Endogenous Repair Signaling after Brain Injury and Complementary Bioengineering Approaches to Enhance Neural Regeneration

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ABSTRACT: Traumatic brain injury (TBI) affects 5.3 million Americans annually. Despite the many long-term deficits associated with TBI, there currently are no clinically available therapies that directly address the underlying pathologies contributing to these deficits. Preclinical studies have investigated various therapeutic approaches for TBI: two such approaches are stem cell transplantation and delivery of bioactive factors to mitigate the biochemical insult affiliated with TBI. However, success with either of these approaches has been limited largely due to the complexity of the injury microenvironment. As such, this review outlines the many factors of the injury microenvironment that mediate endogenous neural regeneration after TBI and the corresponding bioengineering approaches that harness these inherent signaling mechanisms to further amplify regenerative efforts.

KEYWORDS: stem cells, traumatic brain injury, transplantation, controlled release

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Introduction: Traumatic Brain Injury

Within the United States, approximately 5.3 million individuals are affected by traumatic brain injury (TBI) annually,[1–3] and 43% of TBI survivors report having sustained disabilities 1 year after injury.[4] The incidence of TBI has been on the rise in recent years, with wars in Iraq and Afghanistan significantly contributing to their increased numbers.[2,5] As such, TBI accounts for an estimated $76.5 billion strain on US healthcare and economy each year.[6] Given the societal and financial expense, coupled with its increase in incidence, TBI represents a substantial public heath concern that has garnered public attention in recent years. This review will briefly touch on the general pathophysiological signaling after brain injury, and then specifically focus on injury-induced signaling, its relationship to endogenous regenerative efforts, and how bioengineering approaches may exploit the inherent signaling to better facilitate repair and regeneration after TBI.

Pathophysiology after TBI is dependent on several parameters related to how the injury was sustained (ie, focal vs diffuse, mild vs severe), leading to variability among injury phenotypes. However, the pathological progression of all injuries typically encompasses a primary injury due to an initial mechanical insult and a secondary injury that is a result of self-propagating cascades at the cellular and subcellular levels.[7,8] Primary injuries often lead immediately to contusion, laceration, and intracranial hemorrhaging at the tissue level and substantial neuronal death (by necrosis) at the cellular level.[5,9] The hemorrhaging and swelling induced during the primary injury contribute to increases in intracranial pressure and subsequently to limited cerebral blood flow, thus creating an ischemic injury microenvironment.[5,10,11]

Concomitant to ischemic injury, there is a significant cellular influx of Ca^{2+}, Na^{+}, and K^{+} ions,[11,12] where Ca^{2+} influx is thought to be primarily responsible for activation of reactive astrocytes in brain injury.[13] Activated astrocytes, together with activated resident microglial cells and infiltrating systemic leukocytes and macrophages, mediate neuroinflammation following TBI.[14–16] An understanding of how these cells mediate the mounted inflammatory response is critical to effectively develop intervention therapies for TBI. In relation to neural regenerative efforts, understanding how injury-induced inflammatory signaling directly or indirectly influences endogenous repair efforts (ie, adult neural stem cell recruitment) at various temporal and spatial points throughout the injury response is a first step toward developing effective bioengineering approaches targeting neural regeneration for TBI treatment. This review will detail the relevant physiological components of the injury-induced signaling milieu, including neuroinflammatory mediators, and how these factors play direct or indirect roles in the modulation of endogenous

neural stem recruitment. The second half of this review will explore two main bioengineering approaches aimed at further amplifying/modulating the inherent signals to tip the scales to further promote regeneration as opposed to degeneration. This review admittedly focuses on extracellularly present factors and cytokines that have been linked directly or indirectly to neural progenitor/stem cell behavior. For a more thorough and detailed overview of the pathophysiology of TBI and neuroinflammation, readers are encouraged to refer to other reviews.[17,18]

Endogenous Repair Response to Neural Injury and Its Mediating Factors

The capacity for endogenous repair within the adult central nervous system (CNS) has only recently been realized through discoveries of neurogenesis concentrated within regions identified as neural niches – the subgranular zone (SGZ), which lines the dentate gyrus within the hippocampus, and the subventricular zone (SVZ), which lines the lateral ventricle.[19–23] While alterations in both the SGZ and SVZ progenitor populations have been reported after TBI, this review largely focuses on the SVZ neural niche to highlight the effects of injury-induced signaling on adult neural progenitor/stem cell (NPSCs) populations.

The subventricular zone. The SVZ is closely approximated with vasculature,[24–26] where a single cell (type B cells) spans its width, extending processes to contact vasculature on one side and the lateral ventricle on the other.[26,27] Type B cells thus have access to both ventricular and vascular signaling, which is critical to niche maintenance.[23,27] Neural stem cell phenotype is thought to be more effectively maintained in close proximity to endothelial cells, and proliferation within the SVZ arises within 10–15 μm away from blood vessels.[25,28] Proliferation within the SVZ is typically observed in type C cells, the highly proliferative transit-amplifying cells that arise from type B cells and in turn will give rise to type A cells.[10,29] Type A cells are GFAP+ neuroblasts or NPSCs that exit the niche by migrating along the nearby vasculature. Under normal physiological conditions, migration occurs along the rostral migratory stream to the olfactory bulb where they become interneurons.[30] However, following injury, the fate of cells derived from the SVZ is altered.

NPSC response to neural injury. *Injury-induced changes within the niche.* Evidence suggests that the cells of the SVZ undergo proliferative and phenotypic changes following a neural injury, as the SVZ niche has been shown to increase in size, the total number of cells, and the number of proliferative cells.[31–34] It is thought that the type C cells are largely responsible for increased proliferation within the niche after injury.[33,35] However, Thomsen et al have recently proposed a nonproliferative mechanism by which the SVZ increases in size and total cell number in which injury-induced phenotypic subsets of SVZ cells dedifferentiate.[33] While findings of increased cell number and SVZ thickness after injury have been robust across the field, these injury-induced changes within the niche are complex, and there is still much about niche dynamics after injury that has yet to be understood.

NPSC recruitment to site of injury. Neural injury affects not only NPSCs within the niche but also those leaving the niche. Following injury, NPSCs stray from their normal physiological migratory route and home to the site of injury in a vasophilic manner.[31,34,36–39] While the signals driving this behavioral change are still being investigated, it is thought to be driven by chemokines and inflammatory factors secreted by activated cell types in the injury microenvironment.

Injury-induced factors that mediate the endogenous repair response. The active cell types of neural injury create a complex microenvironment through the secretion of a myriad of signaling factors that can facilitate and/or mitigate the injury progression (Fig. 1). These factors range from proinflammatory to neurotrophic in nature, and it is important to note that there is much interplay between signaling molecules, which further complicates their respective roles within the injury sequelae. The cell source and temporal and spatial expression patterns (Figs. 2 and 3) help to inform the nature of each signaling molecule's roles, specifically in mediating the behavior of endogenous NPSCs after injury (see Table 1 for breakdown of specific molecules and their effect on NPSCs). For the purpose of this review, several extracellular factors and cytokines that have been found to affect NPSCs were selected for discussion; however, numerous factors not discussed here, including critical transcription factors, can directly or indirectly affect NPSC behavior and it is important to acknowledge their effect on endogenous behaviors as well. For a more thorough review of signaling factors not described here, interested readers are encouraged to refer to recommended reviews.[40–42]

Stromal cell-derived factor 1α (SDF-1α). Increased expression of the chemokine SDF-1α has been observed within the injury penumbra within 24 hours after neural injury and persists out to 3 days before decreasing.[43,44] Both *in vitro* and *in vivo* data indicate that local increases in SDF-1α after neural injury are generated by reactive astrocytes within the surrounding tissue.[45–47] Unpublished data from our lab also indicate that SDF-1α protein levels peak within the injury penumbra following the controlled cortical impact (CCI) model for TBI at 1 and 3 days, with a decrease at 7 days and a return to baseline at 14 days.

