Nanotechnology for treatment of stroke and spinal cord injury

The use of nanotechnology in cell therapy and tissue engineering offers promising future perspectives for brain and spinal cord injury treatment. Stem cells have been shown to selectively target injured brain and spinal cord tissue and improve functional recovery. To allow cell detection, superparamagnetic iron-oxide nanoparticles can be used to label transplanted cells. MRI is then a suitable method for the *in vivo* tracking of grafted cells in the host organism. CNS, and particularly spinal cord, injury is accompanied by tissue damage and the formation of physical and biochemical barriers that prevent axons from regenerating. One aspect of nanomedicine is the development of biologically compatible nanofiber scaffolds that mimic the structure of the extracellular matrix and can serve as a permissive bridge for axonal regeneration or as a drug-delivery system. The incorporation of biologically active epitopes and/or the utilization of these scaffolds as stem cell carriers may further enhance their therapeutic efficacy.

KEYWORDS: cell therapy ■ contrast agents ■ magnetic resonance ■ nanofibers ■ scaffold ■ spinal cord lesion

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Nanotechnology is a rapidly developing field in contemporary medical research that offers promising future perspectives for the treatment of CNS disorders. To address neural tissue injury, current research involves several distinct areas of application in terms of nanomaterials, comprising nano-enabled drug delivery across the blood–brain barrier, imaging, biocompatible nanocomposites and the future utilization of nanorobots, nanowires and nanochips. This article focuses on the use of nanotechnology in two interconnected aspects of brain and spinal cord injury treatment: cell therapy and tissue engineering.

Cell therapy in the CNS

Cell therapy is one strategy for treating a variety of CNS disorders [1–3], and some successful therapeutic approaches have already been transferred to clinical use [4–6]. Stem and progenitor cells from various sources are used for transplantation in brain or spinal cord injury, including embryonic stem cells (ESCs) [7], neural progenitor cells [8], olfactory ensheathing cells (OECs) [5] and Schwann cells [9]. Beyond this, the use of adult stem cells or adult mesenchymal stem cells (MSCs) presents several advantages that highlight their promising therapeutic potential in human medicine [3]. MSCs are present in adult tissue, primarily in the bone marrow, but they can be found in fat, skin, liver, peripheral blood and umbilical cord; they are easy to isolate and expand, and their immunomodulatory properties offer potential for their use in cell therapy even in allogeneic settings [10]. MSCs are multipotent cells; in other words, they can differentiate not only into cells of mesenchymal origin, but also into nonmesenchymal cell phenotypes. Despite the fact that the transdifferentiation of MSCs into cells of the neuronal lineages has been shown *in vitro*, there is currently no proof that MSCs give rise to functional neurons *in vivo* [3,11]. Nevertheless, MSCs produce a variety of growth factors, chemokines and other bioactive factors supporting regeneration and, furthermore, they can be genetically modified and can serve as carriers for drug delivery [11]. After transplantation into the nervous tissue or intravenous administration, MSCs have been shown to respond to intrinsic signals and migrate toward the site of injury, reduce the lesion volume and elicit functional improvement [12].

Magnetic nanoparticles for labeling stem cells

A crucial aspect of successful cell transplantation is tracking and monitoring the grafted cells in the transplant recipient. To screen cells *in vivo*, several techniques have been described using nanoparticles (quantum dots, pebbles and superparamagnetic iron-oxide [SPIO] nanoparticles) [13,14], but only SPIO nanoparticles visualized by MRI are suitable in human medicine. SPIO nanoparticles are negative contrast agents that selectively shorten T_2-relaxation time and, thus, can be detected in the tissue as a hypointense (dark) signal. MRI, as a

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noninvasive method, may then be used not only to evaluate whether the cells have been successfully engrafted, but also monitor the time course of cell migration and their survival in the targeted tissue. This information may further help to optimize the transplantation procedure in terms of the number of required cells, the method or site of cell administration and the therapeutic time window after injury during which transplantation will be most effective.

