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The best of both worlds: mastering nerve regeneration combining biological and nanotechnological tools

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Over the last decade, remarkable developments in nanotechnology have powered medical research, unveiling new approaches for the solution of public health issues such as the treatment of traumatic peripheral neuropathies.

With an estimated incidence of 13 to 23 cases per 100,000 people per year in developed countries, traumatic nerve injuries constitute the most frequent type of peripheral nervous system (PNS) damage. Peripheral neuropathy results from accidents associated with everyday activities such as sports, work, recreation, and driving. Upon injury, the connection between the neuronal distal axon and soma is interrupted, which triggers the degeneration of the affected nerve with structural and functional loss, known as Wallerian degeneration. Patients suffer symptoms ranging from neuropathic pain to partial or even total sensory and motor limitations, depending on the degree of injury severity. The lack of nerve responsiveness to regeneration – in terms of axon regrowth and remyelination – and unsuccessful target organ reinnervation are the main obstacles hindering complete nerve recovery.

Strategies commonly available for peripheral nerve lesion treatment are chosen according to the severity of the damage. Interventions include conventional approaches such as microsurgery to join nerve ends in the case of a transection, artificial and non-artificial nerve grafts, transplantation of conduits made of different biocompatible materials, polymer conduits coated with cells or growth factors that mimic a graft, and even therapies combining electrostimulation (Su et al., 2018) and physical exercise to promote nerve regeneration (Armada-da-Silva et al., 2013). Currently, the treatment of choice for the most severe transection injuries is microsurgery, although the functional outcome is often limited by inflammation, scar formation, and the pruning of sensory and motor axons.

Less severe lesions in the PNS may undergo spontaneous regeneration. A cascade of cellular events associated with neuroinflammation (**Figure 1A**) takes place in the distal stump of the injured nerve and plays a critical role in regeneration, although a chronic inflammatory reaction may be an obstacle to the regenerative process. Among these cellular events, the activation and proliferation of Schwann cells (SCs) – due to their high plasticity – are key to nerve regeneration. After the injury, SCs lose contact with axons, acquire a dedifferentiated repair phenotype, and, together with resident macrophages, secrete a pool of cytokines in the early degeneration phase (**Figure 1B**). This event promotes the recruitment of hematogenous macrophages which contribute to the removal of axon and myelin debris, an essential step for peripheral nerve regeneration. Repair SCs generate Büngner bands to guide axonal regrowth and, finally, the regenerated axon triggers a switch through which repair SC recover their mature myelinating phenotype. In more severe lesions, the crosstalk between SCs and axons cannot be reestablished, and the regenerative process ultimately fails, causing cellular and axonal death. Unlike peripheral nerve lesions, traumatic injuries to the central nervous system (CNS) show poor regeneration ability. The low rate of myelin removal in the CNS generates a hostile environment for regeneration, as myelin and axonal debris contribute to the formation of the glial scar, which hampers axon regrowth and brings about loss of function.

The optimal temporal window for therapeutic interventions strongly depends on the severity of peripheral damage. The most severe cases, such as nerve transection, require the immediate restoration of nerve connection by microsurgery. If the gap between the two ends of the transected nerve is wide and does not allow suturing, acellular conduits are used to connect the two ends and guide the axonal path for efficient regeneration. Nevertheless, bioengineered conduits may recreate the extracellular matrix of the nerve and even be supplemented with trophic factors, but they still lack the critical cellular component. In cases of less severe transection or even severe non-transection injuries, the connective tissue remains intact, which bypasses the need for surgical intervention to allow the reinnervation of the target organ. On the other hand, non-surgical approaches such as medication and physical exercise can be a suitable option all along treatment (Armada-da-Silva et al., 2013), but electrical stimulation seems to have an optimum time window – between the first 21 days after damage – to yield good clinical results (Su et al., 2018). Even so, functional recovery remains a clinical challenge.