There is compelling evidence that the chemokine SDF-1α plays a critical role in recruiting endogenous NPSCs to the site of injury in that the local SDF-1α source within the injury microenvironment is thought to be chemottractive to NPSCs leaving the niche.[44,46] NPSCs' chemotactic response to SDF-1α has been well characterized *in vitro*[48–50] and has been shown to work synergistically with the vascular basement membrane protein laminin to increase NPSC migration,[48] implicating its relevance to vasophilic mechanisms of endogenous NPSC recruitment after injury. Moreover, blocking the activity of the

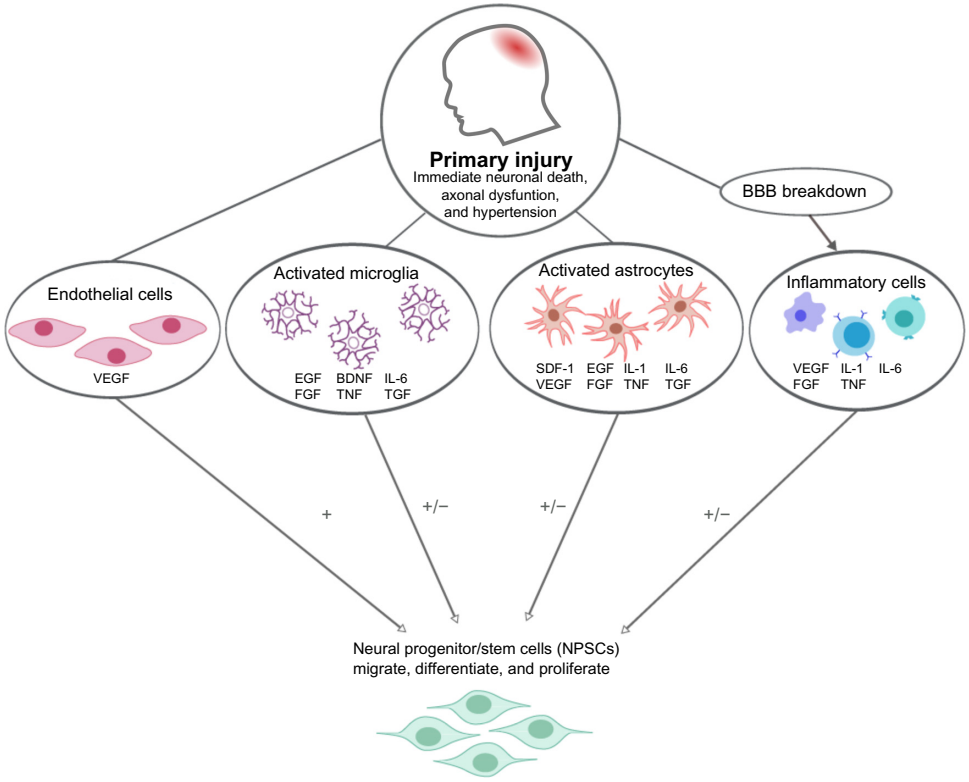

Figure 1. Schematic depicting the active cell types and their role in the pathophysiology of TBI.

SDF-1α receptor CXCR4 attenuated the migration of NPSCs to the injury environment following a stroke.[51]

SDF-1α may also play a role in increased NPSC proliferation observed within the SVZ niche after injury, as *in vitro* studies have shown that SDF-1α promotes NPSC proliferation.[48,50] However, this relationship has yet to be fully elucidated within the context of TBI.

Vascular endothelial growth factor (VEGF). Increased expression of VEGF has been observed in several models of TBI. Much like SDF-1α, VEGF secretion is associated with reactive astrocytes and endothelial cells within the injury penumbra; however, infiltrating inflammatory cell types also contribute significantly to early elevated VEGF levels.[52–56] Neutrophil-derived VEGF is elevated within four hours after injury and persists out to 2 days.[52,53] At approximately 1 day after injury, endothelial cells begin to contribute significantly to elevated VEGF levels within the injury penumbra, and their contribution persists out to 5 days after injury.[52] Between 3 and 7 days after injury, reactive astrocytes appear to secrete VEGF within the penumbra,[52,54–56] coinciding with macrophage VEGF secretion, which peaks from 4–6 days after injury.[52,54]

VEGF may be chemottractive to NPSCs after injury through both direct and indirect mechanisms. *In vitro*, VEGF has been shown to increase NPSC migration after direct stimulation[57] and to promote NPSC migration indirectly through endothelial cells and/or other growth factors.[58,59] The concept of indirect VEGF NPSC stimulation further

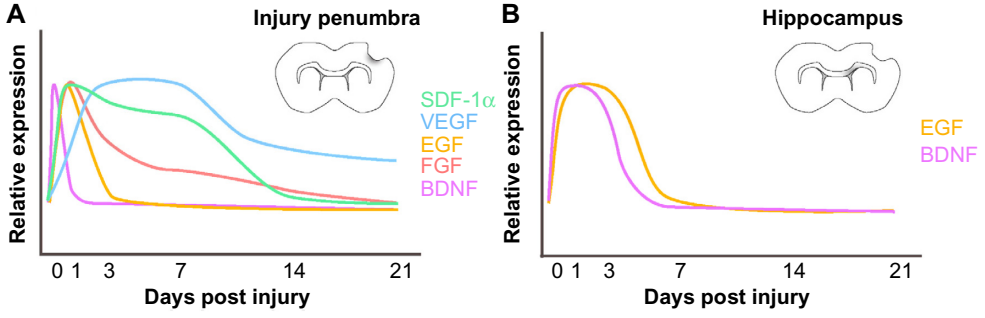

Figure 2. Temporal and spatial pro-regenerative signaling patterns following TBI. (**A**) Within the injury penumbra, expression increases for SDF-1α, VEGF, EGF, FGF, and BDNF in unique temporal patterns. (**B**) Expression of EGF and BDNF has also been observed to increase in the hippocampus after injury.

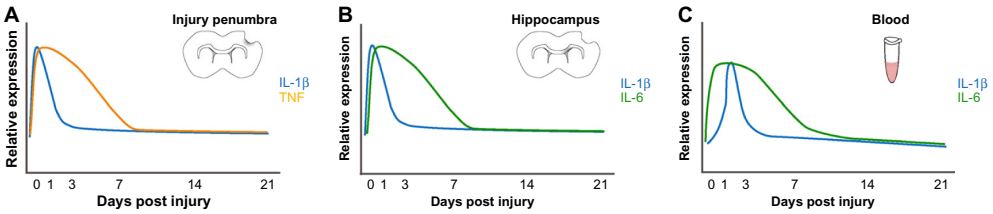

Figure 3. Temporal and spatial inflammatory cytokine signaling patterns following TBI. (**A**) TNF expression increases for 1 week after injury in the injury penumbra, while (**B, C**) IL-6 expression has been shown to increase in the hippocampus and blood for 1 week after injury. (**A**–**C**) A more acute increase in expression has been observed for IL-1β in the injury penumbra, hippocampus, and blood.

underlines the importance of the niche's close proximity to vasculature. Moreover, VEGF overexpression in transgenic mice has shown to increase NPSC recruitment to ischemic areas after a stroke.[60]