Superparamagnetic iron-oxide nanoparticles usually consist of a crystalline iron oxide core and a polymer shell (Figure 1A). In order to prevent aggregation, dextran is the most commonly used surface coating. Dextran-coated SPIO ferumoxide nanoparticles are commercially available and have been approved as contrast agents by the US FDA as Feridex® and Endorem® or as ultrasmall SPIO nanoparticles (Combidex®, Sinerem®). To facilitate the uptake of nanoparticles into cells, a common labeling approach is to combine commercially available contrast agents and transfection agents (e.g., Superfect, poly-L-lysine, Lipofectamine™, Fugene® or protamine). The advantage of Endorem [7], as well as of carboxydextran-coated ferucarbotran (Resovist®) [15], is that they have been shown to be suitable contrast agents for labeling rat or human MSCs (Figure 1B & C), ESCs and OECs, and do not require the use of a transfection agent, however, they have a lower labeling efficiency than newly developed coatings [16,17]. Various strategies for optimizing the delivery of magnetic nanoparticles into cells have been developed, such as the specific targeting and endocytosis of nanoparticles through the use of transferrin receptors [18], magnetodendrimers [19], transduction agents such as HIV-derived TAT protein [20] or by the use of electroporation [21].

Superparamagnetic iron-oxide nanoparticles coated with poly-L-lysine [16,101], D-mannose, or poly(*N*,*N*-dimethylacrylamide) [17] display better cell labeling efficiency and easier MRI detection, along with a lower concentration of iron within the cells, when compared with Endorem. SPIO nanoparticles coated with polyvinyl pyrrolidone [22] or mesoporous silica nanoparticles [23] have also been developed as efficient contrast agents.

For clinical use in particular, it needs to be shown that SPIO nanoparticles are nontoxic and biodegradable and do not affect the proliferation and differentiation potential of MSCs or other cells *in vitro* [24]. The concentration of iron in the culture media has to be optimized for each cell culture, as a lower concentration may result in insufficient cell uptake, whereas a higher concentration may induce the precipitation of complexes or may be toxic to the cells.

In addition to the use of SPIO nanoparticles for labeling transplanted cells, another strategy, the systemic injection of MRI contrast agents and their subsequent preferential phagocytosis by monocytes and macrophages, has been used as a tool for the *in vivo* visualization of the inflammatory response after stroke and other CNS pathologies [25]. For *in vivo* monitoring of macrophages after traumatic brain injury or in an ischemic lesion, the intravenous administration of ultra-SPIO (USPIO) particles [26,27], micron-sized paramagnetic iron-oxide (MPIO) particles [28] as well as SPIO nanoparticles [29] have been used.

In vivo tracking of stem cells in brain & spinal cord lesions

Several studies have demonstrated that SPIO-labeled stem cells migrate into a brain or spinal cord lesion after transplantation; MRI detection was confirmed by histological analysis with iron staining.

Zhang *et al.* showed the migration of iron-labeled neural progenitor cells transplanted into the cisterna magna toward the ischemic area in rats with middle cerebral artery occlusion [30]. Subsequently, it was shown that intracisternally transplanted labeled neural progenitor cells migrated towards the ischemic boundary regions, where they promoted angiogenesis [31]. The use of MRI to visualize the migration of transplanted stem cells to the infarcted area has also been reported by other authors [32–35]. Transplantation of magnetically labeled neurospheres into the ventricles of experimental allergic encephalomyelitic rats revealed the migration of glial precursors into white matter structures [36]. OECs labeled with magnetodendrimers were implanted and monitored in the transected rat spinal cord [37]. Dextran-coated SPIO-labeled Schwann cells and OECs have been shown to remyelinate axons after transplantation into demyelinated lesions in the rat spinal cord [38]. Intravenously injected SPIO-labeled neural stem/precursor cells accumulated within focal inflammatory demyelinating brain lesions in a mouse model of chronic multiple sclerosis [39].