PNS lesions thus exhibit a complex pathophysiology, and current surgical and pharmacological therapies are only partially effective. These reasons have led neuroregeneration research to develop and adopt hybrid therapeutic strategies that combine biological and nanotechnological methodologies (**Figure 1C**).

Among biological strategies, adult multipotent mesenchymal stem cells (MSC) have received special attention and have been extensively studied due to their potential application in the field of regenerative and reconstructive medicine. MSC are adult stem cells originally described in the bone marrow but later found in different adult tissues. MSC regenerative ability exceeds that of other stem cells as a result of advantages such as self-renewal rates, high plasticity, and low immunogenicity and tumorigenicity. Most relevant, the use of MSC avoids ethical concerns usually associated with embryonic stem cells (Bacakova et al., 2018).

Among the most popular MSCs, adipose-derived MSCs (AdMSCs) emerged as an interesting cell population for regenerative therapies, as they rapidly migrate to and integrate into the target tissue after systemic administration. AdMSCs mediate regenerative processes through mechanisms such as transdifferentiation, the induction of angiogenesis, and the secretion of growth factors and extracellular vesicles (LeBlanc and Uchida, 2019). Also, AdMSC has been proven to be more active in the autocrine production of trophic and immunomodulatory factors than other cell types.

AdMSC has been thoroughly studied for their potential therapeutic application in a variety of pathologies with diverse etiology. Safety and absence of adverse effects have been demonstrated after AdMSC transplantation for the treatment of acute respiratory syndrome (Zheng et al., 2014) and secondary progressive multiple sclerosis (Fernández et al., 2018). In addition, good clinical results and an improvement in joint function have been observed in osteoarthritis treatment (Lu et al., 2019).

It is noteworthy that the success of cell transplantation treatment depends on factors such as the number of transplanted cells, the administration route, and the number of cells that arrive and are retained at the target site. In this scenario, the incorporation of nanotechnological tools appears as a promising strategy to enhance treatment outcomes. Magnetic nanoparticles (MNP), particularly iron oxide ones in the maghemite and magnetite phase (γ-Fe_2O_3 and Fe_3O_4), are the most frequently proposed nano-developments in medical research due to their outstanding properties. Their great response to magnetic fields allows remote and non-invasive manipulation, enabling cells loaded with MNP to be magnetically targeted to a specific tissue or organ.

One of the key properties of iron oxide MNP which makes them excellent candidates for biomedical use is their high biocompatibility. Indeed, iron plays a fundamental role in numerous cellular processes such as oxygen storage and transport, electron transport and energy metabolism, and antioxidant and prooxidant reactions. The regulation of iron metabolism and homeostasis are complex processes, and an imbalance caused by excessive exogenous iron administration may trigger toxicity. Furthermore, proper iron uptake is essential for SC maturation in PNS myelination and remyelination along with development and after injury (Santiago González et al., 2019), as iron is an indispensable cofactor for several enzymes involved in the production and maintenance of the myelin sheath.

Even though the use of MNP for different applications is widely disseminated, their toxicological profile has not been well defined yet and remains controversial. When MNP internalization by cells is required, nano-cytotoxicity must be carefully considered for MNP validation as a biomedical tool. Moreover, MNP cytotoxicity is dose-dependent, and the generation of reactive oxygen species is chiefly responsible for cell damage. For these reasons, the evaluation of labile iron levels is of great interest for the use of MNP in regenerative medicine applications.

In addition, special attention should be given to the analysis of the MNP degradation process. MNP degradation depends on cell factors such as type, metabolism, and proliferation rate, and inherent MNP factors such as size, coating, and shape. Iron oxide MNP degradation produces free iron ions which either are incorporated into hemoglobin or join the native iron pool of the body and are degraded by normal iron-recycling pathways. However, excessive iron doses could affect cell morphology, signaling processes, and differentiation potential. Nevertheless, MNP use in controlled conditions allows their efficient internalization by cells without affecting cell functions or differentiation capacity.