Much like SDF-1α, VEGF may also contribute to NPSC proliferation within the SVZ after injury. *In vitro*, both direct and indirect evidence of VEGF-mediated NPSC proliferation have been observed,[61,62] and Wang et al found SVZ proliferation after a stroke to increase in VEGF-overexpressing transgenic mice.[60] VEGF may not only promote proliferation but may also reduce apoptosis in NPSCs, thus contributing to increased SVZ size and survival after neural injury, as reduced NPSC apoptosis following a stroke within neurogenic regions (ie, SVZ, dentate gyrus, rostral migratory stream, olfactory bulb) was observed by Schänzer et al after VEGF intraventricular perfusions.[62]

Epidermal growth factor (EGF). Increases in EGF after neural injury are relatively short-lived, peaking within the first 24 hours in the injury penumbra, CA3, and dentate gyrus regions and returning to baseline levels by 3 days.[43,63] A more sustained EGF increase is observed in the hippocampus (CA1 region), increasing from 24 hours to 3 days and returning to basal levels by 7 days.[63] Early increases in EGF have been attributed to neuronal upregulation, while the sustained response in the CA1 past 24 hours has been attributed to glial cells.[63]

It is important to point out that, given the proliferative response to injury within the SVZ and the known mitogenic effects of EGF on NPSCs,[64,65] one might anticipate increases in EGF expression to spatially coincide with this proliferative zone. However, it has been proposed that rather than an increased level of EGF, TBI induces increased sensitivity of endogenous cell types to EGF signaling by upregulating its receptor, EGFR.[35,66] These data paint a complex picture when taken together with those of Thomsen et al, which describe a new lineage of EGFR+ neural stem cells that appear to arise from dedifferentiating neuroblasts after TBI.[51] Regardless of the mechanism by which NPSCs respond to EGF after an injury, studies in EGF knock-out mice have illustrated that EGF plays a critical role in promoting proliferation within the SVZ and in mitigating apoptosis within the SVZ and injury penumbra.[67]

Fibroblast growth factor (FGF). Increases in FGF have been shown to occur as early as four hours after injury and persist for 14 days following TBI in several models; however, increased FGF expression remains spatially restricted to the injury region.[68–72] Early upregulation of FGF is due to macrophages and microglia (four hours to 3 days), while late FGF upregulation originates from reactive astrocytes (7–14 days), alluding to its potential to play multiple critical roles during the endogenous repair response.[69,72]

Much like EGF, FGF is a well-characterized mitogen *in vitro*, where EGF and FGF are often used in combination to maintain NPSCs in culture.[65,73] Increased FGF following an ischemic insult has been observed to increase hippocampal NPSC proliferation *in vitro*, and this effect was attenuated in FGF knock-out mice *in vivo*.[74] Moreover, NPSC proliferation following injury was reduced to basal levels upon inhibition of FGF, indicating that even in the presence of other injury-relevant signaling molecules, FGF appears to play a critical role in regulating injury-induced NPSC proliferation.[75] In the same study, injury-conditioned media increased NPSC neuronal differentiation; however, FGF inhibition was not observed to significantly reduce neuronal differentiation,[75] suggesting that there are other factors that contribute to neuronal differentiation following injury. Nonetheless, FGF is not completely inactive in mediating neuronal differentiation, as studies have demonstrated its role in neurogenesis.[11,21]

Brain-derived neurotrophic factor (BDNF). While there is evidence in ischemic injury models of BDNF upregulation within the injury penumbra, increased BDNF within the injury region has not yet been observed in TBI models.[43,76,77] Following stroke, BDNF in the hypoxic core increased by two hours and remained elevated out to 3 days.[76,78] However, following a fluid percussion model of TBI, BDNF showed no significant increase within acute time points after injury.[77] The most robust increases in BDNF after TBI have instead been observed in the dentate gyrus and the CA3 regions of the hippocampus.[16,79,80] Hicks et al observed an ipsilateral increase in BDNF within the hippocampus 3–6 hours following a mild fluid percussion injury model (FPI) and found this trend to extend bilaterally after severe FPI in which BDNF increased at 1 hour and was sustained out to 72 hours.[79] While studies have been in agreement regarding hippocampal BDNF expression, increases within the injury penumbra appear to be largely dependent on the injury model, with more severe

Table 1. Studies implicating the role of the soluble factors and cytokines discussed in the modulation of NPSC behavior. ↑ indicates an increase in the respective variable, NE indicates that the signaling molecule had no effect on the respective variable, and ↓ indicates a decrease in the respective variable.

MOLECULE	LITERATURE SOURCE	MODEL	BRAIN REGION	EFFECT ON NPSCs				
				PROLIFERATION	DIFFERENTIATION	APOPTOSIS	MIGRATION	NEUROGENESIS	SURVIVAL
SDF-1α	39, 41, 43, 45	*In vitro*	SVZ	↑			↑		
VEGF	46–50; 51–56	*In vitro*, transgenic mice overexpression, intraventricular perfusion	SVZ	↑		↓	↑		
EGF	58, 59, 62	EGF knock-out mice	SVZ	↑		↓			
FGF	69, 70	*In vitro*, *in vivo*	SGZ; SVZ	↑	NE				
BDNF	77–83	*In vitro*, BDNF conditional knock-out mice		↓	↑				↑
IL-1β	92, 96	*In vitro*, *in vivo*, acute stress	SGZ, SVZ	↓/NE					
TNF-α	95, 106, 107	*In vitro*		↑/↓	↑/NE/↓	↑/↓	↑		
IL-6	114–117	*In vitro*, *in vivo*	SGZ, SVZ						↑/↓
TGF-β	129, 130	Transgenic mice overepression, stroke lesion	SGZ, SVZ	↑/↓				↑/↓	

models more likely to elicit a cortical BDNF response.[77,81] Regardless of location, BDNF appears to originate from granule cells and activated microglia.[77,80,81]

BDNF plays a critical role in mediating both the differentiation and survival of new neurons. Several studies *in vitro* have demonstrated that BDNF both suppresses the proliferation of undifferentiated NPSCs and promotes the neuronal differentiation of NPSCs.[82–85] Moreover, BDNF has been shown to promote the survival of new neurons,[86–88] a critical characteristic in the context of TBI in which endogenous NPSCs face a complex injury microenvironment upon recruitment to the lesion. Gao et al convincingly elucidated this critical role for BDNF after TBI using BDNF conditional knock-out mice in which the death of new neurons within the dentate gyrus was significantly increased compared to wild-type mice after injury.[87]

Interleukin-1β (IL-1β). A number of studies have recorded a significant increase in both IL-1β mRNA and protein in the injury site, penumbral region, and cerebrospinal fluid (CSF) within 15 minutes post injury in various TBI models.[89–92] IL-1β reportedly reaches maximum concentrations as early as 3–8 hours in CCI and moderate FPI,[90,91,93] and as late as 48 hours in an FPI model.[94] Regardless of the injury type, IL-1β is primarily secreted by activated astrocytes, macrophages, lymphocytes, and neutrophils.[95,96]

Both IL-1β and its receptor (IL-1R1) are expressed by NPSCs in the dentate gyrus of the hippocampus[97–99]; however, IL-1R1 has not been detected in progenitor cells derived from the SVZ.[100] In a murine model of acute stress, exogenous IL-1β decreased hippocampal cell proliferation in the SGZ; however, IL-1β had no effect on NPSC proliferation in the SVZ,[97] indicating interactions between IL-1β and NPSCs of the SGZ, but not the SVZ (ie, IL-1β may regiospecifically mediate NPSC proliferation). Additionally, both *in vitro* and *in vivo* experiments found that IL-1β inhibited the proliferation of NPSCs in a dose-dependent manner.[101] In a recent *in vitro* study, IL-1β was shown to directly inhibit rat hippocampal NPSC proliferation and neurosphere growth.[102] These data provide evidence for a direct, largely negative, and regiospecific effect of IL-1β on NPSC proliferation.