In our experiments, Endorem-labeled human MSCs, rat MSCs or mouse ESCs transfected with enhanced green fluorescent protein (GFP) were grafted into rats with a cortical photochemical lesion, either intracerebrally into the hemisphere contralateral to the lesion or intravenously. A

hypointense MRI signal was found in the lesion 1 week after grafting (FIGURE 1G), and persisted for more than 50 days (FIGURE 1H), regardless of the route of administration; its intensity corresponded to iron (Prussian blue) staining (FIGURE 1I) and electron microscopy observations [7,40]. In other experiments, the intravenous injection of MSCs significantly improved the recovery of hindlimb motor function in rats with a spinal compression lesion [41]. The fate of transplanted MSCs labeled with Endorem was followed by *in vivo* as well as *ex vivo* MRI; staining for Prussian blue revealed many cells labeled with nanoparticles in the lesion site, and the lesion cavities were significantly smaller than in control animals (FIGURE 1J–M) [41].

Aside from SPIO nanoparticles, other types of contrast agents, such as gadolinium or MPIO particles, are used for *in vivo* cell detection. As a bimodal contrast agent, a gadolinium–rhodamine–dextran conjugate was used for mapping transplanted neural stem cell migration after a stroke [33,34]. MPIO particles with enhanced magnetic resonance (MR) signal intensity enable the detection of single cells and can also be coupled to various fluorescent dyes for histological detection [42]. As an alternative route for *in situ* cell contrast agent application, MPIO particles were injected intraventricularly into 7-day-old neonatal rats to track the migration of endogenous stem/progenitor cells from the subventricular zone in the developing brain with and without a hypoxic–ischemic insult; the stem cells' migratory pathways were analyzed by high-resolution MRI and correlated with histological analysis [43].

To replace or regenerate damaged tissue, transplanted cells can be administered directly in suspension or transported by a carrier or scaffold that additionally forms a tissue bridge. Hydrogels or other biomaterials seeded with stem cells may serve as an alternative to the conventional grafting of dissociated cells, benefiting from advances in surface chemistry that support cell–matrix interactions. To facilitate the regeneration of injured spinal cord, a cell–polymer construct was developed using Endorem-labeled MSCs seeded onto a poly(2-hydroxyethyl methacrylate) hydrogel, and implanted into a spinal cord hemisection. The hydrogel re-established the anatomic continuity of the tissue 6 weeks after implantation with axonal ingrowth into the hydrogel. MRI and Prussian blue staining confirmed the presence of positively stained cells within the hydrogel [44].

Although the SPIO-labeling method is widely used in preclinical and also clinical trials of cell therapy, there are, nonetheless, some