As mentioned above, a crucial step in PNS regeneration is the successful delivery of cells to the target tissue and their retention at the lesion site. To overcome these obstacles, our group has improved cell therapy outcomes through the implementation of magnetic targeting of AdMSC loaded with responsive MNP (Soto et al., 2021). Magnetic targeting is an encouraging method for the treatment of CNS and PNS lesions, as it optimizes the rates at which systemically transplanted cells arrive and remain at the site of injury. This brings about an increased concentration of trophic factors secreted by AdMSC, which improves cell immunomodulatory effects. Moreover, magnetic targeting allows the *in vivo* remote manipulation of transplanted cells using small magnets. This approach avoids invasive methods of cell transplantation or an extra surgical procedure that could *per se* promote an inflammatory reaction and the exacerbation of neuropathic pain. This strategy, including AdMSC magnetic targeting, also allows cell transplantation in the acute phase after lesion. Given that the therapeutic time window for traumatic lesions in the PNS is short and represents a critical point in neuroregeneration processes, acute phase transplantation may contribute to more satisfactory results.

Good results using magnetic targeting have been reported for the treatment of CNS lesions (Grayston et al., 2022). Also, employing spinal cord injury models, different authors have demonstrated that MSC labeled with MNP respond to the applied magnetic field and, more relevant, improve motor function (Vaněček et al., 2012). Furthermore, some studies have reported the use of magnetic targeting to deliver growth factors to the injured sciatic nerve (Marcus et al., 2018), while others have documented the use of MSC in the treatment of peripheral nerve injuries (Fernandes et al., 2018). Recently, in a rat model of sciatic nerve lesion, our group demonstrated the efficacy of AdMSC magnetic targeting to speed up nerve regeneration from a morphological and functional point of view. The proposed strategy uses a hybrid material consisting of AdMSC loaded with 10 nm iron oxide MNP in their endosomes and submitted to the force produced by a permanent external Neodymium Iron Boron magnet, which warrants animal comfort and full mobility during treatment (Soto et al., 2021). Therefore, to the best of our knowledge and despite the substantial evidence provided in previous studies, a hybrid approach combining the two strategies for *in vivo* sciatic nerve regeneration has so far only been reported by our group.