Tissue necrosis factor-α (TNF-α). Preclinical CCI and traumatic lesion models of TNF-α release have recorded measurable concentrations as early as 1 hour post injury, peaking at 2–4 hours, and declining thereafter.[91,103,104] Other CCI models measured cerebral lysate and CSF concentrations of TNF-α to increase from 3 to 6 hours post injury and peak at 24 hours.[104,105] TNF-α is generally localized near the injury penumbral regions,[92,106] although global TNF-α mRNA increase four hours after moderate and severe TBI has also been reported.[107] Regardless of injury type, TNF-α production primarily stems from activated microglia, astrocytes, and T cells.[108]

TNF-α signals via two distinct receptors: TNF-α receptor 1 (TNFR1), which is responsible for the pro-inflammatory

and pro-apoptotic functions of TNF-α; and TNF-α receptor 2 (TNFR2), which activates pro-growth and survival pathways as well.[109–111] Although the function of each receptor is well understood, the major functions of TNF-α in the brain has remained elusive, with groups recording conflicting information with respect to its effect on NPSC proliferation and differentiation. An *in vitro* study using adult SVZ NPSCs showed that TNF-α activated proliferation and inhibited differentiation.[112] Conversely, in a separate *in vitro* study using NPSCs derived from the striatum, TNF-α was shown to induce migration and inhibit NPSC proliferation, but had no effect on differentiation.[100] This inconsistency could be due to variation in cell type, concentration, or time of TNF-α treatment, or to the differential expression of TNFR1 and TNFR2. TNF-α may be an important factor in inflammation-induced death of NPSCs in the adult brain after an insult. In a model using LPS-activated microglial cells, TNF-α was found to significantly exacerbate hippocampal progenitor cell death.[113] However, dose-dependent effects of TNF-α on NPSC death and behavior have also been observed.[111] Experiments performed with murine organotypic hippocampal slice cultures demonstrated either apoptosis at high concentrations or neuroprotection at low concentrations of TNF-α.[111] These findings were later corroborated in a murine neonatal SVZ stem cell model, with low concentrations stimulating NPSC proliferation and differentiation.[111] These data provide the context to hypothesize that the function of TNF-α in general is dependent not only on the region in which it is acting (likely via specific TNFR binding) but also on the local concentration of TNF-α.

Interleukin-6 (IL-6). The temporal distribution of IL-6 after brain injury varies for both mRNA and protein depending on the brain region and model. For example, using the diffuse FPI model, IL-6 mRNA expression in the whole brain was increased after 4 hours,[107] while IL-6 mRNA expression in the hippocampus increased after 48 hours post injury in the CCI model.[114] Other studies found measurable levels of IL-6 protein in the first 2–4 hours post injury, which peaked at 8 hours (FPI, CCI, closed head injury model).[91,103] IL-6 also remained elevated in the impact region, CSF, and blood samples from 24 hours to 7 days post injury in multiple brain injury models.[90–92,94,103] In spite of the complex nature of IL-6 expression in TBI, this cytokine is primarily expressed by microglia, astrocytes, and T cells.[115] Together, this information implies a complex role for IL-6 in both chronic and acute TBI in various regions of the brain.

Although IL-6 has been largely classified as a pro-inflammatory cytokine, IL-6 has been associated with neuroprotection with respect to TBI,[15,116] as raised levels of IL-6 in the CSF post TBI have been correlated with improved outcome.[117] Nonetheless, current theory holds that acute exposure to IL-6 is detrimental for NPSC survival in the SGZ[118–120] and beneficial in the SVZ.[121] In general, these studies provide evidence of a complex role for IL-6 in the inflammatory milieu, which is highly dependent on the region, concentration, and cell type on which IL-6 is acting.

Other critical cytokines: Interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). In the acute injury phase, T cells, macrophages, and monocytes also secrete IL-10, an anti-inflammatory cytokine associated with better outcome after brain trauma.[122–124] In clinical studies, IL-10 is detectable in blood samples from patients who experienced severe TBI within the first few hours after trauma, with maximal concentration 3 hours post injury.[125–127] Although IL-10 has little effect on NPSCs directly, it plays an important role in modulating the injury microenvironment, which will be discussed in the next section.

TGF-β, another anti-inflammatory cytokine, is primarily produced by microglia and astrocytes and expressed in the hippocampus (dentate gyrus).[128,129] Preclinical ischemia models have found that TGF-β mRNA is significantly upregulated within the first day of injury, as early as six hours post injury, generalized around the lesion areas and mediated by glial cell activation.[130–132] Contrary to studies using ischemia models, a cryolesion model of brain injury found that TGF-β reached peak expression at 6 hours post injury and remained detectable for 1 week in the cortex and 12 hours to 1 week in the hippocampus,[68] suggesting a region-dependent expression of TGF-β.

Similar to IL-6, TGF-β has been primarily associated with neuroprotection with respect to TBI, but has altering functions depending on the region of the brain where the cytokine is acting. For example, studies in transgenic TGF-β overexpression and exogenous TGF-β infusion exhibited a potent decrease in NPSC proliferation and a decrease in the neurogenesis and astrocytosis in the hippocampus.[133,134] Contrary to these findings, in the SVZ, a stroke lesion model study of intranasally administered TGF-β reported a trend of augmented neurogenesis and proliferation.[135] Together, these studies provide evidence that TGF-β inhibits the proliferation and differentiation of NPSCs in the SGZ and provides a foundation to explore the action of TGF-β on NPSCs in the SVZ.

Cytokines influence NPSC behavior by modulating the injury microenvironment. The previous subsection focused on the direct effects that specific injury-induced soluble factors and cytokines may impose on NPSC behavior. This subsection explores the potential pathways by which the inflammatory cytokines that modulate injury microenvironment may indirectly affect NPSC populations. The interplay between the injury microenvironment and efforts for endogenous repair is indisputable and too complicated to begin to tease out thoroughly in this review. Here, a few key inflammatory cytokines are discussed, but this review admittedly only scratches the surface of this topic. Table 2 provides a summary of the effects that the cytokines discussed have on the injury microenvironment that may indirectly affect NPSC behavior.

IL-1β. IL-1β-mediated vascular remodeling plays an important role in NPSC migration and neurogenesis post

Table 2. Studies implicating the role of cytokines in modulating the inflammatory response.

CYTOKINE	LITERATURE SOURCE	MAJOR ROLES IN MODULATING THE INFLAMMATORY MILIEU
IL-1β	132, 133	– Breakdown and regeneration of BBB
	134–137	– Promotes astrogliosis or activates astrocytes
	136, 138, 139	– Activates various cell types to augment self and other cytokine production
	143–149	– Modulates expression of FGF, BDNF, and SDF-1α
TNF-α	138, 154	– Synergistically acts with IL-1β to stimulate growth factor production and cytokine gene expression
IL-6	155–158	– Promotes angiogenesis/vasculogenesis
IL-10	118, 120, 123,159, 160	– Inhibits TNF-α and IL-1β production
TGF-β	161, 162	– Inhibits TNF-α production
	163, 164	– Implicated in astrocyte activation and proliferation

trauma. IL-1β has a hand in both the breakdown[136] and regeneration of the blood–brain barrier (BBB).[137] IL-1β has the most profound effect on astrocytes, either promoting astrogliosis[138] and initiating an array of anti-neurogenic responses[139] or activating astrocytes and promoting angiogenesis, neurogenesis, and leukocyte infiltration.[140,141] The effect of IL-1β on astrocytes is potentiated by its concomitant action on microglial and endothelial cells; all the cell types together produce a variety of cytokines[140,142,143] such as IL-6, IL-10,[142,144] TNF-α,[145] and TGF-β.[146] IL-1β also modulates the expression of various growth factors such as FGF[147] and BDNF[148–151] and the chemokine SDF-1α.[152]

Specifically, in studies in LPS-stimulated macrophages and multiple sclerosis models, IL-1β was shown to significantly induce astrocyte production of SDF-1α.[152,153] Similarly, an *in vitro* study assessing the direct effect of various cytokines on glial cell secretion of FGF found that IL-1β increased FGF secretion in astroglia and microglia.[147] Furthermore, IL-1β has been associated with both downregulation of BDNF concentration and interruption of BDNF signaling pathways. Specifically, a study in rat hippocampal formation showed that the direct administration of IL-1β or LPS (which potentiates IL-1β) was sufficient to decrease the BDNF mRNA levels.[148] In more recent studies, Tong et al have implicated IL-1β with interrupting the neuroprotective properties of BDNF signal transduction by studying the direct effect of IL-1β on specific BDNF pathway proteins.[149,150] Although little research has been conducted on this relationship in TBI models, these data provide promising evidence that similar results would be found in different models.