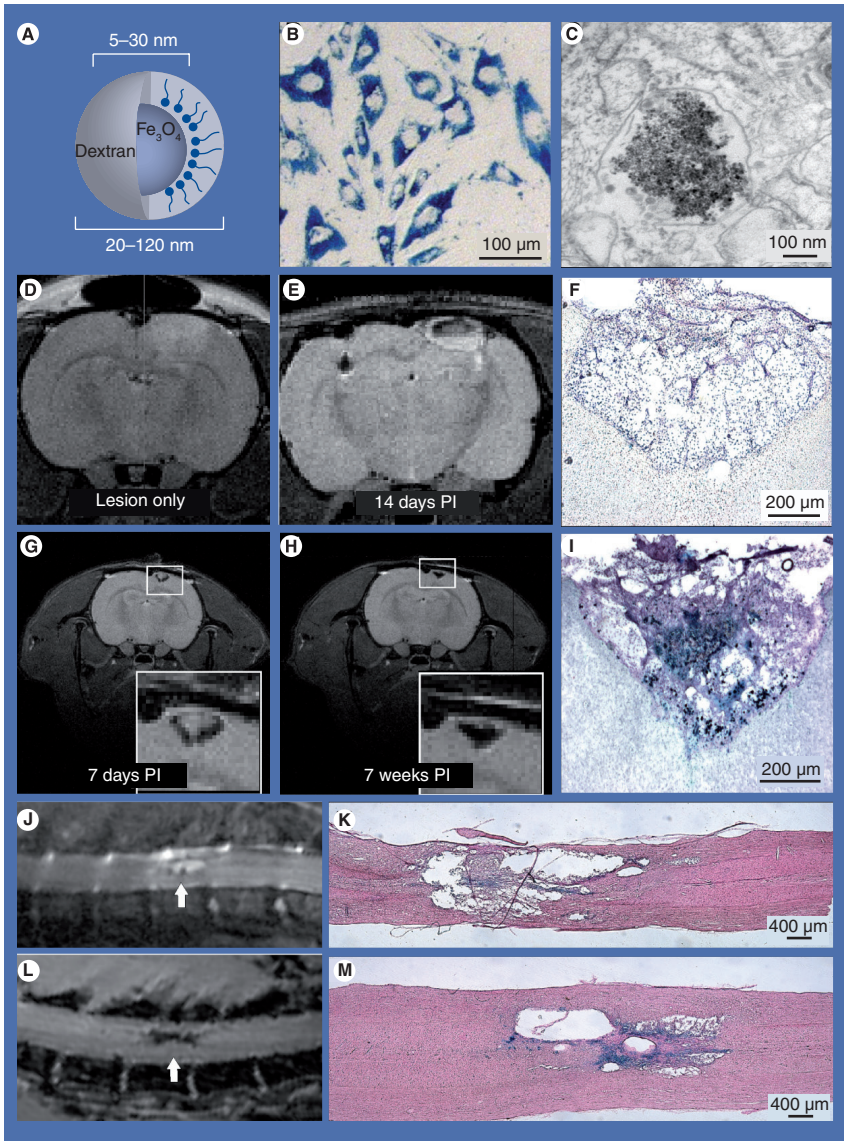

Figure 1. MRI tracking of SPIO-labeled stem cells. (A) Scheme of an iron nanoparticle. The contrast agent Endorem® consists of a superparamagnetic Fe_3O_4 core coated by a dextran shell. **(B)** Rat mesenchymal stem cell (MSC) culture labeled with superparamagnetic iron-oxide nanoparticles and stained for Prussian blue. **(C)** Transmission-electron micrograph of a cluster of iron nanoparticles surrounded by a cell membrane. **(D)** Cortical photochemical lesion visible on MRI 2 weeks after induction as a hyperintensive (light) area. **(E)** Both the cell implant (MSCs in the hemisphere contralateral to the lesion) and the lesion are hypointensive (dark) 2 weeks after implantation. **(F)** A few cells weakly stained for Prussian blue were found in the photochemical lesion in animals without implanted cells. **(G & H)** A hypointense signal in the lesion was seen 7 days after the intravenous injection of Endorem-labeled rat MSCs **(G)** and persisted for 7 weeks **(H)**. Insets show a higher magnification view of the lesion. **(I)** Massive invasion of rat MSCs (Prussian blue staining counterstained with hematoxylin) into a photochemical lesion 7 weeks after intravenous injection. **(J)** Longitudinal section of a rat spinal cord compression lesion on MRI 5 weeks after compression. The lesion is seen as a hyperintensive area (arrow). **(K)** Prussian blue staining of a spinal cord compression lesion (control animal). **(L)** Longitudinal MRI of a spinal cord compression lesion populated with intravenously injected nanoparticle-labeled MSCs 4 weeks after implantation. The lesion with nanoparticle-labeled cells is visible as a dark hypointensive area (arrow). **(M)** Prussian blue staining of a spinal cord lesion with intravenously injected nanoparticle-labeled MSCs.
PI: Postimplantation.
Modified with permission from [7,40].

