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Using Biomaterials to Modulate Chemotactic Signaling for Central Nervous System Repair

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Abstract

Chemotaxis enables cellular communication and movement within the body. This review focuses on exploiting chemotaxis as a tool for repair of the central nervous system (CNS) damaged from injury and/or degenerative diseases. Chemokines and factors alone may initiate repair following CNS injury/disease, but exogenous administration may enhance repair and promote regeneration. This review will discuss critical chemotactic molecules and factors that may promote neural regeneration. Additionally, this review highlights how biomaterials can impact the presentation and delivery of chemokines and growth factors to alter the regenerative response.

1. Chemotaxis: Basic Overview

Dating back to 1891, Theodor Leber first observed the prominent leukocyte migration from the blood vessels in response to certain chemical substances. These seminal experiments discovered active, directed leukocyte migration through the vasculature towards the location where certain chemical substances were introduced [1]. For example, rabbits exposure to mercury at finite locations intravascularly yielded infiltration of leukocytes, yet such response was completely absent with saline exposures [1]. In a follow-up experiment, researchers injected various chemicals intra-abdominally in frogs and observed selective leukocyte infiltration with alkali albuminate, but not with urea [1]. Leber first coined this selective migration phenomena as chemotaxis [1]. Since these seminal experiments, chemotaxis has been implicated in several biological processes such as inflammation, stem cell recruitment, development, and tumor progression. Admittedly, we cannot cover all aspects of chemotaxis thoroughly and therefore the focus of our review centers on chemotactic cell recruitment in the context of central nervous system (CNS) injury repair. More in-depth overview on other chemotaxis processes can be found in previously published reviews [2–5].

Chemokines, or chemoattractant ligands, are small proteins that bind to G-protein coupled receptors (GPCR; also known as seven-transmembrane domain receptors) and activate the phosphatidylinositol 3-kinase (PIP3) and phospholipase A₂ (PLA₂), or rapamycin complex 2 (TorC2) intracellular signaling pathways to subsequently induce cellular movement [2].

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Chemokines are divided into four groups based upon their structure. The four groups are named C, CC, CXC, CX₃C. Classification of a specific receptor or ligand is based upon which group or family it belongs followed by the letter L or R, denoting ligand or receptor, and lastly a unique number [3]. It is important to note that one chemokine receptor may engage different chemotactic ligands, for example CXCL12 binds to both CXCR4 and CXCR7 [3, 4]. The binding of these ligands to their receptors leads to movement via directed cell migration along a chemical concentration gradient. Although not labeled as classic chemokines, neurotrophic factors and growth factors may also employ a gradient mechanism to direct cell migration and thus will be included in this review [5–8]. Cells use temporal and/or spatial sensing to determine a chemical gradient and the direction/location to migrate [9, 10]. Temporal sensing describes the method whereby the cell compares the number of bound receptors over time as the cell moves in any direction. In contrast, spatial sensing utilizes receptors distributed across the cell perimeter and correlates bound receptor location on the cell body to movement in a particular direction [11]. Temporal sensing tells the cell whether or not it is moving with a concentration gradient as it travels while spatial sensing allows for the cell to compare concentrations of ligands around the cell body and have a more directed movement towards the gradient [11]. These mechanisms allow directed cellular recruitment to discrete regions facilitating development, repair, inflammation, or disease processes [9, 12–15].

Engagement of a chemokine ligand with its corresponding GPCR leads to a conformational change and activation of a G protein subunit [12]. Once bound, intracellular signaling cascades induce F-actin polymerization and activation of phosphatidylinositol 3-kinase (PI3K) [13, 15]. The newly created F-actin helps guide PI3K and its products, PIP₃, to the cell membrane, or leading edge, of where the chemoattractant has bound [13, 16]. The local accumulation of PIP₃ amplifies the chemoattractant signal intracellularly by creating a steep gradient of PI3Ks to one region of the cell based upon the small difference in bound receptors around the cell body [13, 15, 16]. The F-actin concentrated to the leading edge then protrudes and creates a polarized cell [15]. Rho family proteins then tune the migration by controlling protrusion and adhesion of the leading edge, and detachment of the non-leading portions [15]. Ras family proteins are able to initiate a positive feedback loop through PI3K activation directly at the leading edge upon receptor binding and further enhance the PIP₃ gradient intracellularly (Fig. 1A) [15]. In addition to this dominate pathway, two other intracellular pathways, TorC2 and PLA₂, elicit cytoskeletal rearrangement and chemotactic cell migration. While additional studies are needed to fully elucidate these pathways, evidence supports the notion that the TorC2 pathway (Fig. 1B) works independently of PIP₃, whereas the PLA₂ pathway works in parallel to PIP₃ [13, 14]. In summary, the GPCR transmembrane receptor binds chemokines to initiate multiple signaling pathways that result in directed cytoskeletal reorganization in response to a chemotactic gradient.

2. Chemotaxis in the Central Nervous System

Chemokine ligands, growth factors, and their receptors are a critical part of the development, maintenance, and repair process of the CNS. Developmental events mediated by chemokine ligand and receptors include formation of the cerebellum [17–19], spinal cord [20], cortical

layers [21], and hippocampus [22]. For example, developing cortical interneuron migration is regulated by CXCL12 and receptor CXCR4 [23]. A CXCL12 gradient attracts interneurons to form a migratory stream traveling to the marginal zone (MZ) [23]. In addition, the tangential migration of interneurons out of the MZ and into the appropriate cortical layer during development coincides with the loss of responsiveness to CXCL12 [23]. Exit of interneurons from the MZ can be replicated *in vivo* by elimination of CXCR4 expression on interneurons, suggesting the ligand-receptor interaction strongly regulates interneuron placement [23]. Further examination of the chemoattractant effects of CXCL12 on interneurons has shown that CXCL12, when ectopically expressed in the cortex during development, leads to the irregular interneuron distribution due to local accumulation at the region of expression [23]. The role of chemotaxis during development is also apparent in other CNS regions. Specifically, secretion of the chemokine CXCL12 by meningeal cells during cerebellar layer development guides neural stem cells to the external granule layer (EGL), while no effect on internal granule cells is observed (Fig. 2A) [18]. This selective response is due to CXCL12 secretion by the meningeal cells in the EGL and homing of neural stem cells expressing the corresponding receptor (CXCR4) while the subpopulation of post-mitotic granule cells that lie below the proliferative region of the EGL do not express CXCR4 and remain in the internal granule cell layer [18]. Further, mice deficient of CXCL12 have abnormal cerebellar layers with reduced thickness of the EGL [17]. Additional studies have suggested that brain derived neurotrophic factor (BDNF) also mediates cerebellar granule cell layer development as migration of cerebellar granule cells out of the EGL is significantly reduced in BDNF knockout mice [19, 24]. Altogether, these developmental events demonstrate the necessity for growth factors and chemokines to direct cellular organization and maturation during development in the CNS.

Secreted factors and chemokines not only attribute to development, but also play a major role in the maintenance of the CNS. For example, the subventricular zone (SVZ) of the adult brain contains progenitor cells that migrate along the rostral migratory stream (RMS) to the olfactory bulb to create new interneurons (Fig. 2B) [25]. While several migratory cues work together to direct the movement of the neural progenitor cells, we will only touch on two prominent molecules as they have direct roles in chemotaxis. In rodents, a concentration gradient of the protein Slit acts as a chemorepulsive, repelling neural progenitor cells from its area of highest concentration, the SVZ, to the olfactory bulb along the RMS [26]. In addition, vascular endothelial growth factor (VEGF) secretion from the olfactory bulb has been reported as a chemoattractant that further enables homing of neural progenitor cells to the olfactory bulb [27]. The Slit and VEGF directed migration of progenitor cells through the RMS exemplifies the ability chemokines possess in replenishing cells of the CNS. Interestingly, these progenitor cells of the RMS also express CXCR4 and thus are capable of directional migration outside of the RMS in response to the chemotactic ligand, CXCL12 [28, 29]. Neural progenitor populations may be able to respond to other chemokines as Tran et al. reported the expression of multiple chemokine receptors that correspond to CXCL12, CCL25, CCL27, CCL5, and CCL8.[30] As eluded to above, SVZ progenitor cells use similar chemotactic mechanisms to respond to injury-induced chemotactic signals [31]. Gordon et al. used retroviral labeling techniques to eloquently demonstrate that quinolinic acid-induced striatal loss led to an increase in neural progenitor cell proliferation in the SVZ

followed by cell migration to the striatum [32]. The migration of SVZ cells to the lesion area was suggested to be due to microglial secretion of CXCL12 and CCL2, as previous studies showed that quinolinic acid upregulated expression in astrocytes [32, 33]. These results are supported by another study, where a cortical lesion led to increased proliferation of SVZ cells and increased expression of CXCL12 near the lesion followed by migration of SVZ cells to the lesion area (Fig 1C) [34]. When CXCR4 was blocked by AMD3100 antagonist, there was a significant reduction in the number of neural progenitor cells that migrated to the lesion alongside astrocytes [34]. Collectively, these examples begin to reveal the critical importance of chemotaxis in the CNS for controlled and coordinated cellular proliferation, migration, and differentiation during development, maintenance, and injury.

In the context of CNS injury or disease processes, many studies have reported transient and insufficient effects of secreted chemokines within an injury or that a diseased microenvironment may inhibit chemotactic repair [32, 34–37]. Therefore, it is not surprising that researchers have turned to tuning chemotactic signaling mechanisms to control the recruitment of cellular players for CNS repair. Here in this review, we will take the opportunity to first highlight discovered chemokines and factors essential for CNS repair. Then, we will discuss biomaterials-based strategies employed to tune different chemotactic signals.