TNF-α. Although TNF-α produces an array of outcomes for NPSCs in an inflammatory environment independently, it is well known that TNF-α and IL-1β are intimately connected in some aspects of neurological development and inflammation,[154,155] and have been shown to act synergistically in various cell culture models.[142,156–158] In particular, TNF-α and IL-1β synergistically stimulate growth factor production from murine astrocytes and human microglial cells.[142,158] In one study, IL-1β and TNF-α were shown to differentially induce cytokine gene expression, including IL-6, from endothelial cells when added alone, or synergistically when added together.[144]

IL–6. IL-6 has been linked to angiogenesis/vasculogenesis after brain injury, as it reportedly acts as a VEGF agonist.[159,160] Fee et al measured IL-6 mRNA expression in a cryolesion injury model for the developing brain, and found marked upregulation up to 4 days post injury and recession by 16 days post injury, in line with tissue regeneration timelines.[160] A handful of studies have similarly observed a positive correlation between IL-6 and increased endothelial cell proliferation *in vitro*.[161,162] Collectively, the influence of IL-6 on angiogenesis/vasculogenesis after brain injury may indirectly influence migrating/recruited NPSCs, which commonly employ vasophilic migration patterns to home to the injury penumbra.

IL-10. The role of IL-10 after brain injury can largely be classified as anti-inflammatory. An early study found time- and dose-dependent inhibition of both TNF-α and IL-1β in rat TBI models as a result of intravenous IL-10 administration,[124] whereas other studies corroborate this effect with both *in vitro* and *in vivo* models of LPS-stimulated secretion of TNF-α and IL-1β from microglia, macrophages, and leukocytes.[122,127,163,164] These findings were further corroborated in a study of IL-10 effects on LPS-activated microglia, where IL-10 inhibited the expression of both IL-1β and TNF-α and decreased the expression of the IL-6 receptor.[164] Together, these data provide evidence for the role of IL-10 as a powerful anti-inflammatory molecule and a foundation for research groups to pursue potential effects of IL-10 on creating a hospitable microenvironment for endogenous NPSC survival after TBI.

TGF-β. While TGF-β is primarily associated with neuroprotection in the injury environment, as it has been recorded to inhibit the production of pro-inflammatory cytokines in the injury environment, it has also been linked to stimulating reactive astrocytosis. Researchers have recorded TGF-β inhibiting induction of TNF-α from primary rat astrocytes at both the protein and the mRNA level.[165] More recently, TGF-β was reported to inhibit the production of TNF-α

and other inflammatory molecules from macrophages,[166] providing evidence that TGF-β may play an important role in ameliorating the injury microenvironment. In spite of these findings, TGF-β has been implicated in the activation of astrocytes using *in vitro* studies,[167,168] and further that TGF-β augmented astrocyte proliferation.[168]

Bioengineering Approaches to Modulate Endogenous Stem Cell Behavior After TBI

A myriad of bioengineering approaches have emerged attempting to manipulate the brain injury microenvironment to elicit more robust neural regeneration via providing exogenous support and/or stimulating the endogenous response. The two main bioengineering approaches are 1) stem cell transplantation and 2) controlled release of bioactive factors. It is important to note the potential crossover between these "exogenous"-derived therapeutic approaches and the innate endogenous repair signaling mechanisms discussed in the previous section.

Stem cell transplantation. One approach many have taken to enhance the endogenous repair response after injury is the introduction of exogenous stem cells. There are still many questions surrounding the mechanisms by which stem cell transplants may function to mitigate the secondary injury of TBI as well as the fate of these cells (ie, viability, phenotypic fate) after transplantation. In part, these questions arise from the modulation of many different transplant parameters and metrics of success in the literature. One of the parameters often modulated is the stem cell type, as both NPSCs and mesenchymal stem cells (MSCs) have been observed to differentiate into cells of a neural lineage under appropriate conditions.[73,164–174] For this reason, there have been numerous studies investigating the efficacy of NPSC and/or MSC transplantation following neural injury; several approaches to transplanting both NPSCs and MSCs will be discussed for the purpose of this review.

Bolus stem cell transplantation. Several critical parameters have been observed to influence the survival, phenotype, and functional benefits of bolus stem cell transplantation following TBI, including the cell type, injury model, severity, and transplant timing and location, among others. Studies in MSCs have indicated that they are capable of expressing neuronal lineage markers after transplantation into animals that have sustained a TBI[169–171] and that MSC transplantation may facilitate motor function recovery out to 1 month after injury.[169,171] However, concerns have arisen regarding the safety of transplanting MSCs into the brain, as MSCs have been observed to form masses that elicit a significant inflammatory response, whereas NPSCs transplanted under the same conditions did not form such masses.[172,173] Therefore, NPSC transplantation is appealing for both its perceived relative safety and the innate neuronal differentiation capacity of NPSCs.

Several studies have been performed using NPSCs to determine the effect of timing and location on transplant fate. Shear et al found that NPSC transplant survival after TBI was significantly higher at more acute time points after injury compared to the later time points, presumably due to glial scarring.[174,175] With respect to transplant location, transplant survival and migration into the surrounding tissue were significantly greater in the ipsilateral compared to contralateral hemisphere, in some cases, accompanied by greater motor and cognitive function recovery.[176,177] Taken together, these data illustrate some of the many factors that can modulate the therapeutic benefit of stem cell transplantation after TBI.

One common thread among the many bolus transplant studies is that very few transplants differentiate into new neurons (<5%).[174,176–179] Most studies have observed differentiation into GFAP + astrocytes to a greater extent than neuronal differentiation (~5–10%); however, many transplanted cells sustain undifferentiated/unidentified phenotypes.[169,178,179] These findings have led researchers to hypothesize that the benefit of stem cell therapy might lie in the trophic support that they provide to the local degenerating neurons.[180–182] As such, a line of research has emerged in which stem cell transplants are designed to capitalize on their capacity to provide trophic support to cells within the injury microenvironment.