confounding factors that can lead to the misinterpretation of MR signal and thus require further analysis [38]. In many cases, a hypointense signal can have a physiological origin, such as iron dissociated from hemoglobin, or a pathological origin, such as hemorrhage, tumor or experimental traumatic procedures (e.g., injection). An alternative method is the use of contrast agents that create opposite contrast, for example manganese-oxide nanoparticles [21]. The presence of phagocytic cells such as macrophages in the lesion site, which may accumulate iron nanoparticles and give rise to a false MR signal, is another potential limitation of this technique [45]. Therefore, the contribution of transplanted SPIO-labeled cells must be clearly considered in MRI interpretation. On the other hand, the loss of MRI signal can be caused by the biodegradation of the nanoparticles or by their dilution due to cell proliferation. Yano *et al.* showed that 3 months after the transplantation of SPIO-labeled GFP-MSCs into the striatum with focal ischemia, only 2.7% of GFP-positive cells were still labeled with SPIO [46]. One approach to overcoming the dilution of the iron label due to cell division is to use MR reporter genes that rely on the enzymatic or metabolic production of a MR contrast agent or the expression of MR contrast surface receptors [47].

Another aspect of cell labeling with contrast agents is their effect on the regenerative potential of stem cells after transplantation. Guzman *et al.* showed that labeling human neural stem cells with magnetic nanoparticles does not adversely affect their survival, migration or differentiation, or alter neuronal electrophysiological characteristics [48]. By contrast, a recent study by Modo *et al.* showed that gadolinium–rhodamine–dextran-labeled neural stem cells, when transplanted into the contralateral hemisphere to a middle cerebral artery occlusion, migrated to the peri-infarct area, but did not significantly improve behavior compared with cells labeled with a fluorescent dye and led to a slight increase in lesion size 1 year following engraftment. This study highlights the necessity of long-term studies, since most contrast agents were not originally designed for cellular imaging [34].

Nanofibers as scaffolds in spinal cord repair

Spinal cord injury often results in permanent paralysis as a consequence of the inability of axons to regenerate across the lesion. Although the adult spinal cord is currently known to be capable of functional reorganization and axonal sprouting and, similarly as other regions of the adult CNS, the adult spinal cord contains neural stem and progenitor cells [49], there are several factors that hinder the intrinsic regenerative capacity of the spinal cord.

First, the process of tissue remodeling after spinal cord injury leads to the formation of a dense glial scar that develops days to weeks after the injury onset. Reactive astrocytes migrate to and accumulate at the lesion site and further contribute to the formation of a physical barrier with their increased expression of extracellular matrix molecules such as chondroitin sulfate proteoglycans, which have been shown to inhibit neurite outgrowth [50]. The scar barrier, which hinders axonal regeneration, is additionally potentiated by the formation of cysts, characteristic of the chronic phase of injury [51], and by endogenous factors that inhibit axon growth in the mature mammalian CNS such as myelin-associated inhibitors, semaphoring 3 and ephrin-B2 [50]. Together, these events lead to the formation of a 'communication barrier' with an adverse effect on the reconnection between the cranial and caudal stumps of the spinal cord.

There are various experimental approaches to the reconstruction and regeneration of the damaged spinal cord, including the application of neurotrophic or neuroprotective agents, the elimination of scar-inhibitory molecules [50] or cell therapy using various types of stem or progenitor cells [7]. A promising strategy for dealing with this impermeable obstacle is 'bridging' the lesion with a permissive environment that would support axonal re-growth and the re-establishment of the damaged connections. The development of such biological constructs or scaffolds aims to repair neural tissue with a 'tissue friendly' replacement that may serve simultaneously as a substrate for cell transplantation and as a drug-delivery system. Generally, the structure of the scaffold is designed to reduce and/or fill the gap left after injury and to promote new tissue formation and functional recovery; thus, the scaffold design must consider parameters such as biocompatibility, controlled porosity and permeability, suitable mechanical properties comparable with the tissue, biodegradability and, additionally, support for cell attachment, growth and differentiation.

