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Nanomedicine Approaches to Modulate Neural Stem Cells in Brain Repair

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We explore the concept of modulating neural stem cells and their niches for brain repair using nanotechnology-based approaches. These approaches include stimulating cell proliferation, recruitment, and differentiation to functionally recover damaged areas. Nanoscale-engineered materials potentially overcome limited crossing of the blood–brain barrier, deficient drug delivery, and cell targeting.

From Neural Stem Cells to Nanomedicine

No therapies are yet available to fully restore loss of brain function. One of the therapies explored in several preclinical trials is the transplantation of neural stem cells (NSCs) or their progenies [1]. However, cell survival is poor, and the integration of transplanted cells into the neural circuitry is uncertain. An alternative is to manipulate endogenous NSCs (Box 1). In this case, the patient would benefit from his own cells, bypassing the use of invasive and costly transplantation procedures. Nanoparticulate systems for brain delivery (e.g., anticancer drugs, analgesics, anti-Alzheimer's and anti-Parkinson's drugs) have been described in the past 25 years [2]. However, modulation of the activity/differentiation of NSCs by nanoparticulate systems releasing small molecules was only recently demonstrated [3,4]. Current progress in the nanomedicine field has been stimulated by better understanding the biology of NSCs and by identifying molecular players capable of modulating their activity, proliferation, migration, and differentiation. This new avenue of research requires the development of advanced nanomaterials that, in some cases, have the capacity to transport drugs across the blood–brain barrier (BBB), target NSCs, deliver their payloads at the cell cytoplasm, and efficiently activate membrane receptors. Ideally, these materials should combine multiple features such as targeting, traceability, high cellular internalization and endolysosomal escape, release of multiple biomolecules at different timepoints and dosages, and biodegradation.

Modulation of Endogenous NSCs

Brain delivery of nanostructured materials can be performed by intracerebroventricular/intracerebral infusion or disruption of the BBB (e.g., by ultrasound). These approaches maximize the amount of drug that reaches the target site. Alternatively, systemic delivery by intravenous (i.v.) or intraperitoneal (i.p.) injections circumvents the need for invasive stereotaxic surgery. To maximize the BBB permeation, nanostructured materials, such as nanoparticles (NPs), can be coated with ligands or antibodies recognized by receptors/transporters or epitopes on brain endothelial cells. Few reports explore the biodistribution of NPs injected intravenously in distinct brain regions (that can be specifically affected by neurodegenerative diseases) and the ability of NPs to escape from vasculature into the brain parenchyma. Additionally, nanomaterials can incorporate peptide ligands to specifically target endogenous NSCs. Some ligands have been identified by phage display peptide libraries [5]. Importantly, functionalized nanostructured hydrogels were shown to successfully induce NSC differentiation while supporting tissue regeneration [6]. Recently, we have successfully developed a NP formulation capable of controlling NSC differentiation both *in vitro* and *in vivo* [3,4]. Contrary to soluble retinoic acid (RA), our results demonstrated that RA–NPs injected intracerebroventricularly contributed to the successful neuronal commitment of mouse subventricular zone (SVZ) NSCs.

Nanomedicine Approaches for Stroke

Stroke is the consequence of blood supply disruption to the brain. In the human brain, stroke stimulates neurogenesis and neuroblast migration to the site of injury. However, the number of neurons generated by NSCs in the postischemic brain is insufficient (approximately 0.2% of the cells lost), and their survival is minimal. Therefore, enhancing NSC activity provides a promising therapeutic platform for stroke. For example, polymeric NPs containing epidermal growth factor (EGF, which stimulates NSC proliferation) or erythropoietin (EPO, which reduces apoptosis of NSC-derived differentiated cells) have been encapsulated in a hyaluronan methylcellulose hydrogel and implanted in the epicortical region of the brain (2–3 mm from the SVZ region). In these conditions, EGF was completely released within the first week, while EPO was released for 3 weeks. The role of NP was to control the release of bioactive agents within the gel without any targeting role to the stem cell population. The sequential release of both molecules regenerated the peri-infarct region, which was correlated with an increase in SVZ NSC proliferation [7]. This biomaterial approach may be an alternative to intracerebroventricular infusion by a catheter/minipump system, as demonstrated in preclinical tests. Additionally, self-assembling peptide nanofiber scaffolds tested in a rat model of hemorrhagic stroke reduced brain injury and supported the secretion of neuroprotective trophic factors while being disassembled [8]. It would also be interesting to further explore this outcome on the neurogenic niches.

Nanomedicine Approaches for Parkinson's Disease

Parkinson's disease (PD) is a disorder characterized by the degeneration of

Box 1. Neural Stem Cell Biology

The discovery that neural stem cells (NSCs) are present in the adult mammalian brain throughout life raised many expectations among the medical and scientific community. NSCs have the ability to produce new neurons, astrocytes, and oligodendrocytes and are located in two main neurogenic niches: the subventricular zone (SVZ) lining the walls of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus. These niches are formed by NSCs and surrounding support cells, such as progenitor cells, neurons, astrocytes, endothelial cells, and microglia. In physiological conditions, new neurons derived from the SVZ, the largest NSC pool, migrate towards the olfactory bulb in rodents, originating interneurons, or towards the striatum in humans, originating striatal neurons. Neurogenesis also persists throughout life in the hippocampal SGZ, however, with a smaller pool of NSCs. In response to injury, NSCs proliferate and migrate to the lesioned site, and differentiate into new neurons. Owing to these unique characteristics, targeting NSCs rather than somatic neural cells might be beneficial from a regenerative perspective since NSCs are considered to be an inexhaustible source of new neurons. Nevertheless, this endogenous regenerative program is inefficient and only few NSC-derived newborn neurons are able to survive under the injured environment. For that reason, nanoparticulate systems are a promising tool to modulate NSC activity, self-renewal, survival, proliferation, migration, differentiation, and functional integration into neural circuits in the context of neurodegeneration.