Modified stem cell transplants following neural injury. In recent years, several groups have looked at either preconditioning or genetically modifying cell transplants to prepare them for the cytotoxic injury microenvironment and/or increase their capacity for trophic support. This is an appealing option, as cell transplants can theoretically be used to deliver neuroprotective factors while simultaneously providing the benefits of stem cell therapy. This approach has shown some promise, as BDNF-expressing NSPCs have been observed to enhance neuronal differentiation, synaptic plasticity, and the number of transplants retained in the lesion site after TBI out to 8 weeks compared to normal NPSCs.[183] These findings were accompanied by an improvement in motor function recovery at 7 days compared to normal NPSCs; however, this increase was insignificant by 4 weeks.[183] BDNF-expressing MSCs have also been shown to significantly improve neurological function compared to normal MSCs out to 90 days after a moderate TBI.[184]

Another approach has been to increase transplant sensitivity to the injury-relevant factors that have been shown to promote survival, proliferation, migration, and/or differentiation through overexpression of the appropriate receptor. For example, Wang et al observed attenuation of the inflammatory response after transplanting MSCs overexpressing CXCR4, the receptor for SDF-1α, into a lesion area after moderate TBI, and, interestingly, also observed local increases in VEGF and BDNF expression.[185] Similar work in stroke models has shown that CXCR4-expressing MSCs result in increased neuronal differentiation, migration into the host tissue, and improved neurological function.[186]

The method of "priming" transplants to encourage neuronal differentiation upon transplantation is yet another

modification that may improve transplant efficacy. Gao et al primed NPSCs by exposing them to laminin, heparin, and FGF for several days prior to transplantation, and observed a post-transplant population that was ~96% positive for early neuronal markers, a marked increase over previous work.[187] However, the functional integration and neurological benefit have yet to be determined for methods such as this.

Soluble signaling, such as neurotrophic factors and chemokines, is critically important, as evidenced by the promise demonstrated in these stem cell modification techniques; however, it is also critically important to consider the mechanical and integrin-centric signaling that transplants are exposed to. Therefore, another route taken for transplant improvement has been the development of scaffolds and novel neurotransplantation systems.

Scaffolds for stem cell transplantation following neural injury. Given the importance of both mechanical and integrin signaling in regulating cell behavior, much effort has been made to mimic native neural tissue in the construction of transplant scaffolds. While there have been some purely synthetic polymeric scaffolds, such as the woven poly(glycolic acid) scaffold used for NPSC transplantation by Park et al.[188], significant attention has been given to the incorporation of extracellular matrix (ECM) components within scaffolds to provide integrin signaling to transplants. Moreover, these scaffolds are frequently hydrogels, as they can be easily tuned to mimic the mechanical properties of native brain.[189,190] Chopp et al have extensively investigated the use of collagen I gels as transplant scaffolds for MSCs, and have found the scaffolds to increase transplant retention within the lesion site and migration into the host tissue, decrease the lesion volume, promote synaptic plasticity within the surrounding tissue, and promote cognitive function recovery when compared to bolus transplantation.[191–194] Guan et al have also found MSCs within collagen I gels to display increased viability and neurite outgrowth following transplantation into the injury microenvironment.[195]

While these data are promising, collagen I is not native to neural tissue, and, as such, other groups have looked to incorporating ECM components or binding motifs that are less foreign to NPSCs, such as laminin. Given the vascular nature of the SVZ, it is logical that NPSCs respond favorably to laminin substrates and, as mentioned previously, laminin has the capacity to promote neuronal differentiation of NPSCs.[48,187] Indeed, increased neuronal differentiation was observed in NPSCs transplanted in a self-assembling peptide gel modified with the laminin binding motif IKVAV.[196] Moreover, Tate et al found that NPSC transplants migrated further into the host tissue and displayed increased long-term survival when transplanted in collagen I gels modified with laminin compared to both collagen I only gels and collagen I gels modified with fibronectin.[197] Several other ECM-based hydrogels have been investigated *in vitro* for their capacity to promote various NPSC behaviors; however, their efficacy within the TBI microenvironment has yet to be determined.[198] While these approaches all rely on endogenous signaling events to elicit a response from exogenous stem cell transplants, another viable approach is the delivery of these critical signals as a means to enhance the endogenous stem cell response.

Bioactive factors to modulate endogenous NPSC activity. A cursory look at the pathophysiology of TBI indicates that increased bioavailability and modulation of specific signaling mediators may be exploited to regulate biochemical cascades linked endogenous repair mechanisms. As discussed earlier, the cellular mechanisms for endogenous neurotrophic support and neurogenesis exist even in non-neurogenic areas of the brain (ie, cortical tissue) after TBI or stroke. One major focus for tissue engineering/regenerative medicine is thus to modulate/amplify this innate capacity of NPSCs for directed, long-distance migration, ability to provide trophic support in the injury area, and capacity for neuronal differentiation and integration. A primary limiting factor for this approach, however, is the BBB, made of specialized brain microvessel endothelial cells (BMEC), pericytes, and glial cells such as astrocytes, which work to maintain exquisite control over all forms of molecular transport between blood and the CNS. The most common routes of delivery to the CNS (intravenous, intracerebroventricular, intracortical, intrathecal, and intranasal, Fig. 4) all subject proteins to one or more factors such as 1) rapid clearance from the serum/CSF, 2) degradation and/or loss of activity due to protein half-life, and 3) limited to no penetration of the BBB, especially of large proteins/peptides.[199] Moreover, for cases where the BBB is bypassed, diffusion is usually the rate-limiting means of transport with injected agents penetrating only in the order of millimeters (or less) from the source in the brain parenchyma.[200] As a result, maintaining an appropriate local concentration of a therapeutic agent over a desired time window is especially challenging in the CNS. Interested readers are encouraged to refer to referenced reviews for further details regarding the BBB and routes of entry to the CNS.[199,201,202]

Notwithstanding the above limitations, delivery of proteins/peptides, receptor agonists/antagonists, and soluble receptors have been proposed for a variety of applications such as Parkinson's disease and injury to the CNS. The cumulative data from such studies indicate that exogenous delivery of bioactive components can 1) elicit desired biological responses in the CNS and 2) produce positive therapeutic outcomes as measured by histological and/or behavioral outcomes. This section will focus on individual soluble factors and summarize their biochemical effects following delivery into the CNS after injury.

SDF-1α. In the context of brain injury, exogenous SDF-1α delivery has been largely focused on angiogenic alterations and not direct evaluation of endogenous NPSC recruitment. For example, intracortical delivery of SDF-1α after lateral FPI in a rodent model showed some efficacy in inducing angiogenesis.[203] Additionally, blocking SDF-1α/CXCR4

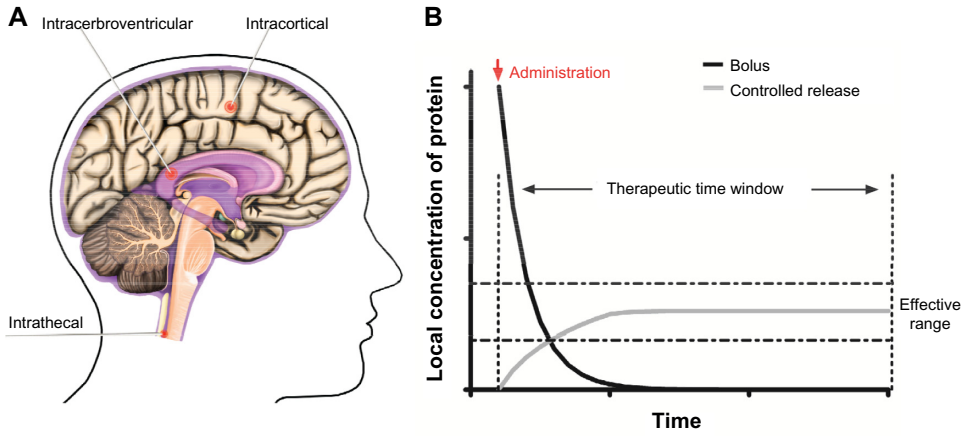

Figure 4. The route of delivery to the central nervous system plays a critical role in determining the spatial and temporal distribution of infused agents. (**A**) Demonstrates conventional means of bypassing the blood–brain barrier, which includes the intracortical, intracerebroventricular, and intrathecal routes. Each route of delivery has its own strengths and weaknesses, and thus outcome of therapy depends heavily on proper selection of the means of administering the therapeutic agent/construct. Intrathecal injections are made directly in the subarachnoid space of the spinal cord, whereas intracerebroventricular and intracortical injections refer to infusion of drugs directly into the ventricles or into the cortical interstitium, respectively. Efficiency of drug accumulation in the CNS is very low, even in the cases where the blood–brain barrier is bypassed. This is especially a challenge for applications where high drug concentrations are required in a specific portion of the brain. (**B**) Bolus injections of a therapeutic have rather transient effects with minimal time in the therapeutic threshold window; however, (idealized) controlled release of bioactive molecules may achieve sustained biochemical effects throughout the therapeutic time window.

signaling through injection of a soluble antibody significantly decreased microvessel density and aggravated functional outcome relative to both vehicle and SDF-1α treated groups.[204] In a different study, intracortical administration of soluble SDF-1α 24 hours post TBI led to a lower degree of BBB disruption, lower expression of pro-inflammatory cytokines, and attenuated neuronal apoptosis in the injury penumbra.[205] However, the authors did not specifically look at histological data or behavior of NPSCs, or determine whether the therapeutic intervention also led to a functional benefit.