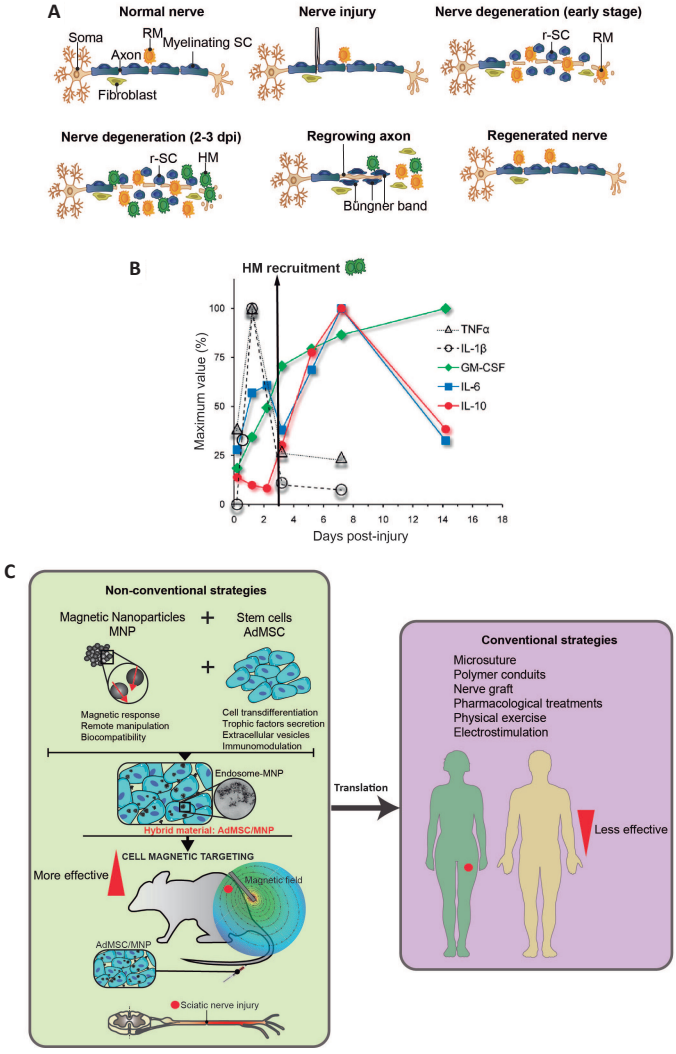

Figure 1 | **Summary of the relevant molecular events associated with the Wallerian degeneration process in the peripheral nervous system and therapeutic strategies for traumatic injuries.**
Scheme of the *in vivo* degenerative-regenerative process. Sciatic nerve scheme showing a control nerve made up mainly of axons, connective tissue, myelinating Schwann cells (SC), and resident macrophages (RM). After the injury, the proximal stump of the nerve remains connected to the neuronal soma and preserves its structure; otherwise, the distal stump suffers a degenerative process after the lesion. SC lose their myelinating phenotype and proliferate, becoming repair Schwann cells (r-SC) which, together with RM, secrete cytokines and recruit hematogenous macrophages (HM). HM contributed to the removal of axon and myelin debris, while Büngner bands generated by r-SC guide axon regrowth to reinnervate the target site. At the same time, axons and SC reestablish the crosstalk and SC recovers their mature myelinating phenotype. (B) Temporal progression of cytokine secretion during degeneration. Within the first hours after injury, SC began to secrete pro-inflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-1β] which, together with factors secreted by fibroblasts, recruit HM which arrive at the site of injury approximately 2-3 days post-injury. After the arrival of HM, the second stage of the inflammatory response begins, characterized by the second peak in IL-6 and a peak in IL-10, an anti-inflammatory cytokine, around 7 days post-injury. This event coincides with the peak in HM recruitment. Adapted from Rotshenker, (2011). (C) Therapeutic strategies for traumatic peripheral nerve injuries. Conventional strategies (right) range from microsuture to nerve grafts and polymer conduits in more severe injuries where the nerve is completely transected. In less severe lesions, pharmacological therapy is commonly used for neuropathic pain treatment. In both cases, physical exercise and electrostimulation are used to improve regeneration and alleviate pain symptoms. Conventional strategies sometimes fail in restoring normal function and morphology of the nerve, which is why non-conventional strategies (left) such as cell therapy alone or combined with magnetic targeting have been developed over the last decades to improve cell recruitment to the injured nerve. The innovative hybrid therapeutic strategy appears as a promising tool to reach complete neuroregeneration after traumatic injuries. This technique has proven useful to improve and accelerate remyelination and distal latency recovery after injury in an animal model. AdMSC: Adipose-derived mesenchymal stem cell; GM-CSF: granulocyte-macrophage colony-stimulating factor; MNP: magnetic nanoparticles.

Hybrid materials have proven useful, and the combination of different properties improves the therapeutic outcomes for numerous CNS and PNS injuries. Beyond the encouraging results, several challenges must be met prior to the translation of magnetic targeting to clinical applications. Some of them are optimal conditions for cell magnetic targeting – including thorough studies of the bio-nano interphase (MNP and biological environment interaction) – and the interaction between magnetically loaded cells and the magnetic fields applied once cells are intravenously administered. Another pressing need is the evaluation of MNP degradation and clearance in patients receiving magnetically loaded cells. Further experiments are necessary to fully elucidate this process, which depends on the metabolism and proliferation rate of the cell type involved.

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