3. Chemokines for CNS Repair

3.1. CXCL12/Stromal Cell Derived Factor-1 α (SDF-1 α)

CXCL12, SDF-1 α , is a chemokine that plays pivotal roles in neural development, induction of stem cells to neuronal fate [38], neural progenitor cell migration [30, 39], initiation of neurite growth and neurogenesis [40, 41], inflammation [42], and cancer cell migration and tumor growth [43]. In the context of CNS injury, CXCL12 is upregulated suggesting a key function in neural repair [35, 40]. Specifically, CXCL12 has been directly correlated to chemotactic migration of neural progenitor cells (NPCs) from neurogenic niches *in vivo* [39, 44]. This chemotactic response has been confirmed with controlled *in vitro* experiments demonstrating that NPCs increase proliferation and chemotaxis in a dose-dependent manner when exposed to CXCL12 gradients [45–47]. Additionally, Barkho et al. reported that CXCL12 significantly increased neuronal and astrocyte differentiation of adult neural progenitor cells *in vitro* [48]. Based on these studies that demonstrate CXCL12 as a potent chemotactic signal, it is not surprising that CXCL12 is a target therapeutic mechanism for promoting CNS repair and regeneration.

Recent therapies utilizing CXCL12 include both direct exogenous delivery of CXCL12 and also exploiting endogenous secretion of CXCL12 within regions of neural injury as a homing mechanism for transplanted cells. For example, Filippo et al. demonstrated that intracortical administration of an N-terminal CXCL12 peptide increased SVZ neuroblast migration from the SVZ niche towards regions of neural injury following traumatic brain injury in mice [39]. The CXCL12/CXCR4 signaling axis has been used as a homing mechanism for a number of transplantation studies. Our group reported an increased chemotactic response to CXCL12 when neural progenitor cells were transplanted within hyaluronic-acid (HA) hydrogel that increased CXCR4 expression [49]. In a mouse kainic

acid seizure model, Hartman et al. demonstrated that there was upregulated CXCL12 in the dentate gyrus. Subsequent, transplantation of CXCR4+ embryonic stem cell derived-neural progenitors (ESNPs) following kainic acid induced seizure led to ESNP migration to the upper blade of the dentate gyrus, a response that was abolished with administration of the CXCR4 antagonist, AMD3100 [50]. Similarly, *in vivo* studies in CNS injury models (ischemia and traumatic brain injury) have demonstrated that injury-induced upregulation of CXCL12 promotes chemotaxis of transplanted immature cells towards injury sites [35, 51]. Wang et al. found that elevated CXCL12 levels following middle cerebral artery occlusion correlated with migration of transplanted bone marrow stromal cells (BMSCs) to the ischemic penumbra [35]. The role of CXCL12 was confirmed as incubation of the BMSCs with the CXCR4 antagonist, AMD3100, prior to transplantation markedly decreased directed migration to the ischemic penumbra compared to native non-treated BMSCs [35]. These results indicate that chemotaxis promoted by CXCL12 and its receptor is a viable mechanism to increase cell recruitment to injury areas in the CNS.

Beyond endogenous progenitor cell and transplanted stem cell recruitment, CXCL12 also appears to influence angiogenesis *in vivo* [51]. CXCL12 intracortical injection following a brain injury in the rat resulted in increased microvessel density compared to vehicle injection, which was abolished in the presence of a CXCL12 function blocking antibody [51]. The microvessels in the injury area were both positive for CD34 and CXCR4, suggesting recruitment of immature endothelial cells via CXCL12 [51]. Spatial learning via a Morris water maze and probe trial kept the same trend as the microvessel density assessment, where administration of CXCL12 led to the best outcome and blocking antibody administration, the lowest [51]. Overall, these studies indicate not only that CXCL12 can promote chemotaxis within the CNS during injury, but also exogenous application leads to enhanced functional outcomes.

3.2. CCL2/Monocyte Chemoattractant Protein-1 (MCP-1)

CCL2, also known as MCP-1 is produced by astrocytes, microglia, and neurons of the CNS under various pathological conditions [37, 52]. Particularly, CCL2 expression increases in astrocytes following traumatic brain injury [53] and spinal cord contusion injury [54]. Previous *in vitro* characterization of Nestin+ and Notch-2+ neural stem/progenitor cells isolated from the SVZ of adults rats migrate towards CCL2 via expression of CCR2 [37, 55]. The ability of CCL2 to attract SVZ cells has led to the investigation of CCL2 levels and effects after CNS injury. Yan et al. examined the role of CCL2 in recruiting neuroblasts from the SVZ following middle cerebral artery occlusion in rats [37]. Results indicated that increased CCL2 expression in the ipsilateral cortex and striatum within the first 7 days post-injury in concert with increased doublecortin positive (Dcx+) neuroblasts within these regions [37]. This study further probed this chemotactic response by infusing CCL2 into the striatum of injured animals for 7 days via an osmotic minipump [37]. CCL2 treatment markedly increased the number of Dcx+/CCR2+ neuroblasts that migrated to the infused striatum [37]. Moreover, mice deficient for the ligand or receptor significantly reduced the number of cells in the ischemic striatum [37]. This study demonstrated the chemotactic potential of CCL2 with respect to SVZ cells during injury and highlighted how exogenous administration may enhance the cellular recruitment to sites of injury.

3.3. CX3CL1/Fractalkine

CX3CL1, commonly referred to as fractalkine, is expressed by neurons of the CNS and interacts primarily with CX3CR1 positive microglia and astrocytes [56, 57]. Regionally, fractalkine is expressed most prominently in the cortex, hippocampus, caudate putamen, thalamus, and olfactory bulb [56]. In the context of injury, CX3CL1 is upregulated after stroke [58] and experimental autoimmune encephalomyelitis, suggesting involvement in the injury process [59]. In addition, *in vitro* chemotaxis assays have demonstrated microglia migration towards CX3CL1 in a dose-dependent manner. Due to upregulation during injury and chemotaxis of microglia, the broader chemotactic effect of CX3CL1 on other cells types has been explored. Zhu et al. determined that CX3CL1 directed the migration of human bone marrow stromal cells (hMSCs) to the lesion site of a middle cerebral artery occlusion rat model [60]. Subsequent knockdown of CX3CR1 on hMSCs prior to injection significantly reduced homing to the ischemic penumbra thereby implicated CX3CL1 as a potent cell recruiting tool for CNS repair [60]. Exogenous administration of CX3CL1 to enhance endogenous repair has also been examined following neural injury. Qin et al. administered exogenous CX3CL1 following the middle cerebral artery occlusion in the rat and reported a significant increase in the number of endothelial progenitor cells at the infarct region [61]. Moreover, the measured infarct area and modified neurological severity score were also significantly lower [61]. These studies suggest that CX3CL1 can promote the migration of progenitor cells after injury and enhance repair through supplemental exogenous administration.

3.4. Vascular Endothelial Growth Factor (VEGF)

VEGF is a neurotrophic factor that is involved in development, cell proliferation, injury repair, and most notably, angiogenesis [5, 62–65]. Developmental angiogenesis of the RMS is mediated by VEGF secreting astrocytes [66]. Bozoyan et al. illustrated that downregulation of VEGF leads to significant decrease in blood vessel density on the outer border of the RMS (evaluated in P14 mice) [66]. Moreover, VEGF-mediated angiogenesis may also directly impact neurogenesis in neural progenitor cell niches of the CNS [64, 67]. Sun et al. identified a direct relationship between VEGF expression and angiogenesis in the SVZ of the rat brain, but also noted a relationship between VEGF levels and the organization of SVZ neural progenitor cells located in close proximity to blood vessels [67]. Additionally, immature neuronal doublecortin cells of the subgranular zone (SGZ) and SVZ of the rat brain express VEGF receptor 2, suggesting that VEGF stimulation is not solely implicated in angiogenesis and plays a role in the development and organization of the neurogenic niches [64]. As mentioned previously, VEGF does play a chemotactic role in the migration of SVZ cells to the olfactory bulb for neuronal replenishment in rodent models [27]. These migratory effects are not limited to development and maintenance of proliferative CNS niches, but have been examined during CNS injury as well. For instance, Wang et al. evaluated neurogenesis following a middle cerebral artery occlusion induced cerebral ischemia in conjunction with injection of VEGF expressing plasmids in the lateral ventricle of the rat brain. The upregulated expression of VEGF began in the lateral ventricle and traveled to the cortex [62]. The presence of VEGF in these areas significantly reduced infarct volume in the ipsilateral hemisphere and increased the number of proliferative cells in the cortex that ultimately stained positive for immature neuronal markers (Tuj1) two

weeks after injury [62]. Although the origin of the increased neuronal population was not determined, this study provides evidence that enhanced VEGF presence in the CNS can increase proliferative cells near sites of injury. Similar outcomes have been discovered when exogenous VEGF is infused into the lateral ventricle of mice for 7 days after traumatic brain injury [68]. Like the previous study, administration of VEGF in the ventricles led to a significant increase in the number of proliferative cells in the SVZ and the injured cortex compared to vehicle infusion [68]. After 90 days, the TBI lesion volume of mice infused with VEGF was half the volume of the control group, suggesting VEGF had a protective and/or regenerative role [68]. Again, the origin of the cells that were more populated around the injury in the VEGF group and labeled with neuronal marker NeuN, was not determined [68]. The authors however speculate that the elevated population of proliferative cells in the VEGF group across the SVZ, corpus callosum, and injured cortex suggest VEGF may promote migration through white matter to the injured region [68]. The capability of VEGF to incite cell migration *in vitro* combined with the known protective and regenerative effects *in vivo* makes VEGF a factor to be further investigated in the context of CNS repair.