As scaffolds in neural tissue engineering, various synthetic as well as natural biomaterials have been used to test their potential *in vitro* as well as *in vivo*. Synthetic biodegradable or nondegradable porous hydrogels were developed from poly(2-hydroxyethyl methacrylate),

poly (2-hydroxypropyl)-methacrylamide (PHPMA) [44], biodegradable poly-β-hydroxybutyrate [52], polyethylene glycol or poly(lactic-co-glycolic acid) [53]. Various modifications of the polymers have been employed to improve their properties in terms of enhanced cell adhesion, growth or differentiation through the use of laminin-derived peptide epitopes containing integrin receptor-binding sites [44], various functional groups with positive or negative charges [54], the cholesterol hydrophobic moiety [55], the immobilization of neurotrophic factors [56] or the construction of aligned pore orientation [57]. PHPMA-RGD hydrogels implanted into a chronic spinal cord lesion, either with or without seeded MSCs, can successfully bridge a spinal cord cavity or provide a scaffold for tissue regeneration. Behavioral analysis showed a statistically significant improvement in rats with combined treatment, hydrogel and MSCs, 6 months after implantation compared with the control group and, furthermore, this therapy prevented tissue atrophy [44,58].

In addition to polymeric hydrogels, a promising innovation in tissue regeneration has been the development of nanofiber materials [59]. The porous structure of the nanofibrous network mimics the architecture of the extracellular matrix, and the high surface area to volume ratio has been shown to support the adhesion, proliferation and differentiation of various cells [59]. Although the potential of nanofibers encompasses a broad scope of nonbiological applications, such as filtration, acoustics and energy, the use of nanofibers in biomedicine is currently under intensive study. Nanofibers have been developed not only as scaffolds for wound dressings [60] and for bone, cartilage, skeletal and smooth muscle, blood vessel and neural tissue engineering [59], but also for enzyme immobilization [61] and drug-delivery systems [62].

There have been various processing techniques used to produce nanofibers: electrospinning [63,64], molecular self-assembly [65], drawing out [66] or thermally induced phase separation [67], and catalytic synthesis [68]. Currently, electrospun, self-assembled and carbon nanofibers have been studied as potential scaffolds for neural tissue engineering.

Electrospun nanofibers

In the electrospinning process, nanofibers are created as polymeric jets from the surface of a polymeric solution in a very high intensity electrostatic field (10–100 kV). The nanofibers can be formed either at the tip of a capillary tube (needle or capillary spinners) or from liquid surfaces on a rotating spinning electrode (needleless technology) [102]. Compared with needle electrospinning, the needleless technology enables the production of nanofibers on an industrial scale. Our results confirm that nanofibers produced by the needleless Nanospider™ technology support stem cell growth and proliferation (**Figure 2A & B**) [69]. As potential scaffolds for neural tissue engineering, various electrospun nanofibers have been studied as substrates for neuronal cell cultivation [37,70]. Modification of the electrospinning technique enables the production of nanofibrous substrates with parallel-aligned fibers. Aligned poly(L-lactic acid) nanofibers supported an increased rate of neural stem cell differentiation and neurite outgrowth along the direction of the fiber orientation, compared with randomly oriented nanofibers [71]. Similarly, oriented neurite outgrowth and glial migration from dorsal root ganglia explants were found with aligned electrospun nanofibers of poly-ε-caprolactone, a collagen/poly-ε-caprolactone blend [72] or poly-L-lactate [73].