dopaminergic neurons in the substantia nigra pars compacta, which leads to the depletion of dopaminergic fibers in the striatum and consequently originates motor symptoms. Several contradictory reports showed that neurogenesis is altered in PD. By modulating NSCs, new dopaminergic neurons could repopulate the lesioned striatum. The combination of hepatocyte growth factor (HGF)-loaded hydrogels with leukemia inhibitory factor (LIF)-loaded NPs significantly mobilized human NSCs *in vitro* [9]. Both molecules induced NSC migration according to their release profile: HGF and LIF induced migration for approximately 2 and 5 weeks, respectively. A separate study has shown that a multifunctional biomaterial comprising an injectable multifunctional gelatin–hydroxyphenylpropionic acid hydrogel and dextran sulfate/chitosan polyelectrolyte complex NP loaded with stromal cell-derived factor 1α (SDF-1α) is a very promising therapy for cavity brain lesions by recruiting endogenous NSCs and enhancing neural tissue regeneration [10]. The role of the NPs was to provide the desired release kinetics of SDF-1α to recruit NSCs and progenitor cells, while the gel was to provide a compatible structural support for cell homing before matrix remodeling. Therefore, the combination of nanomedicine with tissue engineering may be suitable for PD brain repair. Further preclinical studies are needed to show the relevance of this approach.

Nanomedicine Approaches for Alzheimer's Disease

Alzheimer's disease (AD) is the most prevalent type of dementia characterized by synaptic and neuronal loss in brain areas such as the entorhinal cortex, hippocampus, and neocortex, which are essential for memory and other mental abilities. The transplantation of NSCs, genetically altered or stimulated with pro-neurogenic factors, were able to rescue spatial and memory deficits in several AD animal models [11,12]. However, cell replacement can also be achieved by modulating the endogenous neurogenic niche of the hippocampus. Recently, it was demonstrated that poly(lactic-co-glycolic acid) (PLGA) NPs containing curcumin are capable of inducing neurogenesis and reverse learning and memory impairments in a rat model of AD [13]. The role of NPs was to increase the bioactive agent neuroprotective efficacy, reduce the required dose, and facilitate the transport through the BBB without compromising its integrity. Moreover, polymeric conjugates (100 nm) based on hyaluronic acid conjugated with multiple molecules of ephrin-B2 and sonic hedgehog per polymer chain have also been successfully used to stimulate neurogenesis in normally quiescent regions of the brain [14]. The multivalent ligand induced a sixfold increase in the fraction of newly divided neuronal precursors in the adult rat striatum.

Future Outlook

The modulation of NSC activity by nanomedicine approaches would represent a significant step towards the development of new brain repair therapies. However, several issues remain to be solved. An

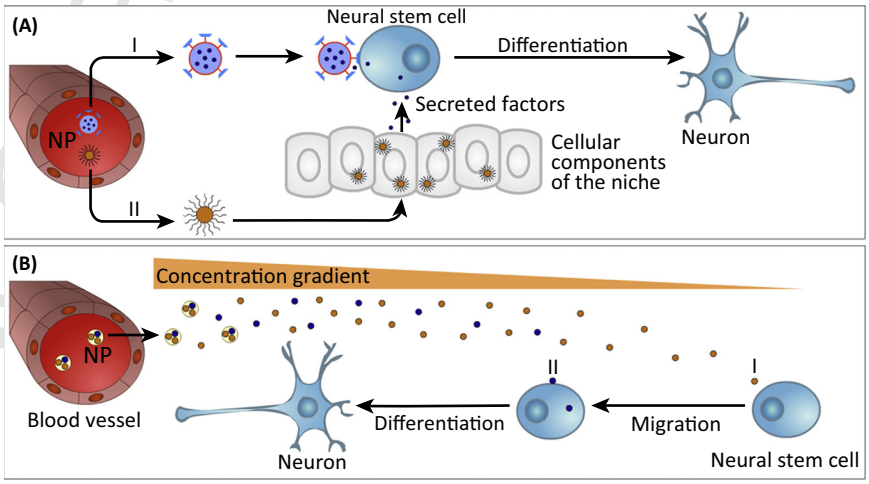

Figure 1. Modulation of Neural Stem Cells (NSCs) Either at the Neurogenic Niche or at Non-Neurogenic Regions, such as the Striatum or Cortex. In the neurogenic niche (A), nanoparticles (NPs) can specifically target receptors expressed by NSCs (I). Alternatively, NPs may target other cellular components of the stem cell niche, which in turn secrete factors that modulate the activity/differentiation of NSCs (II). In a non-neurogenic region (B), NPs may initially release factors to recruit NSCs (I) and subsequently to differentiate them (II), acting extra- or intracellularly.

important subject is to identify NSC epitopes to specifically target this cell population *in vivo* (Figure 1A). This effort will increase both the specificity and the efficiency of nanomedicine platforms while reducing potential side effects. Another important concern is to better understand the NSC niche upon injury. It is possible that the manipulation of actively proliferating progenitor cells rather than quiescent cells would be more efficient. Importantly, an understanding of the pathology processes and signaling pathways involved are critical elements to consider when selecting an appropriate nanomaterial for brain therapy.

Although the great majority of published studies acted on neurogenic regions, nanostructured materials might also boost neurogenesis in non-neurogenic regions (e.g., striatum, cortex). In this case, biomaterials should first recruit NSC or progenitor cells and then induce their differentiation by the delivery of neurogenic factors (Figure 1B). Accomplishing this goal requires the development of materials able to release multiple bioactive molecules at different times and dosages.

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