3.5. Brain-derived Neurotrophic Factor (BDNF)

BDNF is a neurotrophic factor secreted by neurons and endothelial cells of the brain that has roles in development, maintenance, and injury repair [19, 69, 70]. BDNF is essential for proper development of cerebellar granule cell neurons and like CXCL12, is also needed for proper cerebral granule cell migration to the internal layer through chemotaxis [24]. Furthermore, Bath et al. examined the effects of BDNF haploinsufficient mice by injecting BrdU to track proliferative cells and found a significant reduction in the density of BrdU labeled cells in the olfactory bulb granule cell layer after 28 days, suggesting BDNF haploinsufficient mice may have a reduced chemotactic cue needed for proper migration or survival [71]. BDNF has also been investigated for its role in axon formation in hippocampal neurons, where neurons secrete BDNF locally to initiate a positive feedback loop to enhance axon growth and differentiation [69]. Outside of these roles, BDNF is a critical chemotactic signal during CNS injury with transient secretion of BDNF from endothelial cells and neurons [70, 72]. Grade et al. labeled SVZ cells prior to induction of ischemia in mice and used time-lapse imaging of *ex vivo* brain slices to measure SVZ progenitor cell migration toward ischemic tissue. The results demonstrated a direct relationship between SVZ progenitor cell migration and BDNF expression from the ischemic tissue as the application of a BDNF scavenging molecule markedly decreased migration relative to the control [72]. Similarly, Cook et al. examined the injection of soluble BDNF compared to BDNF released slowly from an injected hydrogel following induced striatal stroke in mice [73]. Nine weeks after stroke, then number of recruited neuroblasts was significantly increased only when BDNF was released slowly from an injected hydrogel, suggesting BDNF is capable of recruiting neuroblasts to the injury region when presented appropriately [73]. Therefore, it may be beneficial to sustain BDNF in the injury area in order to enhance the functional outcome. Together, these studies suggest BDNF is not only involved in directing developmental migration, but also initiating CNS injury repair and chemotaxis.

4. Challenges and Biomaterial-based Opportunities in Chemokine Delivery

While chemokines initiate robust cellular recruitment responses inherently in the body, harnessing that process as a repair tool has proved challenging. Previous studies exploring administration of chemokines have reported transient and insufficient therapeutic outcome [74, 80]. We have highlighted several studies that make use of chemotactic molecules for CNS repair in the section above and also in Table 1. Short half-life [81], rapid clearance *in vivo*, proteolytic degradation [82, 83], and receptor internalization [84] all limit the therapeutic potential of delivered chemokines. Moreover, a better understanding of how the body responds to administration of exogenous chemokines is necessary to elicit desired cellular response for repair and regeneration. This section will focus on the challenges of chemokine and growth factor delivery and how biomaterials-based strategies may address some of these challenges. Although we cannot discuss all biomaterials based strategies for maximizing chemotaxis and repair, we summarized seminal studies in Table 2 that demonstrate key findings within the field. Finally, we highlight the additional benefits of biomaterials in CNS repair beyond serving as carriers for chemotactic factors.

4.1 Challenges in Chemokine Delivery

4.1.1 Half-life and Clearance—One key limitation in delivering any biological molecule, including chemokines, is the maximizing the bioactivity lifespan. Once delivered, proteins are susceptible to a myriad of degradation and clearance mechanisms, including proteolytic degradation and endocytosis. Together, these processes result in rapid clearance of bioactive chemokines or the loss of bioactivity. For instance, Emerich et al. reported that VEGF bolus administration into the rat striatum had a half-life of about 90 minutes and was not able to be detected between 4–8 hours after injection [85]. Adding further complexities, the injury microenvironment exacerbates degradation with increased expression of proteases from activated inflammatory cells. Proteases break down chemokines via N-terminal processing, which truncates the chemokine and can alter bioactivity (Fig. 3A) [86]. One subset of proteases, matrix metalloproteinases (MMPs), are found throughout the CNS and are markedly elevated following traumatic brain injury, stroke, spinal cord injury, and CNS diseases [87–90]. Adelita et al. reported that MMPs cleave CXCL12, leading to both a loss of chemotactic potential to adult neural stem cells isolated from the mouse SVZs and induction of neural stem cell apoptosis by activating the intrinsic caspase 9 pathway when treated with the truncated form *in vitro* [82]. Moreover, MMPs actions are not limited to degradation, some MMPs such as MMP-1 are capable of breaking down ligands and producing chemokine receptor antagonists [83]. Serine proteases such as cathepsin G [91] and neutrophil elastase [92] also contribute to cleavage of chemokines. For example, Delgado et al. found that cathepsin G, which is produced by neutrophils and monocytes, was able to cleave CXCL12 thereby eliminating chemotaxis of T lymphocytes *in vitro* [91]. Alternatively, cleavage of certain ligands such as CCL15 and CCL23 by several MMPs can, in fact, increase chemotaxis [93]. While these chemokines are not known to be expressed in the CNS, MMP processing is very potent during CNS injury and should be considered when developing a chemotaxis strategy [93]. From a therapeutic delivery standpoint, one obvious mechanism to compensate for rapid proteolytic breakdown is to deliver a larger amount of the chemoattractant at one time; however, specific gradients are required for optimal effect

as chemotaxis often exhibits a biphasic dose-dependent response [38, 44]. For example, Widera et al. examined neural stem cell migration *in vitro* toward a CCL2 gradient and discovered the optimal chemotactic response was at a moderate level of CCL2 both above and below that amount resulted in a much muted chemotactic response [55]. These results have also been demonstrated by our lab with NSCs response to CXCL12 and further suggest optimal chemokine concentration gradients are necessary to produce maximal chemotaxis [47, 94]. The invasive nature of osmotic pumps, the lack of control over bolus delivery combined with the specific concentration gradients required for chemotaxis and their rapid inactivation demands better more controlled delivery mechanisms. Therefore, biomaterials have emerged as a means to sustain controlled therapeutic levels of chemokines in the CNS following injury all the while also preventing and/or protecting chemokines from rapid inactivation and elimination from prominent degradation mechanisms.

4.1.2 Receptor Internalization—Receptor internalization may decrease the sensitivity of cells to respond to ligand presence. Depending upon the cell type, specific receptor, and surrounding ligand concentration, the internalization half-life of chemokine receptors varies between 10 minutes to 8 hours [95–97]. In addition, different chemokine receptors have different pathways and therefore rates of internalization. For example, CXCR4 is more rapidly internalized when compared to CCR5 due to CCR5 containing fatty acids bound to its cysteine residues [82]. Due to variable receptor half-lives, a single ligand delivery rate may not be beneficial to all chemokine receptors and modification of the release profile may reduce receptor internalization. Ligands bind to their receptor to initiate the signaling pathways associated with chemotaxis. However, overstimulation by ligands may desensitize this process via internalization and lysosomal receptor degradation (Fig. 3B) [7]. Upon ligand binding to receptors, the receptors internalize and may follow a pathway to be either recycled to the surface or degraded [98]. Studies have shown that prolonged exposure time to ligands increases receptor degradation compared to shorter term administration [95, 99]. Studies have also shown that the dose of chemokines and factors significantly impacts receptor presence [7, 95, 97]. For example, Lopez et al. studied ligand induced endocytosis and reported a ligand dose-dependence on the percentage of receptor endocytosis [7]. This study suggests that smaller, controlled amounts of ligand over time may have the ability to reduce receptor internalization and increase intracellular signaling, chemotaxis, and ultimately repair. Biomaterials can generate slow and concentration-controlled release rates that reduce the possibility for receptor overstimulation.

4.2. Biomaterials for Altered Presentation and Release of Chemokines

Inherent differences in chemokine half-life, internalization rates, host microenvironment, and pathology-specific demands implies the need to tune delivery systems/approaches for each specific application. Below we outline various approaches that researchers have used to address this need through distinct biomaterials systems. We note that only a handful of neural biomaterial approaches have directly measured chemotaxis as an outcome, therefore the following sections will include seminal biomaterial examples that may serve as platforms for future chemokine delivery systems.

4.2.1 Physical Encapsulation within Biomaterials for Sustained Release—

Many diffusion-based systems have been created to release chemokine over a prolonged period. Hydrogels are a prominent materials approach to encapsulate chemotactic molecules within their porous network [100]. The encapsulated molecules are then free to diffuse throughout the gel matrix to provide a sustained release rather than single bolus injection. The hydrogel composition may be tuned by modifying many different properties (e.g. blends of multiple polymers, varying extents of crosslinking, charged sidechains) to ultimately influence the release rate [101, 102]. Examples of using hydrogel platforms for prolonging chemotactic factor release include an injectable gelatin-hydroxyphenylpropionic acid hydrogel containing polyelectrolyte nanoparticles sequestering CXCL12 [103]. This complex platform prolonged the release such that less than 10% was delivered over 7 days and resulted in significantly enhanced chemotactic *in vitro* migration of adult neural stem cells when compared to CXCL12 alone [103]. In a similar fashion, Silva and Mooney compared bolus delivery of the chemokine VEGF versus VEGF delivered in an injectable alginate hydrogel in an ischemic hindlimb mouse model. The study found that while bolus delivery of VEGF persisted for 72 hours *in vivo*, hydrogel delivery enabled VEGF detection for 15 days leading to significantly increased blood vessel density [104]. This approach has also proved successful for VEGF delivery to the CNS, where alginate hydrogel sustained levels of VEGF contributed to reduced lesion volume and neurologic impairment score in a rat cerebral ischemia model [85]. While these studies did not measure chemotaxis, the ability for the hydrogel systems to sustain VEGF delivery and lead to increased CNS repair may be applied to future chemotaxis studies. Altogether, these studies highlight the impact hydrogel-based delivery systems can have on preventing rapid clearance of bioactive factors *in vivo* thereby enhancing their therapeutic effect. We emphasized that the optimal hydrogel design varies according to the application as many different gels have shown positive outcomes for different CNS applications. Some studies focus on *in situ* gelling to allow for hydrogels to fill an irregularly shaped injury cavity [105], while others have developed fast-gelling blends that remain able to be injected due to their shear-thinning properties [106]. Similarly, hydrogel platforms range in composition including chitosan [107], hyaluronan-methylcellulose blends [108], poly(ethylene glycol) diacrylate [109], and agarose [105]. Overall, the simplistic but effective means by which hydrogels sustain molecule delivery *in vivo* lends them to be one of the most well studied biomaterial systems for chemotactic repair.

