

Current Concepts in Neural Regeneration After Traumatic Brain Injury

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TBI poses unique difficulties in terms of developing effective therapeutic modalities. The inflammatory cascade that results from TBI is distinct from that which follows other forms of injury to the CNS. This difference poses unique challenges to developing potential mechanisms of treatment based on the concepts of neural regeneration and repair.

Key Words: neural transplantation, neuroregeneration, trauma, traumatic brain injury

Abbreviations Used: BrdU, bromodeoxyuridine; CNS, central nervous system; DiO, dioctadecyloxycarbocyanine, DNA, deoxyribonucleic acid; GFAP, glial fibrillary acidic protein; MSC, marrow-derived stromal cells; NeuN, neuronal nuclei marker; TBI, traumatic brain injury

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Each year more than a million Americans are treated for TBI and released from emergency rooms. More than 5 million people live with permanent disabilities from TBI-related injuries, including behavioral disorders, cognitive difficulties, and motor impairment.⁶ TBI is the leading cause of death in young people in America and costs billions of healthcare dollars each year. For many years, therapies aimed at regenerating lost brain tissue have been explored. The replacement of dead neurons by new cells derived from precursor cells, which has been the focus of recent efforts, remains a controversial ethical issue.

The primary damage caused by TBI is the result of the initial impact. Intracellular edema develops, and the electrochemical processes inherent to neural function are disrupted.^{1,16,28} The secondary or delayed damage associated with TBI is less well understood but involves a complex cascade of events and pathophysiological interactions. Research once emphasized pharmacological therapies and early surgical intervention intended to limit this secondary constellation of events.

In the last decade, however, TBI has been recharacterized as a chronic, progressive disease involving the delayed death of neurons,²⁴ and the focus of research has shifted concomitantly. Recently, stimulation of latent neural progenitor cells and the introduction of exogenous precursor cells have been topics of investigation (Fig. 1).²¹ Dead and damaged neural tissues have been replaced both *in vitro* and *in vivo* in rodents and nonhuman primates, but an effective and reproducible treatment for humans has yet to be developed. This article reviews current

concepts in the utilization of stem cell populations and their potential therapeutic role in the treatment of TBI.

Endogenous Stem Cells

Cellular replacement strategies have involved both endogenous and exogenous sources of progenitor cells. Endogenous neurogenesis has been a long-standing goal to minimize further trauma to the brain from transplantation and to avoid the subsequent need for immune suppressive therapies. Efforts to stimulate endogenous precursor cells to migrate to areas of injury have allowed the regions where these cells are found to be characterized. Adult mammals have neural stem cells that can be activated and that cause remodeling in the event of injury.¹⁰ After TBI these proliferating stem cells continue this remodeling process.⁵

Remodeling occurs at two specific areas. The area of cortical injury nearest the site of impact involves glial remodeling. GFAP staining has shown that reactive astrocytes are upregulated and form a glial scar 3 weeks after injury. Second, staining with NeuN, a neuronal marker, has shown hypertrophy of neurons in the ipsilateral granular cell layer.²³

Stem cells that supply the astrocytes originate in the subventricular zone. An intraventricular injection of the lipophilic tracer DiO reliably demonstrates labeling of this zone. These labeled cells migrate from the subventricular zone into the damaged area of cortex.²³ These cells colocalize with nestin, an intermediate filament specific to neuronal precursors. DiO labeling in the area of injured cortex persists 3 weeks, indicating that these cells survive. GFAP co-localizes with the DiO in this region. Morphologically, these

cells appear to be the same reactive astrocytes that form the glial scar. Under normal circumstances, these cells supply replacement cells to the olfactory bulb, where they become glomerular neurons.⁴ Therefore, these cells have the *potential* to differentiate along neuronal or glial cell lines. Recent studies have focused on delivering growth factors to this cell population to influence them to become new neurons in the presence of brain injury.

The adult nervous system hosts multiple regions of stem cells. Neural crest stem cells can be found in the peripheral nervous system. In the CNS, the retina, olfactory bulb, forebrain, hippocampus, and spinal cord contain stem cells (Fig. 2).^{3,8,9,14} The largest repositories of neural stem cells are found in the subventricular zone and dentate gyrus of the hippocampus.¹⁹