A growing body of evidence supports the therapeutic efficacy of prolonged SDF-1α delivery for various applications such as wound healing,[206,207] skeletal regeneration,[208,209] and myocardial infarctions.[210,211] These studies demonstrate the viability of recruiting progenitor cells through exogenous infusion of SDF-1α. However, there is a lack of studies that specifically elucidate the *in vivo* effects and feasibility of local sustained release of SDF-1α in the context of neural injury. A number of devices have been proposed that hope to achieve controlled release of SDF-1α over different time periods, which include SDF-1α-loaded star PEG-heparin hydrogels,[212] poly(lactic-*co*-glycolic)acid (PLGA) microparticles,[213] chitosan-tripolyphosphate-based nanoparticles,[214] poly(lactide ethylene oxide furmate) hydrogels,[215] and a composite of gelatin/dextran and poly(*N*-isopropylacrylamide)-based stimuli-sensitive hydrogel.[196] The above systems have been characterized *in vitro* and seem to maintain bioactivity of encapsulated SDF-1α to varying degrees.

VEGF. Multiple studies have investigated the effects of VEGF infusion after TBI. For example, continuous infusion of VEGF in the lateral ventricles in murine models has shown significant neural and angiogenic effects.[216] Specifically, VEGF administration significantly reduced lesion size, increased SVZ cell proliferation, increased microvessel density, and increased proliferative cell markers in the perilesion area.[217] Another similar study for VEGF delivery to the lateral ventricle after ischemic insult found similar effects on infarct volume and angio/neurogenesis in the injury area.[218] However, the mechanism(s) involved in increased bioavailability of VEGF leading to functional recovery is still unclear. Some evidence suggests that VEGF infusions help in promoting the survival of immature neurons rather than directly affecting the proliferation of neuroblasts.[219]

Other means of VEGF delivery include the intranasal route, which can significantly increase concentrations in the olfactory bulb, frontal cortex, and thalamus when compared to intravenous injections.[220,221] In order to garner sustained local release of VEGF, Emerich et al utilized alginate hydrogels and tested its effects on a rodent model of cerebral ischemia.[222] The authors determined that *in vivo* VEGF concentration levels were undetectable in the striatum 10 hours after bolus VEGF injection, compared to ~100 hours for animals receiving alginate +VEGF. As a result, measured lesion volumes were lower, and functional tests showed significant improvements relative to stroke + bolus VEGF and stroke-only controls. Other studies have demonstrated the feasibility of VEGF release in Huntington's disease[222] as well as gene transfer for cerebral ischemia[223] and Parkinson's disease.[224]

FGF. Infusion of FGF both subcutaneously and through the intracerebroventricular route has been reported to increase the proliferation of NPSCs in the dentate gyrus and the SVZ,[225,226] suggesting some ability for FGF transport through

the BBB and the ependymal lining.[227] In an attempt to increase the efficiency of intravenous transport to the CNS, Wu et al described a biotinylted FGF molecule covalently modified with a monoclonal antibody for the transferrin receptor (TfR).[228] This "Trojan Horse" strategy usually targets the TfR and undergoes receptor-mediated transport to the brain parenchyma.[228] In this case, brain uptake was increased by a factor of 5, although total uptake was only about 0.05% of the total injected dose. Yet, the increased bioavailability of FGF [after middle cerebral artery occlusion (MCAO) in rodent models] was associated with a decrease in infarct volume and correlated with significant improvements in functional outcome relative to vehicle and nonconjugated FGF.[229] However, a limited amount of evidence exists for long-term functional benefits using this strategy. Intracerebroventricular infusion of FGF over 7 days after FPI in rodent models led to cognitive recovery through significantly increased proliferation and neurogenesis in the SVZ and the SGZ.[230] Some studies[230,231] have reported cognitive improvements in the absence of significant neuroprotective effects on existing neurons in both fluid percussion and focal injury models. On the other hand, contradicting results from Dietrich et al showed decreased lesion volume, indicating neuroprotection, after intravenous administration of FGF.[232] More studies are needed to elucidate the relationship between neuroprotection and increased bioavailability of FGF. Additionally, FGF has also been correlated with augmented neurogenesis in the hippocampus and the SVZ following infusion in aged rodents.[233]

As opposed to bolus injections, devices that achieve controlled release of FGF have also been characterized. Controlled release of FGF leads to higher proliferation and phenotypic preservation of NPSCs cultured *in vitro* as well as lower sensitivity to oxidative stress, and thus a lower propensity to undergo apoptosis.[234] Chitosan microspheres covalently modified with heparin were synthesized that electrostatically immobilized FGF in the matrix, which exhibited increased NPSC cell attachment relative to groups with no FGF.[235] Devices proposed for controlled release for FGF include poly(lactic-*co*-glycolic)acid nanospheres and[236] high-density collagen,[237] as well as induced FGF expression through adenoviral transfection.[238]

EGF. Effects of EGF on neural cell types have demonstrated enhanced survival, proliferation, and differentiation of neural precursors into neurons and astrocytes *in vitro*.[65] More recently, *in vivo* data for infusion into the ipsilateral lateral ventricle after FPI indicated that the EGF-administered groups had higher proliferation rates in the SVZ, SGZ, and the hilus, in addition to affording neuroprotection for existing neurons relative to the vehicle.[239] However, unlike FGF infusion,[230] EGF administration for 7 days failed to show any long-term increase in NPSC proliferation in the dentate gyrus. Histological studies indicate preferential differentiation of NPSCs toward an astroglial cell fate in the dentate gyrus and the SVZ.[240] Regardless of the rather transient effects of EGF seen in this model, the authors still report a significant improvement in functional outcome. *In vitro* results for NPSCs exposed to EGF also support the results in the above study, in which increases in NPSC proliferation and preferential differentiation toward astrocytes were observed.[241] However, another study showed controlled release of EGF from a hyaluronan and methylcellulose hydrogel after cortical ischemia led to a higher number of NPSCs differentiating into neuronal precursors relative to stroke-only controls in the SVZ.[242] This may be an example of how the delivery method of therapeutics, in addition to the injury model, can measurably change the biological response.[240] Other types of EGF delivery include an adenoviral-mediated gene delivery of heparin-binding EGF (HB-EGF; member of the EGF family), which increased NPSC proliferation and the extent of neurogenesis observed after cerebral ischemia.[243]

There is also substantial evidence that EGFR antagonism has possible therapeutic benefits.[244] After injury, EGFR is upregulated primarily in astrocytes, and upon interaction with its ligands (EGF, TGF-α and others) can activate the process of astrogliosis.[244,245] Results from a TBI (CCI) study indicated increased neuroprotection, attenuation of astrogliosis, and decreased levels of IL-1β after administration of simvastatin.[246] Simvastatin inhibits the formation of lipid rafts (essential for EGFR function) but does not specifically inhibit EGFR function. Specific EGFR reversible and irreversible antagonists have also been studied in the case of spinal cord injury[203,247] and optic nerve regeneration,[248] and shown inhibition of astrogliosis, downregulation of pro-inflammatory cytokines, and improved functional outcome.