Nanoparticles and microspheres also enable sustained delivery of chemotactic molecules through diffusion and degradation. The release of chemotactic molecules from biodegradable polyester-based particles differs from hydrogels in that the particles encapsulate the molecules thereby enabling a more complex release profile. Diffusion of the payload molecule through the polymer matrix is followed by degradation of the particle itself, leading to sustained diffusion over a longer period of time [110]. Nanoparticles are desirable for CNS applications due to their size and potential for systemic delivery. For instance, our lab has established the feasibility for systemic nanoparticle delivery to the injured brain following a traumatic brain injury. We found that 20nm and 40nm nanoparticles crossed a dysfunctional blood-brain-barrier for up to 13 hours after injury (controlled cortical impact mouse model) [111]. Moreover, nano- and microparticles also

have facile, minimally invasive local tissue delivery for CNS applications [112]. For example, our lab recently demonstrated significantly increased and sustained presence of CXCL12 when encapsulated within poly(lactic-co-glycolic) acid nanoparticles (PLGA NPs) compared to bolus presentation following intracortical injections in adult mice [112]. Furthermore, this sustained exposure to bioactive CXCL12 lead to upregulation of endogenous CXCL12 expression across the cortex, indicating the presentation of chemokines impacted endogenous chemotactic signaling as shown previously by Enam et al. [109, 112]. Additional examples include Cao and Shoichet demonstrating the release of bioactive nerve growth factor (NGF) from PLGA microspheres up to 91 days [113]. While the sustained release profiles may achieve prolonged delivery windows with polymeric particles, the disadvantages of using biodegradable thermoplastic or elastomeric polymers include low encapsulation efficiency[113] and potential to denature biomolecules during the synthesis process as harsh organic solvents are typically required. To address these shortcomings, recent studies have shifted to composite hydrogel/particle platforms to exploit the positive attributes of each component [114, 115]. For instance, a hyaluronan-methylcellulose hydrogel containing fibroblast growth factor 2 (FGF-2) loaded PLGA NPs stimulated increased blood vessel density in a spinal cord injury model compared to controls [115]. A later study by Khaing et al. used a similar composite delivery system to provide sustained and localized BDNF delivery following spinal cord injury [114]. The study found a significant effect on spinal mediated learning when the composite system was applied [114]. Again, chemotaxis was not directly measured in both of these studies, yet sustained delivery and positive outcomes demonstrate the feasibility of such systems to enable complex chemokine delivery and enhanced CNS repair. Interestingly, particle-based delivery approaches alone are less common than hydrogels or composites for CNS repair, their utility as a chemotactic delivery tool has been well characterized for other physiologic applications. Huang and Liu demonstrated enhanced migration of mesenchymal stem cells (MSCs) *in vitro* when CXCL12 was delivered via chitosan/tripolyphosphate/fucoidan NPs versus CXCL12 alone [116]. In the same fashion, a study by Popova et al. revealed the effects of chemokine loaded NPs *in vivo* on neutrophil infiltration in the lymph nodes of mice [117]. Poly(N-isopropylacrylamide) NPs loaded with both CXCL8 (interleukin-8) and CCL3 (macrophage inflammatory protein 1-alpha) injected into the footpads of mice [117]. The cell counts of neutrophils in the subcapsular region were significantly higher 24 hours post injection with animals receiving NPs compared to bolus [117]. Altogether, employing a solely particle-based strategy may be sufficient to induce enhanced chemotactic responses in CNS applications.

4.2.2 Electrostatic/Affinity/Immobilization-based Biomaterial Strategies—

Electrostatic, affinity, and immobilization-based biomaterial systems are complementary to physical entrapment strategies for providing sustained release profiles. Such systems deviate from primary physical entrapment to exploit electrostatic or affinity-based interactions to control the adsorption and release of chemotactic molecules [108, 118, 119]. These methods result in high protein loading efficiency, often above 90% [120, 121]. Wang and Irvine created anionic alginate microspheres loaded with cationic CCL21 via electrostatic adsorption that successfully stimulated dose-dependent T-cell chemotaxis [122]. Similarly, Gonçalves et al. examined the ability of chitosan/poly(γ -glutamic acid) films to release

CXCL12 through electrostatic adsorption and recruit human MSCs [123]. When CXCL12 was incorporated in between the film layers or adsorbed to the final film layer MSCs were readily recruited compared to the negative control [123]. These studies demonstrate the feasibility and utility for electrostatic-based delivery to provide sustained chemokine release. More recently, researchers have utilized affinity bound CXCL12 to induce myoblast migration *in vitro* [124]. Specifically, Thakar et al. found that heparin sulfate bound CXCL12 induced myoblast movement at a significantly higher velocity compared to biotin immobilized presentation on a gold-supported oligoethylene glycol surface over 4 hours [124]. A study by Lee et al. found that controlled release of affinity bound nerve growth factor (NGF) resulted in enhanced peripheral nerve regeneration [125]. Although we did not highlight NGF in our section discussing relevant chemokines, NGF reportedly stimulates potent chemotactic migration of spinal neuroblasts [126]. While immobilized presentation did not prove superior to affinity-based in the previous study, other studies have found immobilization successful in promoting chemotaxis [127]. A study by DeLong et al. showed that a polyethylene glycol (PEG) immobilized basic fibroblast growth factor (bFGF) gradient resulted in increased migration of smooth muscle cells, which are known to respond chemotactically to bFGF [127].

Similar to the gradient created by immobilized bFGF in the previous study and endogenous chemotactic gradients, more sophisticated presentation of factors may be necessary to initiate specific cell recruitment. A recent novel approach to mimic endogenous gradient spatial complexities is to employ patterning techniques for immobilizing varying concentrations of factors within a material [128, 129]. Specifically, Wylie et al. developed a three-dimensional protein patterned scaffold that immobilized multiple growth factors using two photon patterning of two complementary peptide binding pairs [128]. A barstar-sonic hedgehog (SHH) fusion protein gradient was patterned into the gel using immobilized barnase and neural progenitor stem cells (NPSCs) migrated a greater distance into the gradient gel *in vitro* compared to the gel without SHH, showing that the pattern may have initiated movement along the gradient like native migration [128]. In summary, electrostatic, affinity, and immobilization-based biomaterial strategies are capable of inciting chemotaxis and repair in the CNS and may overcome the limited protein loading of other deliver methods. Further, spatially controlled immobilized factors may allow for fine tuning of chemotactic molecule delivery and recapitulation of complex chemokine niches.

Conclusion

Chemotaxis-based repair remains relevant, as it exists as an inherent tool used throughout CNS development, replenishment, and repair. Here we summarized key molecules relevant to CNS repair, the outcome of their direct application during injury, and the limitations that can be addressed through the use of biomaterials. Chemokines and factors have the potent ability to attract transplanted and endogenous immature cells to sites of injury within the CNS, although that is not their only role. It is necessary to consider that while chemokines and factors can be manipulated in favor of CNS repair, chemokines also regulate inflammatory cell migration and cancer progression [43, 154]. The complex involvement of chemokines and factors in homing cells from various microenvironments further illustrates that tuned delivery through biomaterials may help advance chemotactic repair in the CNS by

reducing chemokine clearance, creating tailored release profiles, and decreasing ligand induced receptor internalization. Finally, we find biomaterial-based chemokine delivery is an area of CNS regenerative research that has yet to be explored to its full potential.

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References

1. Leber T. The Origin of Inflammation. *The Lancet*. 1981; 1:1446–1448.
2. Florio T, Schettini G. Chemokines, their receptors and significance in brain function. *NeuroImmune Biol*. 2008; 6:242–273.
3. Ubogu EE, Cossoy MB, Ransohoff RM. The expression and function of chemokines involved in CNS inflammation. *Trends Pharmacol Sci*. 2006; 27:48–55. [PubMed: 16310865]
4. Balabanian K, Lagane B, Infantino S, et al. The Chemokine SDF-1/CXCL12 Binds to and Signals through the Orphan Receptor RDC1 in T Lymphocytes. *J Biol Chem*. 2005; 280:35760–35766. [PubMed: 16107333]
5. Kranich S, Hattermann K, Specht A, et al. VEGFR-3/Flt-4 mediates proliferation and chemotaxis in glial precursor cells. *Neurochem Int*. 2009; 55:747–753. [PubMed: 19646499]
6. Forstreuter F, Lucius R, Mentlein R. Vascular endothelial growth factor induces chemotaxis and proliferation of microglial cells. *J Neuroimmunol*. 2002; 132:93–98. [PubMed: 12417438]
7. Garcia Lopez M, Aguado Martinez A, Lamaze C, et al. Inhibition of dynamin prevents CCL2-mediated endocytosis of CCR2 and activation of ERK1/2. *Cell Signal*. 2009; 21:1748–1757. [PubMed: 19643177]
8. Zhou P, Porcionatto M, Pilapil M, et al. Polarized Signaling Endosomes Coordinate BDNF-Induced Chemotaxis of Cerebellar Precursors. *Neuron*. 2007; 55:53–68. [PubMed: 17610817]
9. Funamoto S, Meili R, Lee S, et al. Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell*. 2002; 109:611–623. [PubMed: 12062104]
10. Gardiner EM, Pestonjamas KN, Bohl BP, et al. Spatial and temporal analysis of Rac activation during live neutrophil chemotaxis. *Curr Biol*. 2002; 12:2029–2034. [PubMed: 12477392]
11. Iglesias, PA. *Encyclopedia of Cell Biology*. Elsevier; Dynamics of Gradient Sensing and Chemotaxis; p. 4-9.
12. Tan W, Martin D, Gutkind JS. The Gα13-Rho Signaling Axis Is Required for SDF-1-induced Migration through CXCR4. *J Biol Chem*. 2006; 281:39542–39549. [PubMed: 17056591]
13. Chen L, Iijima M, Tang M, et al. PLA2 and PI3K/PTEN Pathways Act in Parallel to Mediate Chemotaxis. *Dev Cell*. 2007; 12:603–614. [PubMed: 17419997]
14. Kamimura Y, Xiong Y, Iglesias PA, et al. PIP3-Independent Activation of TorC2 and PKB at the Cell's Leading Edge Mediates Chemotaxis. *Curr Biol*. 2008; 18:1034–1043. [PubMed: 18635356]
15. Sasaki AT, Firtel RA. Regulation of chemotaxis by the orchestrated activation of Ras, PI3K, and TOR. *Eur J Cell Biol*. 2006; 85:873–895. [PubMed: 16740339]
16. Sasaki AT, Chun C, Takeda K, et al. Localized Ras signaling at the leading edge regulates PI3K, cell polarity, and directional cell movement. *J Cell Biol*. 2004; 167:505–518. [PubMed: 15534002]
17. Ma Q, Jones D, Borghesani PR, et al. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proc Natl Acad Sci*. 1998; 95:9448–9453. [PubMed: 9689100]
18. Reiss K, Mentlein R, Sievers J, et al. Stromal cell-derived factor 1 is secreted by meningeal cells and acts as chemotactic factor on neuronal stem cells of the cerebellar external granular layer. *Neuroscience*. 2002; 115:295–305. [PubMed: 12401342]
19. Borghesani PR, Peyrin JM, Klein R, et al. BDNF stimulates migration of cerebellar granule cells. *Development*. 2002; 129:1435–1442. [PubMed: 11880352]