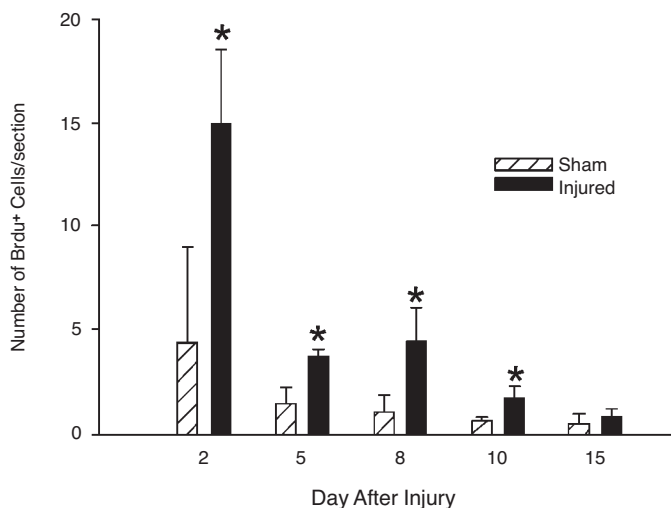


Figure 1. Graphic representation of BrdU positive cells per section in the SGZ (subgranular zone) and SEZ (subventricular zone) of animals injected with BrdU 18 and 20 hours after traumatic brain injury, then sacrificed at various times. The cells have a peak of proliferation at 2 and 8 days after injury. They then degrade or migrate away over 2 weeks or so. Most cells go on to form glial cells. These cells arise from the subgranular zone of the hippocampus and subependymal zones of the lateral ventricles. It is considered to be a normal response of the brain to injury. Modulation in the development of these cells away from an astrocytic (and therefore scar-forming) lineage is a potential key therapeutic target in ameliorating the damage following TBI. Data are presented as the number of cells per section \pm standard deviation (SD). Injured animals in each group (3 to 6 animals) are compared with their respective shams using a *t* test. *Significant at $p < 0.05$. From Rice AC, Khaldi A, Harvey HB, Salman NJ, White F, Fillmore H, Bullock MR: Proliferation and neuronal differentiation of mitotically active cells following traumatic brain injury. *Exp Neurol* 183(2):406-417, 2003. Used with permission from Elsevier Science.

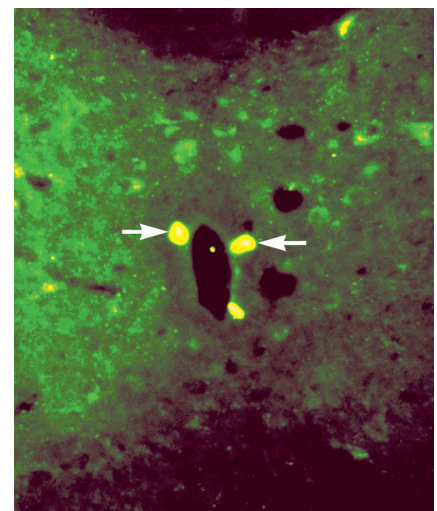


Figure 2. Photomicrograph of BrdU-stained cells dividing around the central canal in the spinal cord of an adult rodent exposed to recombinant Shh after sustaining a demyelinating lesion. These cells represent endogenous neural progenitors stimulated by the combination of demyelination and Shh. Fluorescent stain for BrdU (200x). From Bambakidis NC, Theodore N, Nakaji P, Harvey A, Sonntag VK, Preul MC, Miller RH: Endogenous stem cell proliferation after central nervous system injury: Alternative therapeutic options. *Neurosurg Focus* 19(3):E1, 2005. Used with permission from Journal of Neurosurgery.

Progenitor Cells versus Stem Cells

As an embryo develops, the pluripotent cells that compose the differentiating tissue gradually lose their ability to transdifferentiate. Embryonic stem cells are derived from the inner cell mass.²⁹ Adult stem cells are present in many different organ systems in the body. Tissue restriction limits the possible fates of these cells more than in their embryonic counterparts. This factor may explain the differences seen in experimental models that have tested the fates of cells in *in vitro* animal models. Some studies have shown that even these cells may cross fate lines and generate other tissues. For example, adult neural stem cells can become hematopoietic cells when transplanted into the marrow of irradiated mice. Whether this process represents actual transdifferentiation or cell-cell fusion is debated. The ideal population of adult stem cells would exhibit no *in vitro* or *in vivo* difference in their fate lines.

Stated simply, stem cells can differentiate into many different types of cells (Fig. 3). They have an unlimited capacity for self-renewal and are found in many different kinds of tissue. Progenitor cells also have multiple potential destinies. However, they have far fewer potential fates than stem cells. Although much greater than that of differentiated cells, the capacity of progenitor cells for renewal is more limited than that of stem cells. Stem cells are the best candidates for treating TBI because they are robust and more flexible than progenitor lines. Furthermore, neural progenitor cells must be isolated from embryos, a process with controversial ethical and sociological implications.

