and TBI + scaffold/hMSC groups. Treatment with scaffold/hMSCs reduces lesion volume and prevents tissue loss. Scale bar shown on right = 1mm, original magnification x 200.

Fig. 4.

Bar graph showing changes in lesion volume among different groups. Compared with other groups, treatment with scaffold/hMSCs significantly reduced lesion volume. *= $p < 0.05$.

Fig. 5.

Immunohistochemical staining shows the MAB 1281-positive cells in the lesion boundary zone from hMSCs iv, hMSCs ic and scaffold/hMSCs treated groups. Arrows show the positive cells (pink). Scale bar shown on right = 50 μ m, original magnification x200.

Fig. 6.

Bar graph showing significantly increased MAB1281-positive cells detected in the lesion boundary zone in the scaffold/hMSCs-treated group versus hMSCs ic and hMSCs iv groups (* $p < 0.05$).

Fig. 7. Microphotographs showing DAB staining for vWF for evaluation of vascular structures in hMSCs iv, hMSCs ic and scaffold/hMSCs-treated groups. Scale bar shown on right = 50 μ m. Original magnification, $\times 200$.

Fig. 8.

Bar graphs showing the vascular density to be significantly more in the boundary zone (BZ) and hippocampus (CA3) in the scaffold/hMSCs-treated group compared with that of other groups. * = $p < 0.05$. The vascular density of the group treated with hMSCs delivered intravenously in the boundary zone was higher than that of the hMSCs delivered intracerebrally, with scaffolds-only and saline-treated groups. # = $p < 0.05$