20. Tsai H-H, Frost E, To V, et al. The chemokine receptor CXCR2 controls positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. *Cell*. 2002; 110:373–383. [PubMed: 12176324]
21. Stumm RK, Zhou C, Ara T, et al. CXCR4 regulates interneuron migration in the developing neocortex. *J Neurosci*. 2003; 23:5123–5130. [PubMed: 12832536]
22. Gao X, Smith GM, Chen J. Impaired dendritic development and synaptic formation of postnatal-born dentate gyrus granular neurons in the absence of brain-derived neurotrophic factor signaling. *Exp Neurol*. 2009; 215:178–190. [PubMed: 19014937]
23. Li G, Adesnik H, Li J, et al. Regional Distribution of Cortical Interneurons and Development of Inhibitory Tone Are Regulated by Cxcl12/Cxcr4 Signaling. *J Neurosci*. 2008; 28:1085–1098. [PubMed: 18234887]
24. Kokubo M, Nishio M, Ribar TJ, et al. BDNF-Mediated Cerebellar Granule Cell Development Is Impaired in Mice Null for CaMKK2 or CaMKIV. *J Neurosci*. 2009; 29:8901–8913. [PubMed: 19605628]
25. Lois C, Alvarez-Buylla A. Long-Distance Neuronal Migration in the Adult Mammalian Brain. *Science*. 1994; 264:1145–1148. [PubMed: 8178174]
26. Wu W, Chen Jin-hui, Jiang Zhi-hong, et al. Directional guidance of neuronal migration in the olfactory system by the protein Slit. *Nature*. 1999; 400:331. [PubMed: 10432110]
27. Wittko IM, Schänzer A, Kuzmichev A, et al. VEGFR-1 Regulates Adult Olfactory Bulb Neurogenesis and Migration of Neural Progenitors in the Rostral Migratory Stream In Vivo. *J Neurosci*. 2009; 29:8704–8714. [PubMed: 19587277]
28. Mao W, Yi X, Qin J, et al. CXCL12/CXCR4 Axis Improves Migration of Neuroblasts Along Corpus Callosum by Stimulating MMP-2 Secretion After Traumatic Brain Injury in Rats. *Neurochem Res*. 2016; 41:1315–1322. [PubMed: 26801174]
29. Peng H, Huang Y, Rose J, et al. Stromal cell-derived factor 1-mediated CXCR4 signaling in rat and human cortical neural progenitor cells. *J Neurosci Res*. 2004; 76:35–50. [PubMed: 15048928]
30. Tran PB, Ren D, Veldhouse TJ, et al. Chemokine receptors are expressed widely by embryonic and adult neural progenitor cells. *J Neurosci Res*. 2004; 76:20–34. [PubMed: 15048927]
31. Faiz M, Acarin L, Villapol S, et al. Substantial migration of SVZ cells to the cortex results in the generation of new neurons in the excitotoxically damaged immature rat brain. *Mol Cell Neurosci*. 2008; 38:170–182. [PubMed: 18434192]
32. Gordon RJ, Tattersfield AS, Vazey EM, et al. Temporal profile of subventricular zone progenitor cell migration following quinolinic acid-induced striatal cell loss. *Neuroscience*. 2007; 146:1704–1718. [PubMed: 17459592]
33. Guillemin GJ, Croitoru-Lamourey J, Dormont D, et al. Quinolinic acid upregulates chemokine production and chemokine receptor expression in astrocytes. *Glia*. 2003; 41:371–381. [PubMed: 12555204]
34. Saha B, Peron S, Murray K, et al. Cortical lesion stimulates adult subventricular zone neural progenitor cell proliferation and migration to the site of injury. *Stem Cell Res*. 2013; 11:965–977. [PubMed: 23900166]
35. Wang Y, Deng Y, Zhou G-Q. SDF-1 α /CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. *Brain Res*. 2008; 1195:104–112. [PubMed: 18206136]
36. Imitola J, Raddassi K, Park KI, et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1 α /CXC chemokine receptor 4 pathway. *Proc Natl Acad Sci*. 2004; 101:18117–18122. [PubMed: 15608062]
37. Yan Y-P, Sailor KA, Lang BT. Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. *J Cereb Blood Flow Metab*. 2007; 27:1213–1224. [PubMed: 17191078]
38. Zhang L, Hua Q, Tang K, et al. CXCR4 activation promotes differentiation of human embryonic stem cells to neural stem cells. *Neuroscience*. 2016; 337:88–97. [PubMed: 27615032]
39. Filippo TRM, Galindo LT, Barnabe GF, et al. CXCL12 N-terminal end is sufficient to induce chemotaxis and proliferation of neural stem/progenitor cells. *Stem Cell Res*. 2013; 11:913–925. [PubMed: 23851289]

40. Ardel TA, Bhattacharyya BJ, Belmadani A, et al. Stromal derived growth factor-1 (CXCL12) modulates synaptic transmission to immature neurons during post-ischemic cerebral repair. *Exp Neurol*. 2013; 248:246–253. [PubMed: 23811016]
41. Opatz J, Kury P, Schiwy N, et al. SDF-1 stimulates neurite growth on inhibitory CNS myelin. *Mol Cell Neurosci*. 2009; 40:293–300. [PubMed: 19084600]
42. Liu KKY, Dorovini-Zis K. Regulation of CXCL12 and CXCR4 expression by human brain endothelial cells and their role in CD4+ and CD8+ T cell adhesion and transendothelial migration. *J Neuroimmunol*. 2009; 215:49–64. [PubMed: 19765831]
43. Teng L, Fu H, Deng C, et al. Modulating the SDF-1/CXCL12-induced cancer cell growth and adhesion by sulfated K5 polysaccharides in vitro. *Biomed Pharmacother*. 2015; 73:29–34. [PubMed: 26211579]
44. Belmadani A, Ren D, Bhattacharyya BJ, et al. Identification of a sustained neurogenic zone at the dorsal surface of the adult mouse hippocampus and its regulation by the chemokine SDF-1: SDF-1 Signaling and the Subhippocampal Zone. *Hippocampus*. 2015; 25:1224–1241. [PubMed: 25656357]
45. Gong X, He X, Qi L, et al. Stromal cell derived factor-1 acutely promotes neural progenitor cell proliferation in vitro by a mechanism involving the ERK1/2 and PI-3K signal pathways. *Cell Biol Int*. 2006; 30:466–471. [PubMed: 16630729]
46. Kokovay E, Goderie S, Wang Y, et al. Adult SVZ Lineage Cells Home to and Leave the Vascular Niche via Differential Responses to SDF1/CXCR4 Signaling. *Cell Stem Cell*. 2010; 7:163–173. [PubMed: 20682445]
47. Addington CP, Pauken CM, Caplan MR, et al. The role of SDF-1 α -ECM crosstalk in determining neural stem cell fate. *Biomaterials*. 2014; 35:3263–3272. [PubMed: 24438907]
48. Barkho BZ, Munoz AE, Li X, et al. Endogenous Matrix Metalloproteinase (MMP)-3 and MMP-9 Promote the Differentiation and Migration of Adult Neural Progenitor Cells in Response to Chemokines. *Stem Cells Dayt Ohio*. 2008; 26:3139–3149.
49. Addington CP, Dharmawaj S, Heffernan JM, et al. Hyaluronic acid-laminin hydrogels increase neural stem cell transplant retention and migratory response to SDF-1 α . *Matrix Biol*. 2017; 60–61:206–216.
50. Hartman NW, Carpentino JE, LaMonica K, et al. CXCL12-Mediated Guidance of Migrating Embryonic Stem Cell-Derived Neural Progenitors Transplanted into the Hippocampus. *PLoS ONE*. 2010; 5:e15856. [PubMed: 21209827]
51. Li S, Wei M, Zhou Z, et al. SDF-1 α induces angiogenesis after traumatic brain injury. *Brain Res*. 2012; 1444:76–86. [PubMed: 22330724]
52. Weiss JM, Downie SA, Lyman WD, et al. Astrocyte Derived Monocyte Chemoattractant Protein-1 Directs the Transmigration of Leukocytes Across a Model of the Human Blood-Brain Barrier. *J Immunol*. 1998; 161:6896–6903. [PubMed: 9862722]
53. Glabinski AR, Balasingam V, Tani M, et al. Chemokine monocyte chemoattractant protein-1 is expressed by astrocytes after mechanical injury to the brain. *J Immunol*. 1996; 156:4363–4368. [PubMed: 8666808]
54. Lee YL, Shih K, Bao P, et al. Cytokine chemokine expression in contused rat spinal cord. *Neurochem Int*. 2000; 36:417–425. [PubMed: 10733009]
55. Widera D, Holtkamp W, Entschladen F, et al. MCP-1 induces migration of adult neural stem cells. *Eur J Cell Biol*. 2004; 83:381–387. [PubMed: 15506562]
56. Harrison JK, Jiang Y, Chen S, et al. Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. *Proc Natl Acad Sci*. 1998; 95:10896–10901. [PubMed: 9724801]
57. Maciejewski-Lenoir D, Chen S, Feng L, et al. Characterization of fractalkine in rat brain cells: migratory and activation signals for CX3CR1-expressing microglia. *J Immunol*. 1999; 163:1628–1635. [PubMed: 10415068]
58. Liu Y-Z, Wang C, Wang Q, et al. Role of fractalkine/CX3CR1 signaling pathway in the recovery of neurological function after early ischemic stroke in a rat model. *Life Sci*. 2017; 184:87–94. [PubMed: 28624392]