BDNF. Pencea et al studied the effects of exogenous BDNF introduced through intracerebroventricular infusions into the lateral ventricles in rodent models.[200] The investigators reported increased proliferation in the SVZ and striatum lining the lateral ventricle as well as in the thalamus and the hypothalamus regions lining the third ventricle. In all cases, significant increases in proliferation compared to saline infusions are not seen beyond ~1.8 mm away from the ventricle lining. In addition, the density of newly formed cells also has a direct correlation to the number of TrkB+ (receptor for BDNF) cells in the vicinity. Interestingly, the authors reported a lack of colocalization between proliferating and TrkB+ cells. This may indicate that BDNF has limited direct effects on NPSCs, and, rather, paracrine signaling from surrounding cells may play a more significant role. It is important to note, however, that internalization of the TrkB/BDNF receptor–ligand complex is implicated in initiating the intracellular cascade in neurons.[249] Thus, low TrkB immunopositivity does not necessarily implicate a lack of sensitivity to BDNF. After BDNF infusions, a significant increase in neuronal cell commitment was seen only in the hypothalamus region. Adoption of astroglial cell fate did not change significantly in any of the brain regions relative to saline controls. Although this study showed promising results in terms of increasing cell

proliferation, the invasive process of placing a cannula to reach the ventricles produces an injury itself and leads to a biochemical response.[250] Moreover, the limited regions of the brain that can be accessed through intracerebroventricular infusions are still a challenge that needs to be overcome.

More recently, the effects of continuous release of BDNF have been proposed and evaluated both *in vitro* and *in vivo*. Huang et al investigated the use of collagen hydrogels as a means of controlled delivery *in vitro*.[251] Although the device had a cumulative burst release of about 50%, NPSC viability and proliferation rates were significantly higher in the BDNF-loaded gel group for all time points evaluated (1, 4, and 7 days) relative to collagen by itself. Sustained release of BDNF (relative to supplementation in soluble form) also affected NPSC phenotype, which showed a significant bias toward neurons rather than astrocytes after 1 week, suggesting that the temporal concentration profile of BDNF can be used to modulate NPSC behavior. Controlled release using nano-scale, semi-aligned poly ε-caprolactone (PCL) fibers loaded with a short BDNF-mimetic ligand attempted to physically intercept and redirect NPSCs migrating on the rostral migratory stream (RMS).[252] The authors found that PCL, with and without the BDNF mimetic, allowed a significant increase in NPSC migration into the injury tract (created by the injection needle) at 8 days post injury. However, at 21 days, significant increases in precursor cell population in the injury tract were observed only in the PCL + BDNF-mimetic group. It is important to note that addition of the BDNF-mimetic protein did not improve the depth of NPSC infiltration into the injury tract regardless of time. In a similar study, the orientation of the PCL nanofibers was found to affect NPSC proliferation and differentiation, indicating both physical and chemical cues can be used to modulate NPSC behavior.[253] Other types of delivery devices/systems have also been proposed, which include delivery through the intranasal route [254] and sustained release form PLGA-poly(L-lysine)-PEG microspheres,[255] as well as delivery of adenoviral vectors carrying the gene encoding BDNF.[256]

Delivery of multiple growth factors. Co-delivery and/or orchestrated delivery of bioactive factors is an idea that is gaining traction in the field due to its potential for inducing an enhanced functional recovery, in some cases, through a synergistic effect. For example, Kojima and Tater report that co-delivery of EGF and FGF-2 intrathecally after a spinal cord injury can increase proliferation rates and migration of ependymal cells (which can give rise to NPSCs), whereas delivery of either of the factors alone cannot elicit the same response.[257] Additionally, a collagen-based controlled-release device, also designed to deliver the EGF and FGF-2, reported similar findings after spinal cord injury.[237] Many of the neurotrophins mentioned in this review act to upregulate a similar array of intracellular cascades, and thus it can be challenging to select complementary sets of signals to acquire an additive or synergistic therapeutic outcome. For example, a cocktail of VEGF and FGF delivered to the lateral ventricle after TBI did not appear to provide significantly more benefit than delivery of VEGF alone.[216] Specifically, the VEGF-only group was able to increase angiogenesis and the number of neuronal precursors around the injury area to a greater extent than the VEGF + FGF-treated groups. Additional studies looking at various neurotrophin co-deliveries as well as hydrogel/neurotrophin complexes indicate feasibility in the CNS after injury; however, there is still much to be understood regarding the complex interactions between neurotrophins after a neural injury.[258–260] Interested readers are encouraged to refer to the review by Lee et al for more information.[261]

Summary

Although the administration of injury-relevant signaling factors has long been assessed for its potential to promote neuroprotection and neuroregeneration for a wide variety of CNS conditions, as of now there are no commercially available solutions. Common pitfalls include rapid enzyme-mediated loss in bioactivity, nonspecific adsorption to serum proteins and tissues, as well as the inability to maintain therapeutic concentration levels in the brain regions specific to a given pathology throughout a desired time window. Indeed, the focus in protein therapeutics is beginning to shift toward developing delivery devices that can preserve protein bioactivity and increase its bioavailability in an efficacious, predictable, and controlled fashion.[262] Moreover, such devices can reduce the total dosage of drug required and thus avoid unwanted side effects.[263] As mentioned earlier when discussing EGF delivery, there is evidence that the same signal, when presented differently (spatially and/or temporally), can affect the biochemical response.[240,259,261] However, there is a paucity of studies looking specifically into uncovering these mechanisms, which could be key to developing efficient means for protein delivery for specific applications.

Another important consideration is the duality in function of the majority of signaling mediators after injury.[264] For example, the anti-inflammatory signal, IL-6, on one hand, inhibits TNF-α and stimulates angiogenesis, but, on the other, upregulates chemotactic signaling and adhesion molecule production that promotes recruitment of monocytes from the systemic circulation.[265,266] Another example is SDF-1α, which is a potent chemotactic agent important in regulating the endogenous regeneration after injury but also has tumorogenic potential and has been correlated to neuropathic pain.[267] As a result, a candidate protein must be chosen very carefully in order to elicit the desired response. Otherwise, a substantial improvement in long-term therapeutic outcome is unlikely due to direct and indirect interactions, which can be difficult to predict in such a complex system.

Many of these concerns are also reflected in the future of stem cell therapies for neural injury. As illustrated in this review, there are many parameters that govern the efficacy of stem cell transplantation in mediating repair (ie, cell type,

injury, transplant location and timing, cellular modifications, scaffolding, etc). Therefore, it is critically important to use the knowledge available regarding temporal and spatial signaling patterns after injury to inform future work in developing stem cell therapies for neural injury so as to have a better command of the driving forces behind their outcomes. Bringing together these many moving parts will move the field forward in a more productive and meaningful fashion.

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Author Contributions

Wrote the first draft of the manuscript: CPA, AR, DD. Contributed to the writing of the manuscript: CPA, AR, DD, SES. Agreed with manuscript results and conclusions: CPA, AR, DD, SES. Jointly developed the structure and arguments for the paper: CPA, AR, DD, SES. Made critical revisions and approved final version: CPA, AR, DD, SES. All authors reviewed and approved of the final manuscript.

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