According to numerous *in vitro* studies, nanofibrous scaffolds, and particularly those with oriented fibers, can serve as suitable guidance conduits for cell therapy and nervous tissue repair. However, currently only a few studies have attempted to verify the potential of electrospun nanofibers in *in vivo* experiments. As scaffolds for regeneration in a model of peripheral nerve injury, grafts of nonwoven micro/nanofiber mesh tubes made from electrospun chitosan [74] or from a poly(lactic-co-glycolic acid)/poly-ε-caprolacton blend were developed [75]. These nerve conduits were shown to be promising bioabsorbable scaffolds for stimulating and guiding functional peripheral nerve regeneration in a rat model of sciatic nerve transection, although functional and histological results showed that the nerve regeneration process was delayed in the graft relative to autografts [74]. In the spinal cord, scaffolds based on layers of polymer nanofibers were implanted into the hemisected spinal cord (**Figure 2C & D**), where they integrated into the surrounding tissue and promoted the ingrowth of connective elements, blood vessels and neural cell processes (**Figure 2E–H**). The extent of the ingrowth was dependent on the spatial orientation of the nanofiber layers [44].

As an attempt to integrate cells into a tissue-engineered scaffold, a tubular scaffold, containing bundles of parallel nanofibers made of a biodegradable dextran sulfate–gelatin co-precipiate seeded with human nasal olfactory mucosa cells