59. Pan Y, Lloyd C. Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature*. 1997; 387:611. [PubMed: 9177350]
60. Zhu J, Zhou Z, Liu Y, et al. Fractalkine and CX3CR1 are involved in the migration of intravenously grafted human bone marrow stromal cells toward ischemic brain lesion in rats. *Brain Res*. 2009; 1287:173–183. [PubMed: 19563789]
61. Qin W, Li Z, Luo S, et al. Exogenous fractalkine enhances proliferation of endothelial cells, promotes migration of endothelial progenitor cells and improves neurological deficits in a rat model of ischemic stroke. *Neurosci Lett*. 2014; 569:80–84. [PubMed: 24704182]
62. Wang Y-Q, Cui H-R, Yang S-Z, et al. VEGF enhance cortical newborn neurons and their neurite development in adult rat brain after cerebral ischemia. *Neurochem Int*. 2009; 55:629–636. [PubMed: 19540294]
63. Fournier NM, Lee B, Banasr M, et al. Vascular endothelial growth factor regulates adult hippocampal cell proliferation through MEK/ERK- and PI3K/Akt-dependent signaling. *Neuropharmacology*. 2012; 63:642–652. [PubMed: 22580375]
64. Jin K, Zhu Y, Sun Y, et al. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proc Natl Acad Sci*. 2002; 99:11946–11950. [PubMed: 12181492]
65. Sun Y, Jin K, Xie L, et al. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J Clin Invest*. 2003; 111:1843–1851. [PubMed: 12813020]
66. Bozoyan L, Khlghatyan J, Saghatelian A. Astrocytes Control the Development of the Migration-Promoting Vasculature Scaffold in the Postnatal Brain via VEGF Signaling. *J Neurosci*. 2012; 32:1687–1704. [PubMed: 22302810]
67. Sun J, Sha B, Zhou W, et al. VEGF-mediated angiogenesis stimulates neural stem cell proliferation and differentiation in the premature brain. *Biochem Biophys Res Commun*. 2010; 394:146–152. [PubMed: 20188072]
68. Thau-Zuchman O, Shohami E, Alexandrovich AG, et al. Vascular endothelial growth factor increases neurogenesis after traumatic brain injury. *J Cereb Blood Flow Metab*. 2010; 30:1008–1016. [PubMed: 20068579]
69. Cheng P-L, Song A-H, Wong Y-H, et al. Self-amplifying autocrine actions of BDNF in axon development. *Proc Natl Acad Sci*. 2011; 108:18430–18435. [PubMed: 22025720]
70. Wang H, Ward N, Boswell M, et al. Secretion of brain-derived neurotrophic factor from brain microvascular endothelial cells: BDNF release in brain endothelial cells. *Eur J Neurosci*. 2006; 23:1665–1670. [PubMed: 16553631]
71. Bath KG, Mandairon N, Jing D, et al. Variant Brain-Derived Neurotrophic Factor (Val66Met) Alters Adult Olfactory Bulb Neurogenesis and Spontaneous Olfactory Discrimination. *J Neurosci*. 2008; 28:2383–2393. [PubMed: 18322085]
72. Grade S, Weng YC, Snapyan M, et al. Brain-Derived Neurotrophic Factor Promotes Vasculature-Associated Migration of Neuronal Precursors toward the Ischemic Striatum. *PLoS ONE*. 2013; 8:e55039. [PubMed: 23383048]
73. Cook DJ, Nguyen C, Chun HN, et al. Hydrogel-delivered brain-derived neurotrophic factor promotes tissue repair and recovery after stroke. *J Cereb Blood Flow Metab*. 2017; 37:1030–1045. [PubMed: 27174996]
74. Jin Y. Transplants of Fibroblasts Genetically Modified to Express BDNF Promote Axonal Regeneration from Supraspinal Neurons Following Chronic Spinal Cord Injury. *Exp Neurol*. 2002; 177:265–275. [PubMed: 12429228]
75. Sun D, Bullock MR, Altememi N, et al. The Effect of Epidermal Growth Factor in the Injured Brain after Trauma in Rats. *J Neurotrauma*. 2010; 27:923–938. [PubMed: 20158379]
76. Guaiquil VH, Pan Z, Karagianni N, et al. VEGF-B selectively regenerates injured peripheral neurons and restores sensory and trophic functions. *Proc Natl Acad Sci*. 2014; 111:17272–17277. [PubMed: 25404333]
77. Zendedel A, Nobakht M, Bakhtiyari M, et al. Stromal cell-derived factor-1 alpha (SDF-1 α) improves neural recovery after spinal cord contusion in rats. *Brain Res*. 2012; 1473:214–226. [PubMed: 22842524]

78. Sun W, Liu J, Huan Y, et al. Intracranial injection of recombinant stromal-derived factor-1 alpha (SDF-1 α) attenuates traumatic brain injury in rats. *Inflamm Res*. 2014; 63:287–297. [PubMed: 24352531]
79. Lian D, He D, Wu J, et al. Exogenous BDNF increases neurogenesis in the hippocampus in experimental *Streptococcus pneumoniae* meningitis. *J Neuroimmunol*. 2016; 294:46–55. [PubMed: 27138098]
80. Wang Y, Cooke MJ, Lapitsky Y, et al. Transport of epidermal growth factor in the stroke-injured brain. *J Controlled Release*. 2011; 149:225–235.
81. Cooke MJ, Wang Y, Morshead CM, et al. Controlled epi-cortical delivery of epidermal growth factor for the stimulation of endogenous neural stem cell proliferation in stroke-injured brain. *Biomaterials*. 2011; 32:5688–5697. [PubMed: 21550655]
82. Adelita T, Stilhano RS, Han SW, et al. Proteolytic processed form of CXCL12 abolishes migration and induces apoptosis in neural stem cells in vitro. *Stem Cell Res*. 2017; 22:61–69. [PubMed: 28623786]
83. Dean RA, Cox JH, Bellac CL, et al. Macrophage-specific metalloelastase (MMP-12) truncates and inactivates ELR+ CXC chemokines and generates CCL2-7-8, and-13 antagonists: potential role of the macrophage in terminating polymorphonuclear leukocyte influx. *Blood*. 2008; 112:3455–3464. [PubMed: 18660381]
84. Luker KE, Steele JM, Mihalko LA, et al. Constitutive and chemokine-dependent internalization and recycling of CXCR7 in breast cancer cells to degrade chemokine ligands. *Oncogene*. 2010; 29:4599–4610. [PubMed: 20531309]
85. Emerich DF, Silva E, Ali O, et al. Injectable VEGF Hydrogels Produce Near Complete Neurological and Anatomical Protection following Cerebral Ischemia in Rats. *Cell Transplant*. 2010; 19:1063–1071. [PubMed: 20412616]
86. McQuibban GA, Gong J-H, Wong JP, et al. Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo. *Blood*. 2002; 100:1160–1167. [PubMed: 12149192]
87. Guilfoyle MR, Carpenter KLH, Helmy A, et al. Matrix Metalloproteinase Expression in Contusional Traumatic Brain Injury: A Paired Microdialysis Study. *J Neurotrauma*. 2015; 32:1553–1559. [PubMed: 25858502]
88. Dong X, Song Y-N, Liu W-G, et al. MMP-9, a Potential Target for Cerebral Ischemic Treatment. *Curr Neuroparmacol*. 2009; 7:269–275. [PubMed: 20514206]
89. Leppert D, Ford J, Stabler G, et al. Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis. *Brain*. 1998; 121:2327–2334. [PubMed: 9874483]
90. Leake A, Morris CM, Whateley J. Brain matrix metalloproteinase 1 levels are elevated in Alzheimer's disease. *Neurosci Lett*. 2000; 291:201–203. [PubMed: 10984641]
91. Delgado MB, Clark-Lewis I, Loetscher P, et al. Rapid inactivation of stromal cell-derived factor-1 by cathepsin G associated with lymphocytes. *Eur J Immunol*. 2001; 31:699–707. [PubMed: 11241273]
92. Padrines M, Wolf M, Walz A, et al. Interleukin-8 processing by neutrophil elastase, cathepsin G and proteinase-3. *FEBS Lett*. 1994; 352:231–235. [PubMed: 7925979]
93. Starr AE, Dufour A, Maier J, et al. Biochemical Analysis of Matrix Metalloproteinase Activation of Chemokines CCL15 and CCL23 and Increased Glycosaminoglycan Binding of CCL16. *J Biol Chem*. 2012; 287:5848–5860. [PubMed: 22147696]
94. Dutta D, Fauer C, Mulleneux HL, et al. Tunable controlled release of bioactive SDF-1 α via specific protein interactions within fibrin/nanoparticle composites. *J Mater Chem B*. 2015; 3:7963–7973. [PubMed: 26660666]
95. Zimmermann N, Conkright JJ, Rothenberg ME. CC Chemokine Receptor-3 Undergoes Prolonged Ligand-induced Internalization. *J Biol Chem*. 1999; 274:12611–12618. [PubMed: 10212240]
96. Weber M, Blair E, Simpson CV, et al. The Chemokine Receptor D6 Constitutively Traffics to and from the Cell Surface to Internalize and Degrade Chemokines. *Mol Biol Cell*. 2004; 15:2492–2508. [PubMed: 15004236]