Exogenous Delivery of Cells

Regeneration in the CNS requires one or more of the following processes to occur: neurotrophic factor elaboration, cell replacement, axonal guidance, removal of growth inhibition (characteristic of damage in the CNS), modulation of the immune response, a sub-

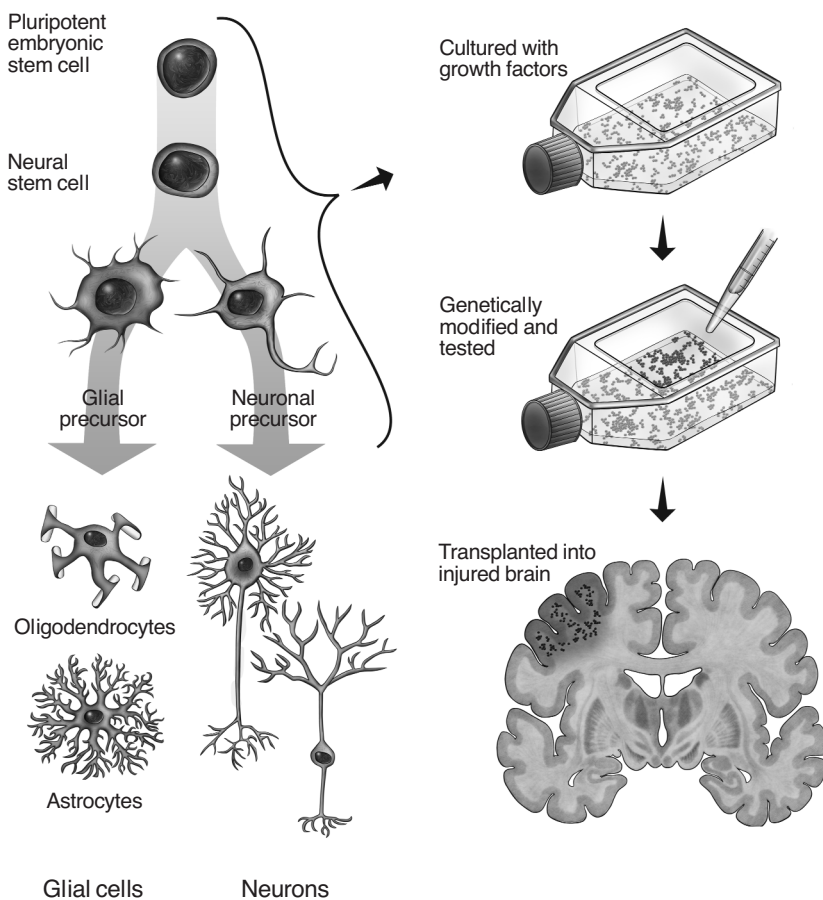


Figure 3. Diagram demonstrating the pluripotent nature of embryonic stem cells as well as their potential therapeutic roles in traumatic brain injury. Pluripotent stem cells develop under the influence of various growth factors into multipotent progenitors which can then form either neuronal, oligodendroglial, or astrocytic cells. This process can be modulated *in vitro* and subsequently can result in cells which can be transplanted at the site of injury with a resultant potential for therapy.

strate for bridging, and cell signaling manipulation.¹⁰ Injections into the bloodstream and intraparenchymal delivery have been used to transplant cells in an attempt to rebuild a damaged nervous system. To be effective, the transplanted cells must be self-renewing and pluripotent. They must exhibit tropism for the damaged area and form functional connections. The survival and integration of these cells depend on both the location of the transplant and the local environment.²⁷

Fetal Tissue Transplantation

The direct implantation of fetal cells into damaged cortex was first evaluated

more than 20 years ago. Recent studies based on the fluid percussion model of TBI found that the optimal window for transplantation is within 2 weeks of injury.²⁶ Transplanted fetal tissue is able to integrate into damaged tissue and thereby restore function. This capability may hold particular promise after an injured brain is exposed to retraining activities. In this case transplanted cells may help re-establish injured neuronal connections.

Keyvani et al. performed DNA microarray analysis of intact and injured brains.¹⁵ They examined the non-necrotic ipsilateral cortex and homotopic contralateral cortex in brain-

injured rats exposed to environmental enrichment after injury. In the brain-injured rats, 59 genes were differentially regulated compared to 17 genes regulated in analogous areas in the brains of intact rats. This finding suggests an increased propensity for enrichment-induced plasticity after injury. Such research is promising, but significant ethical and immunological dilemmas remain.

