Commentary

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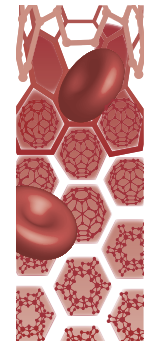

Recent advances in the use of magnetic nanoparticles to promote neuroregeneration

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The complexity of the nervous system is one of the major obstacles for implementing effective strategies of neuroregeneration. During the regeneration process a large number of biological events, including axonal growth, spatial organization of different cell types, cell–cell interactions, cell–matrix interactions, need to be re-assembled. This process requires a cascade of sequential events, which are generally orchestrated by growth factors and signaling cues secreted by specific cell types. The structure reconstruction is often governed by cell migration and creation of a cell niche, which provides the required gradients of biochemical and biophysical signals. Based on the understanding of the basic principles governing a nerve regeneration process, there is a great potential to exploit this knowledge to create synthetic tools to promote or accelerate nerve repair. In this context, nanomedicine is offering extraordinary possibilities to cross biological barriers or to mimic specific components of the regeneration process, such as cell manipulation, cell stimulation, cell homing, spatio-temporally controlled delivery of signaling cues, etc. This commentary focuses on novel perspectives offered by magnetic nanoparticles (MNPs) activated by noninvasive magnetic fields for promoting neuroregeneration or re-innervation in pathological conditions such as neurodegenerative diseases or injuries.

MNPs in biomedicine

Applications of MNPs on biomedical areas generally require the use of nanoparticles with a magnetic core, typically consisting either of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) with superparamagnetic or ferromagnetic properties, and a stabilizing organic or inorganic shell [1]. MNPs share with other nanoparticles the advantages related to the nanometer size and high aspect ratio. For example, MNPs have been used to increase the *in vivo* half-life of neurotrophins and growth factors by protecting them from fast degradation and demonstrating neurite elongation and increased complexity of the neuronal branching trees compared with the free factors [2]. MNPs conjugated with ligands demonstrated receptor-mediated targeted delivery across the blood brain barrier (BBB) in animal models [3] and also the ability for targeting specific neuronal cell population [4]. However, the magnetic behavior of MNPs offers distinctive and unique capabilities compared with other types of nanoparticles; the physical principles behind their common applications are: capability to remotely guide MNPs by external static magnetic fields; heat generation from the MNPs in a magnetic colloid, when submitted to electromagnetic fields and large magnetic moment of single-domain MNPs as a disturbance of proton nuclear resonance, which makes them excellent contrast agents for magnetic resonance imaging (MRI).

Axonal growth

The 'action at distance' exerted by MNPs on molecules, supramolecular complexes, cells and subcellular structures is a relatively new technique that has led to novel and exciting biomedical applications. In this context, MNPs have ability to manipulate axonal growth and guidance. Interestingly, by labeling neurites with MNPs, the extremely low forces generated by MNPs under the effect of the external magnetic field can be used for stretching the neurites, resulting in elongation and growth by a mechanism known as 'stretch growth' [5]. MNP-driven forces can be also used to gain control on axonal guidance, by orientating the direction of growth of neurites [6]. When the

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magnetic field is applied to surface-bound MNPs, filopodia of retinal ganglion cells (RGCs) elongated directionally, providing a tool to study the role of mechanical forces in filopodia dynamics and function [7]. The manipulation ability of MNPs can be also used to remotely control specific cellular components. Particularly, the manipulation of MNP-labeled signaling endosomes by a focal magnetic field was found to modulate growth cone motility and neurite growth [8]. D-Mannose-coated MNPs were used for manipulation of neuron synaptosome by an external magnetic field, without affecting the key characteristics of glutamatergic neurotransmission [9]. MNPs were also used to manipulate the localization of factors whose segregation is responsible of the specification of axonal versus dendritic fate in developing neurons [10]. Ideally, MNPs can be used to manipulate many intracellular signaling pathways. For example, MNPs have been used to bind the Rho-GTPases signaling complexes, whose manipulation by magnetic forces was found to trigger local remodeling of the actin cytoskeleton and morphological changes [11].

Approaches for treating central nervous system injuries

Although central nervous system (CNS) axons have intrinsic regeneration capabilities, the spontaneous regeneration process *in vivo* is very poor. In 1890s Santiago Ramon y Cajal first reported that CNS nerves appear to attempt to regenerate but they cannot, introducing the concept of the 'hostile CNS environment'. 10 years later, the work by Aguayo and colleagues demonstrated that adult mammalian CNS neurons are able to regenerate if grown into the 'permissive environment' of a peripheral nerve graft. During the following years, the molecular cues characterizing the 'permissive' and 'hostile' elements of CNS have been characterized. Indeed, three different strategies have been proposed over the years for treating CNS injuries, which can be summarized as 'fix what we have', 'build around it' or 're-build what we had'. The first approach is based on the current understanding of the cascade of events of inflammation reaction and the physiopathology of the regeneration process. Drug delivery strategies aimed to prevent cell death, promote axon re-growth and remove blockades have been pursued, but the biggest limitation are always the same: short half-life of the drugs and poor accessibility of the CNS to drug delivery. It has been suggested that magnetically guided MNPs can localize neurotrophins and other drugs, and simultaneously, protect them from premature cleavage. The effective and direct passage of systemically injected MNPs across normal BBB in rats under an external magnetic field, with progressive and significant accumulation of MNPs in the cortex near the magnet was recently demonstrated [12]. Another method to enhance the drug transport through the BBB is to transiently disrupt the barrier with MNP-mediated hyperthermia [13]. Interestingly, MNP manipulation can also implement strategies of selective depletion of molecules. For example, MNPs have been functionalized with O-methyl-β-cyclodextrin, a molecule able to bind cholesterol and external magnetic fields have been used for the removal of the bonded cholesterol from the synaptosomes in order to modulate glutamate transport [14].