97. Berchiche YA, Gravel S, Pelletier M-E, et al. Different Effects of the Different Natural CC Chemokine Receptor 2b Ligands on -Arrestin Recruitment, G i Signaling, and Receptor Internalization. *Mol Pharmacol*. 2011; 79:488–498. [PubMed: 21088225]
98. Meiser A, Mueller A, Wise EL, et al. The Chemokine Receptor CXCR3 is Degraded Following Internalization and is Repelenished at the Cell Surface by De Novo Synthesis of Receptor. *J Immunol Baltim Md* 1950. 2008; 180:6713–6724.
99. Fan G-H, Lapiere LA, Goldenring JR, et al. Differential regulation of CXCR2 trafficking by Rab GTPases. *Blood*. 2003; 101:2115–2124. [PubMed: 12411301]
100. Connon, CJ., Hamley, IW., editors. RSC Soft Matter Series. Cambridge: Royal Society of Chemistry; Hydrogels in Cell-Based Therapies; p. P001-P004.
101. Raja WK, Gligorijevic B, Wyckoff J, et al. A new chemotaxis device for cell migration studies. *Integr Biol Quant Biosci Nano Macro*. 2010; 2:696–706.
102. Wu Y, Joseph S, Aluru NR. Effect of Cross-Linking on the Diffusion of Water, Ions, and Small Molecules in Hydrogels. *J Phys Chem B*. 2009; 113:3512–3520. [PubMed: 19239244]
103. Lim TC, Rokkappanavar S, Toh WS, et al. Chemotactic recruitment of adult neural progenitor cells into multifunctional hydrogels providing sustained SDF-1 release and compatible structural support. *FASEB J*. 2013; 27:1023–1033. [PubMed: 23193174]
104. Silva EA, Mooney DJ. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. *J Thromb Haemost*. 2007; 5:590–598. [PubMed: 17229044]
105. Jain A, Kim Y-T, McKeon RJ, et al. In situ gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury. *Biomaterials*. 2006; 27:497–504. [PubMed: 16099038]
106. Gupta D, Tator CH, Shoichet MS. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. *Biomaterials*. 2006; 27:2370–2379. [PubMed: 16325904]
107. Li H, Ham TR, Neill N, et al. A Hydrogel Bridge Incorporating Immobilized Growth Factors and Neural Stem/Progenitor Cells to Treat Spinal Cord Injury. *Adv Healthc Mater*. 2016; 5:802–812. [PubMed: 26913590]
108. Parker J, Mitrousis N, Shoichet MS. Hydrogel for Simultaneous Tunable Growth Factor Delivery and Enhanced Viability of Encapsulated Cells *in Vitro*. *Biomacromolecules*. 2016; 17:476–484. [PubMed: 26762290]
109. Enam SF, Krieger JR, Saxena T, et al. Enrichment of endogenous fractalkine and anti-inflammatory cells via aptamer-functionalized hydrogels. *Biomaterials*. 2017; 142:52–61. [PubMed: 28727998]
110. Singh R, Lillard JW. Nanoparticle-based targeted drug delivery. *Exp Mol Pathol*. 2009; 86:215–223. [PubMed: 19186176]
111. Bharadwaj VN, Lifshitz J, Adelson PD, et al. Temporal assessment of nanoparticle accumulation after experimental brain injury: Effect of particle size. *Sci Rep*. 2016; 6:29988. [PubMed: 27444615]
112. Dutta D, Hickey K, Salifu M, et al. Spatiotemporal presentation of exogenous SDF-1 with PLGA nanoparticles modulates SDF-1/CXCR4 signaling axis in the rodent cortex. *Biomater Sci*. 2017; 5:1640–1651. [PubMed: 28703822]
113. Cao X, Shoichet MS. Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders. *Biomaterials*. 1999; 20:329–339. [PubMed: 10048405]
114. Khaing ZZ, Agrawal NK, Park JH, et al. Localized and sustained release of brain-derived neurotrophic factor from injectable hydrogel/microparticle composites fosters spinal learning after spinal cord injury. *J Mater Chem B*. 2016; 4:7560–7571.
115. Baumann MD, Kang CE, Tator CH, et al. Intrathecal delivery of a polymeric nanocomposite hydrogel after spinal cord injury. *Biomaterials*. 2010; 31:7631–7639. [PubMed: 20656347]
116. Huang Y-C, Liu T-J. Mobilization of mesenchymal stem cells by stromal cell-derived factor-1 released from chitosan/tripolyphosphate/fucoidan nanoparticles. *Acta Biomater*. 2012; 8:1048–1056. [PubMed: 22200609]

117. Popova T, Teunis A, Magni R, et al. Chemokine-Releasing Nanoparticles for Manipulation of the Lymph Node Microenvironment. *Nanomaterials*. 2015; 5:298–320. [PubMed: 25878893]
118. Pakulska MM, Tator CH, Shoichet MS. Local delivery of chondroitinase ABC with or without stromal cell-derived factor 1 α promotes functional repair in the injured rat spinal cord. *Biomaterials*. 2017; 134:13–21. [PubMed: 28453954]
119. Vulic K, Shoichet MS. Affinity-Based Drug Delivery Systems for Tissue Repair and Regeneration. *Biomacromolecules*. 2014; 15:3867–3880. [PubMed: 25230248]
120. Skaat H, Ziv-Polat O, Shahar A, et al. Enhancement of the Growth and Differentiation of Nasal Olfactory Mucosa Cells by the Conjugation of Growth Factors to Functional Nanoparticles. *Bioconjug Chem*. 2011; 22:2600–2610. [PubMed: 22029397]
121. Atterberry PN, Roark TJ, Severt SY, et al. Sustained Delivery of Chemokine CXCL12 from Chemically Modified Silk Hydrogels. *Biomacromolecules*. 2015; 16:1582–1589. [PubMed: 25894928]
122. Wang Y, Irvine DJ. Engineering chemoattractant gradients using chemokine-releasing polysaccharide microspheres. *Biomaterials*. 2011; 32:4903–4913. [PubMed: 21463892]
123. Gonçalves R, Antunes J, Barbosa M. Mesenchymal stem cell recruitment by stromal derived factor-1-delivery systems based on chitosan/poly(γ -glutamic acid) polyelectrolyte complexes. *Eur Cell Mater*. 2012; 23:249–261. [PubMed: 22492017]
124. Thakar D, Dalonneau F, Migliorini E, et al. Binding of the chemokine CXCL12 α to its natural extracellular matrix ligand heparan sulfate enables myoblast adhesion and facilitates cell motility. *Biomaterials*. 2017; 123:24–38. [PubMed: 28152381]
125. Lee AC, Yu VM, Lowe JB, et al. Controlled release of nerve growth factor enhances sciatic nerve regeneration. *Exp Neurol*. 2003; 184:295–303. [PubMed: 14637100]
126. Behar TN, Schaffner AE, Tran HT, et al. Correlation of gp140/rk expression and NGF-induced neuroblast chemotaxis in the embryonic rat spinal cord. *Brain Res*. 1994; 664:155–166. [PubMed: 7895025]
127. DeLong SA, Moon JJ, West JL. Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration. *Biomaterials*. 2005; 26:3227–3234. [PubMed: 15603817]
128. Wylie RG, Ahsan S, Aizawa Y, et al. Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nat Mater*. 2011; 10:799–806. [PubMed: 21874004]
129. Leipzig ND, Wylie RG, Kim H, et al. Differentiation of neural stem cells in three-dimensional growth factor-immobilized chitosan hydrogel scaffolds. *Biomaterials*. 2011; 32:57–64. [PubMed: 20934216]
130. Schnell E, Klinkhammer K, Balzer S, et al. Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-e-caprolactone and a collagen/poly-e-caprolactone blend. *Biomaterials*. 2007; 28:3012–3025. [PubMed: 17408736]
131. Miller C, Jeftinija S, Mallapragada S. Micropatterned Schwann cell-seeded biodegradable polymer substrates significantly enhance neurite alignment and outgrowth. *Tissue Eng*. 2001; 7:705–715. [PubMed: 11749728]
132. Kigerl KA, Gensel JC, Ankeny DP, et al. Identification of Two Distinct Macrophage Subsets with Divergent Effects Causing either Neurotoxicity or Regeneration in the Injured Mouse Spinal Cord. *J Neurosci*. 2009; 29:13435–13444. [PubMed: 19864556]
133. Olive JL, McCully KK, Dudley GA. Blood flow response in individuals with incomplete spinal cord injuries. *Spinal Cord*. 2002; 40:639–645. [PubMed: 12483497]
134. Rosenberg GA. Matrix metalloproteinases in neuroinflammation. *Glia*. 2002; 39:279–291. [PubMed: 12203394]
135. Mahoney M, Saltzman WM. Transplantation of brain cells assembled around a programmable synthetic microenvironment. *Nat Biotechnol*. 2001; 19:934–939. [PubMed: 11581658]
136. Lo C-M, Wang H-B, Dembo M, et al. Cell movement is guided by the rigidity of the substrate. *Biophys J*. 2000; 79:144–152. [PubMed: 10866943]
137. Yim E, Reano R, Pang S, et al. Nanopattern-induced changes in morphology and motility of smooth muscle cells. *Biomaterials*. 2005; 26:5405–5413. [PubMed: 15814139]