Infusional Delivery

In a hemorrhagic brain injury model, injection of blood from human umbilical cords has been found to reduce the effects of neurological damage.¹⁸ Rats injected with human umbilical cord blood 24 hours after intracerebral hemorrhage were evaluated immediately before receiving the injection, 1 day after injury, and then 7 and 14 days after injury. On Days 7 and 14, the neurological severity test scores of these experimental rats were better than those of saline-treated rats. Umbilical cord blood has been used successfully to repopulate depleted immune systems. Therefore, it is thought that hematopoietic stem cells are able to transdifferentiate and to perform a similar function in the nervous system.¹⁸

Mahmood et al.¹⁷ used MSCs as a source of neural growth factors to facilitate intrinsic stem cells to grow and differentiate. MSCs can differentiate into endodermal, mesodermal, and ectodermal derivatives.¹² MSCs were delivered by an intravenous injection of marrow cells. Long bones from normal rats were obtained and cleaned, and the cells were cultured and labeled with BrdU. One day after the rats received a pneumatic injury intended to simulate a concussive impact, the cell mixture was injected. Rats were assessed via standard neurological function tests at various endpoints. Histological analysis was performed when the rats were sacrificed 15 days after injury. Levels of three different growth factors were measured on Days 2, 5, and 8: nerve growth factor, brain-derived nerve growth factor, and basic fibroblast growth factor.

Compared to untreated control animals, the animal receiving intravenous

MSC had significantly better scores on the Rotarod motor test and neurological severity test. Furthermore, MSCs migrated into the injured hemisphere where they formed oligodendrocytes and some neurons. The levels of nerve growth factor peaked on Days 5 and 8. Brain-derived nerve growth factor peaked on Day 8 after injury. Levels of basic fibroblast growth factor were not statistically different at any of the time points. In this model, the cells provided useful growth factors and eventually became functional cells in the damaged hemisphere. The increased level of these factors alone may partially explain the functional recovery seen in experimental models of TBI.¹⁵

Intraparenchymal Injection

Direct injection of stem cells has been the focus of recent research. In 2002 Riess et al.²² concluded that the injection of neural stem cells attenuates motor dysfunction but not cognitive dysfunction after TBI. In 2004 Shear et al.²⁵ directly injected neural progenitor cells into the ipsilateral caudate of injured cortex of rats 1 week after injury. Motor abilities improved as early as 1 week after transplantation and were still present at 1 year. The transplanted cells persisted as long as 14 months and remained in areas of the hippocampus and adjacent cortex. Interestingly, the transplanted cells labeled with NG2, a marker of oligodendrocytic precursors. Neural stem cells and progenitor cells still need to be harvested from embryonic, adult, or dead donors. However, nonneural sources of progenitor cells have also been injected.

MSCs have been used successfully to attenuate the effects of neural injury via intraparenchymal injection. Chen et al.⁷ found that MSCs actually elaborate neurotrophic factors that were measured in the study by Mahmood et al.¹⁷ MSC-derived neurotrophins had a protective effect on the native neurons. Again, nerve growth factor was the most significantly elevated factor and was responsible for the maintenance of cholinergic neurons. Thus, not only do the transplanted cells integrate into the

CNS, they also synthesize diffusible substances that protect uninjured tissue. Cholinergic neurons are partially responsible for memory formation and may play a large role in recall.

MSCs may fuse with neurons rather than undergo transdifferentiation.² Tissue replacement may play a role in this particular model although the maintenance of native cells via the elaboration of neurotrophins is becoming a more central focus in research.

Other nonmurine sources of stem cells have also been studied. Embryonic stem cells from cynomolgus monkeys have also been transplanted successfully into mice after brain injury.¹¹ Embryonic stem cells were preinduced by *in vitro* treatment with retinoic acid and differentiated into neurons. The cells were transplanted 1 week after injury. Mice were evaluated by the Rotarod test and the beam walking test. The scores of the mice receiving the transplants were significantly better than those of the mice without transplants. This study was the first to use nonhuman primates as a source of stem cells. Although the mechanism of injury was cryogenic, the idea of using a genetically similar but nonhuman donor might solve certain ethical problems. Nonetheless, other issues, such as the danger of transplant rejection, persist.

Mechanisms of Action

In the context of cellular transdifferentiation, stem cells act as a source of new neurons. Native stem cells in the subventricular zone contribute to brain remodeling, resulting in the familiar glial scar. Neurotrophins such as nerve growth factor are protective of cholinergic neurons and other neuronal populations. Phillips et al.²⁰ used immortalized embryonic rat hippocampus cells (HiB5) and transfected HiB5 cells that produced mouse nerve growth factor. The performance of both transplant groups on motor and cognitive tests improved significantly compared to control animals. However, the nerve growth factor-secreting transplants were associated with the least cell death in the hip-

pocampal CA3 region. Nerve growth factor may contribute to neuronal plasticity and regeneration and also may act as a suppressor of free radicals.

Conclusion

Although TBI remains a challenging clinical problem, therapeutic interventions based on the concepts of neural regeneration and repair hold promise. Further work is needed to translate such concepts from the bench to the bedside to ameliorate the effects of and to promote recovery from TBI.

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