The 'build around it' strategy is based on the use of stimulation or recording electrodes, which are key parts of devices for deep brain stimulation, an effective treatment modality for several neurological conditions such Parkinson's disease (PD), essential tremor (ET), dystonia, hyperkinetic disorders, etc. However, neuronal stimulation can also be achieved by inducing a localized temperature increase. In this context, the heating of MNPs under the effect of electromagnetic fields has been used to perform wireless deep brain stimulation of well-defined neuronal populations [15]. Moreover, manipulation of neuronal activity with magnetic stimuli has opened a new discipline, namely magnetogenetics that would overpass limitations of optogenetics by activating neurons with noninvasive magnetic stimuli without the need for invasive surgery. For example, the heat dissipated by MNPs when exposed to electromagnetic fields can be used to trigger the reversible firing of thermosensitive channels or the force exerted by MNPs when exposed to static electromagnetic field can be used to modulate mechanosensitive channels [16]. The magnetogenetics approach, combined with the possibility to use endogenous genetically encoded ferritin nanoparticles [17] or the ability to pass the BBB by exploiting the dragging force of a permanent magnet or the thermal energy generated by MNP hyperthermia, represents a revolutionary tool to modulate intrinsic neural electric activity by noninvasive magnetic fields.

The 're-build what we had' strategy is essentially based on cell therapies. Several cell types such as stem cells, Schwann cells (SC), olfactory ensheathing cells (OeCs) have been utilized as transplantable cells in nerve regeneration, but the number of clinical trials that have proved beneficial effects is still limited so far. A big issue in cell therapy is cell engraftment in the target site since only a small percentage of the transplanted cells *in vivo* reaches the desired location. In this context, MNPs can be also used for manipulation of whole cells and remote guidance of MNP-labeled cells has been proposed as a strategy to safely direct cells at the site of pathology, improving the outcome of cell therapies. This procedure also offers the distinct advantage that the efficiency of cell engraftment can be monitored by MRI. For example, MNP-labeled bone marrow MSC were transplanted into the injured

spinal cord via the subarachnoid space and an outer magnetic field was used to successfully guide the labeled cells to the lesion site, showing a superior cell homing and a greater number of complete axons at the lesion site [18]. Many papers on the same subject have been published in the last 10 years, confirming that MNP labeling of transplanted cells coupled with magnetic guidance offers a promising avenue to restore tissue functionality in CNS diseases.

Approaches for treating peripheral nervous system injuries

Axons of the mature mammalian peripheral nervous system (PNS) have higher capabilities to regenerate than those of CNS. The axons of PNS can regenerate *in vivo* but axonal regeneration is intrinsically disordered. If the regeneration is not guided, growth cone branches grow in a disorganized manner producing a neuroma, which can manifest clinically as a painful lump. Nerve gaps longer than 2 cm require a graft to bridge the defect, to guide the neurons and to prevent the formation of the neuroma. The conduit is positioned and sutured between the proximal and the distal stumps, providing an artificial conduit during the nerve regeneration process and limiting injury site infiltration by scar tissue. The graft could be autologous (surgically removed from a patient donor site, e.g., sural nerve) or artificial. Clinical trials suggest that autologous nerve grafts are the gold standard treatment for treating gaps longer than 3 cm. In fact, in artificial conduits, regeneration is sustained only by cells and growth factors secreted by the injured nerve ends, which fail to diffuse and reach the center portion of the conduit for length >3 cm. However, MNPs labeled with growth factors can be immobilized in the center of an artificial nerve guidance conduit by static magnetic fields, providing a spatial gradient of exogenous factors that compensate for the endogenous one; recovery of motor function has been demonstrated by this strategy in a model of rat median nerve lesion [19]. MNPs can be also used to localize the delivery of therapeutic agents at the desired target and to minimize any unwanted exposure to the medication of other sites. For example, the use of anesthetics for local nerve block should be ideally administered only to the desired nerve. A recent study performed on rats demonstrated that the intravenous injection of MNPs conjugated to bupivacaine and the application of a magnet to the rat ankle, is highly efficient in inducing local ankle block [20].

Future perspective

MNPs are nanotools with extraordinary ability for cell therapy and surgery. MNP-mediated manipulation and stimulation have the potential to provide clinicians and scientists with powerful tools to develop new therapeutic approaches for axonal growth and nerve regeneration, as well as to investigate neuron function and molecular signaling pathways for the discovery of novel therapeutic targets. The advances in MNP synthesis, functionalization and manipulation have opened up exciting perspectives for the treatment of nerve injuries that seemed inconceivable only a few years ago. At the tissue level, MNPs can be used to gain access to the BBB; at the cellular level, MNPs can magnetize cells and localize them at the target site; at the subcellular level, MNPs can finely modulate neurite growth, guidance and sprouting by multiple different mechanisms: by inducing stretch-growth; by altering localization/segregation of endogenous factors, and consequently, altering their intracellular signaling cascade; by manipulating organelles that transmit regulatory signals between soma and growth cones; by perturbing intrinsic neural electric activity by activation of mechanosensitive or thermosensitive proteins; at the molecular level, MNP can drive localization of therapeutic factors or depletion of detrimental molecules. Considering that this methodology has the potential for clinical translation as static magnetic fields are extensively used in medicine and several MNPs are approved for human use, one could speculate that neurosurgeons of the future could implant biocompatible FDA-approved magnetic nanomaterials or magnetically actuated cells at the injury site and use magnetic or electromagnetic fields to remotely guide or activate neuroregeneration.

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