138. Jannat RA, Robbins GP, Ricart BG, et al. Neutrophil adhesion and chemotaxis depend on substrate mechanics. *J Phys Condens Matter*. 2010; 22:194117. [PubMed: 20473350]
139. Leipzig ND, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. *Biomaterials*. 2009; 30:6867–6878. [PubMed: 19775749]
140. Ferguson AR, Christensen RN, Gensel JC, et al. Cell Death after Spinal Cord Injury Is Exacerbated by Rapid TNF-Induced Trafficking of GluR2-Lacking AMPARs to the Plasma Membrane. *J Neurosci*. 2008; 28:11391–11400. [PubMed: 18971481]
141. Lehnardt S, Schott E, Trimbuch T, et al. A Vicious Cycle Involving Release of Heat Shock Protein 60 from Injured Cells and Activation of Toll-Like Receptor 4 Mediates Neurodegeneration in the CNS. *J Neurosci*. 2008; 28:2320–2331. [PubMed: 18322079]
142. Rogove AD, Tsirka SE. Neurotoxic responses by microglia elicited by excitotoxic injury in the mouse hippocampus. *Curr Biol*. 1998; 8:19–25. [PubMed: 9427623]
143. Reyes RC, Brennan AM, Shen Y, et al. Activation of neuronal NMDA receptors induces superoxide - mediated oxidative stress in neighboring neurons and astrocytes. *J Neurosci Off J Soc Neurosci*. 2012; 32:12973–12978.
144. Mori S, Sadoshima S, Fujii K, et al. Decrease in cerebral blood flow with blood pressure reductions in patients with chronic stroke. *Stroke*. 1993; 24:1376–1381. [PubMed: 8362434]
145. Ojo JO, Mouzon B, Algamil M, et al. Chronic Repetitive Mild Traumatic Brain Injury Results in Reduced Cerebral Blood Flow, Axonal Injury, Gliosis, and Increased T-Tau and Tau Oligomers. *J Neuropathol Exp Neurol*. 2016; 75:636–655. [PubMed: 27251042]
146. Wang Y, West JD, Bailey JN, et al. Decreased Cerebral Blood Flow in Chronic Pediatric Mild TBI: An MRI Perfusion Study. *Dev Neuropsychol*. 2015; 40:40–44. [PubMed: 25649779]
147. Wang T-Y, Bruggeman KF, Kauhausen JA, et al. Functionalized composite scaffolds improve the engraftment of transplanted dopaminergic progenitors in a mouse model of Parkinson's disease. *Biomaterials*. 2016; 74:89–98. [PubMed: 26454047]
148. Itosaka H, Kuroda S, Shichinohe H, et al. Fibrin matrix provides a suitable scaffold for bone marrow stromal cells transplanted into injured spinal cord: A novel material for CNS tissue engineering. *Neuropathology*. 2009; 29:248–257. [PubMed: 18992011]
149. Guan J, Zhu Z, Zhao RC, et al. Transplantation of human mesenchymal stem cells loaded on collagen scaffolds for the treatment of traumatic brain injury in rats. *Biomaterials*. 2013; 34:5937–5946. [PubMed: 23664090]
150. Fon D, Zhou K, Ercole F, et al. Nanofibrous scaffolds releasing a small molecule BDNF-mimetic for the re-direction of endogenous neuroblast migration in the brain. *Biomaterials*. 2014; 35:2692–2712. [PubMed: 24406218]
151. Zamproni LN, Mundim MV, Porcionatto MA, et al. Injection of SDF-1 loaded nanoparticles following traumatic brain injury stimulates neural stem cell recruitment. *Int J Pharm*. 2017; 519:323–331. [PubMed: 28115261]
152. Dutta D, Hickey K, Salifu M, et al. Spatiotemporal presentation of exogenous SDF-1 with PLGA nanoparticles modulates SDF-1/CXCR4 signaling axis in the rodent cortex. *Biomater Sci*. Epub ahead of print 2017.
153. Kim DH, Seo YK, Thambi T, et al. Enhancing neurogenesis and angiogenesis with target delivery of stromal cell derived factor-1 α using a dual ionic pH-sensitive copolymer. *Biomaterials*. 2015; 61:115–125. [PubMed: 26001076]
154. Benslimane-Ahmim Z, Pereira J, Lokajczyk A, et al. Osteoprotegerin regulates cancer cell migration through SDF-1/CXCR4 axis and promotes tumour development by increasing neovascularization. *Cancer Lett*. 2017; 395:11–19. [PubMed: 28263839]

Figure 1.

Chemotactic Signaling Cascades. The leading edge of a migratory cell (right side) responds to chemotactic molecules initiating intracellular signaling that culminates in F-actin polymerization to protrude the cell towards the source. The trailing edge of the cell (left side) responds with myosin contraction pulling the cell off the substrate with PIP3-phosphatases (PTEN) dephosphorylating PIP3 to PIP2. (A) TorC2 pathway (PI3K independent): A chemotactic ligand binds to the GPCR activating an intermediate G-protein and subsequently the Tor Complex 2 (TorC2). The complex then phosphorylates protein kinase B related kinase (PKBR1) at the membrane leading to phosphorylation of several cytoskeletal proteins including TalinB. (B) The PIP3/PI3K pathway: A chemotactic ligand binds to the GPCR leading to recruitment of PI3K to the membrane. PI3K phosphorylates PIP2 to PIP3, PIP3 then activates Rac initiating F-actin polymerization. The F-actin polymerization stimulates additional recruitment of PIP3 and Rac creating a positive feedback loop at the cell's leading edge.

Figure 2.

Chemotaxis during CNS Development, Replenishment, and Repair. (A) Granule cell migration during development where CXCL12 secretion by the meninges homes CXCR4+ granule cells to the external granule layer (EGL) and CXCR4- cells migrate to the internal granule layer (IGL). (B) Interneuron replenishment to the olfactory bulb (OB) is mediated by chemorepulsive protein Slit originating from the subventricular zone (SVZ) and VEGF chemoattractant originating from the olfactory bulb, directing immature cells to the olfactory bulb along the rostral migratory stream (RMS). (C) CXCL12 induces CNS repair by local secretion near the cortical lesion, causing directed migration of CXCR4+ SVZ cells to the injury site.

Figure 3. Chemokine Inactivation by MMPs and Ligand Induced Receptor Internalization. (A) N-terminal processing by MMPs inactivates CXCL12 by preventing N-terminal binding to CXCR4 and subsequent intracellular signaling. (B) Overstimulation by large concentrations of chemokine ligands induces receptor internalization and degradation.

Figure 4.
Hydrogel releasing chemoattractant sustains delivery over time.

Table 1

Chemokine Delivery and Outcomes.

Ligand/Factor	Associated Receptor	Delivery Method	Injury Model	Outcomes	Reference
CCL2	CCR2	Striatal infusion for 7 days, osmotic pump	Focal cerebral ischemia, rat	↑Migration of Dcx+ neuroblasts to infarction	[37]
CX3CL1	CX3CR1	Intracerebroventricular injection	Middle cerebral artery occlusion, rat	↓ Infarct Volume ↑Endothelial progenitor cells in infarct area	[61]
VEGF	VEGFR	Intraventricular infusion for 7 days, mini-osmotic pump	Close head traumatic brain injury, mouse	↓ Lesion Volume ↑ SVZ cell migration to lesion	[68]
BDNF	trkB	Transplanted BDNF secreting fibroblasts	Spinal segment lesion, rat	↑ Forelimb usage ↑ Axonal regeneration	[74]
EGF	EGFR	Intraventricular infusion for 7 days, mini-osmotic pump	Lateral fluid percussion brain injury, rat	↑ SVZ cell proliferation at 7 days	[75]
CXCL12	CXCR4	Cortical injection	Lateral fluid percussion brain injury, rat	↑ Increase in microvessel density at 14 days	[51]
VEGF	VEGFR	Bolus injection into subconjunctival space	PNS corneal injury model, mouse	↑ Increase in central & peripheral nerve regeneration	[76]
CXCL12	CXCR4	28-day intrathecal osmotic pump	Spinal cord contusion injury, rat	↑ Motor function test ↑ Number of Neurons	[77]
CXCL12	CXCR4	Intracranial injection	Weight drop traumatic brain injury model	↓ Cerebral edema ↑Cortical cell number	[78]
BDNF	trkB	Intraventricular injection	Streptococcus pneumoniae meningitis	↑ Number of Dcx+ neuroblasts at 14 days	[79]

Table 2

Biomaterial Based Approaches to Chemotaxis and Outcomes.

Ligand/Factor	Associated Receptor	Delivery Method	Injury Model	Outcomes	Reference
BDNF	trkB	Hyaluronic acid hydrogel	Focal stroke, mouse	↑ Migrated BrdU/NeuN cells ^a	[73]
BDNF-mimetic	trkB	Nanofibrous scaffold implant	Implant only, rat	↑ Dex+ neuroblast migration from SVZ to implant	[150]
BDNF	trkB	In situ gelling hydrogel implant	Hemisection, spinal cord injury, rat	↑ Spinal neurofilament intensity	[105]
CXCL12	CXCR4	Injected polymer microspheres	Traumatic brain injury, mouse	↑ Dex+ neuroblast migration to lesion area ^a	[151]
CXCL12	CXCR4	Injected polymer nanoparticles	Controlled cortical impact traumatic brain injury, mouse	↑ Endogenous CXCL12 expression ^a ↑ CXCR4 cell density ^a	[152]
VEGF	VEGFR	Injected alginate hydrogels	Vessel ligation ischemic hindlimb, mouse	↑ Angiogenesis in limbs ^a	[104]
CXCL12	CXCR4	Striatal infusion of polymeric micelles	Focal cerebral ischemia, rat	↑ Number of BrdU/Dex+ SVZ cells ^a ↑ vWF+ cells in ischemic border ^a	[153]
VEGF	VEGFR	Striatal injection of alginate	Middle cerebral artery occlusion, rat	↓ Motor asymmetry ^a ↓ Neurologic severity score ^a ↓ Lesion volume ^a	[85]
CXCL12	CXCR4	Gelatin-hydroxyphenylproprionic acid hydrogel containing polyelectrolyte complex NPs	Neural Stem Cells ^b	↑ Migration of cells ^a	[103]
CXCL12	CXCR4	Chitosan/tripolyphosphate/fucoidan NPs	Mesenchymal Stem Cells ^b	↑ Migration of cells ^a	[116]
CXCL8 CCL3	CXCR1 CXCR2 CCR1 CCR4 CCR5	Poly(N-isopropylacrylamide) NPs	Restoration of immune cell chemotaxis, mouse	↑ Migration of neutrophils into lymph nodes ^a	[117]
CCL21	CCR7	Alginate microspheres – electrostatic adsorption	T-Cells ^b	Dose-dependent T-cell migration	[122]

Ligand/Factor	Associated Receptor	Delivery Method	Injury Model	Outcomes	Reference
CXCL12	CXCR4	Poly(γ -glutamic acid) to create multilayered films – electrostatic adsorption	Mesenchymal stem cells ^b	↑ Migration of cells	[123]
CXCL12	CXCR4	Poly(lactic-co-glycolic) acid NPs	Traumatic brain injury, mouse	↑ Number of CXCR4+ cells ^a ↑ Endogenous CXCL12 expression	[152]

^aIncreased/decreased compared to bolus delivery

^b*In vitro* study