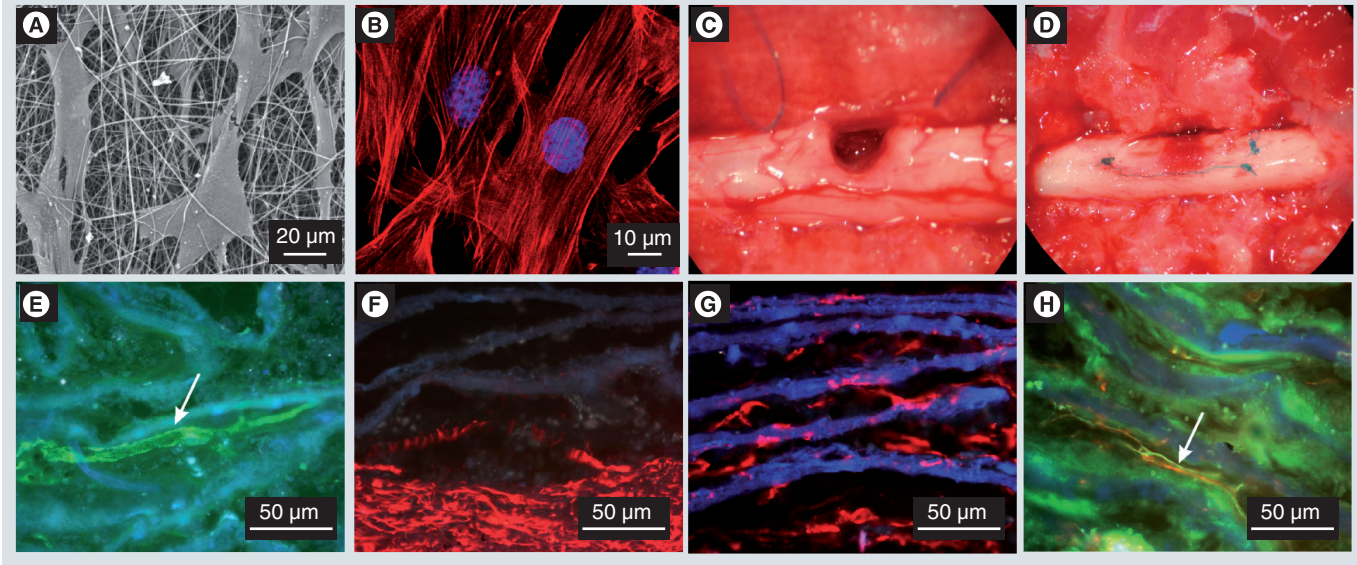

Figure 2. Electrospun nanofibers as scaffolds for spinal cord injury. (A) Representative scanning-electron micrograph of human mesenchymal stem cells cultured on gelatin nanofibers prepared by the needleless Nanospider™ technology. **(B)** Confocal micrograph of human mesenchymal stem cells cultivated on gelatin nanofibers; immunofluorescent staining for F-actin (phalloidin) and cell nuclei (DAPI). **(C)** A rolled nanofiber scaffold was implanted into a hemisection of a rat spinal cord. **(D)** The dura mater, muscles and subcutaneous tissue were sutured in anatomical layers. **(E)** Blood vessel (RECA staining) ingrowth into the nanofibrous scaffold. The layers of the nanofiber scaffold are visible in blue color **(F)** A few astrocytic processes (GFAP staining) grew into the nanofibrous scaffold. **(G)** The ingrowth of Schwann cells (p75 staining) on the layers of the scaffold. **(H)** The ingrowth of NF-160 neurofilaments (green) and Schwann cells (red) into the nanofibrous scaffold. All immunohistological stainings were evaluated 4 weeks after implantation.

or human embryonic spinal cord cells, was used as an implant for the regeneration of rat spinal cord transection [76]. Physiological and behavioral analyses revealed partial recovery of function in one or two limbs 3 months after implantation; recovery was enhanced when human embryonic spinal cord cells were implanted.

Self-assembling peptide nanofibers

Self-assembling nanofibers are based on the synthesis of peptide amphiphile molecules. These peptides are characterized by their periodic repeats of alternating positive and negative L-amino acids, which in the presence of a physiological salt-containing solution spontaneously self-assemble from the aqueous solution into a stable nanofiber matrix [77]. Since the nanofibrous matrix is formed by the ionic strength of the *in vivo* environment, self-assembling nanofibers can be injected into the injured nervous tissue as a liquid without the necessity of an additional surgical procedure. This unique property gives these molecules great therapeutic potential in clinical application.

Several peptide scaffolds have been shown to allow cell attachment and differentiation. These peptides can be chemically designed to incorporate specific functional ligands, such as peptide epitopes containing integrin receptor-binding sites, for example RGD, IKVAV or RADA, which further advance scaffold application. Peptide amphiphile molecules containing IKVAV sequences were found to promote the outgrowth of processes from cultured neurons and to suppress astrocytic differentiation of cultured neural progenitor cells [78]. *In vivo* injection of these nanofibers into a spinal cord compression lesion reduced astrogliosis and cell death and increased the number of oligodendroglia at the site of injury. Furthermore, IKVAV self-assembling nanofibers promoted the regeneration of both descending motor and ascending sensory fibers through the lesion site and resulted in significant behavioral improvement [79]. The injection of self-assembling peptides into the wound site of an optic tract transection not only permitted significant axonal growth through the site of the treated lesion, partially restoring the optic tract, but also resulted in the return of functional vision in brachium transected experimental animals [80]. Another peptide scaffold modified with RADA16-I sequences supported the growth of PC12 cells and the formation of functional synapses *in vitro* using rat primary hippocampal neurons [81]. RADA16-I self-assembling scaffold was also shown to support the attachment and differentiation of neuronal precursor cells and Schwann cells *in vitro* as well as *in vivo*, and to bridge the injured spinal cord of rats after *in vivo* transplantation [82].

Carbon nanofibers are being investigated as candidates for neural tissue engineering owing to their electrical conductivity and also good cytocompatibility. Carbon nanofibers studied as neural implants selectively supported the growth of neuronal cells and reduced the adhesion of astrocytes [68]. Carbon nanofibers have also been tested as a subcellular electrical–neural interface, which is desirable for implantable biomedical devices [83].

Future perspective & conclusion

The development of efficient paramagnetic labeling together with a noninvasive MRI technique enables researchers as well as clinicians to follow the fate and survival of transplanted cells in the host organism. It can allow the effect of cell therapy in patients with various disorders and brain or spinal cord injuries to be evaulated and, consequently, establish the optimal conditions for transplantation in terms of the number of transplanted cells, the route of administration and the therapeutic window. Nevertheless, the *in vivo* assessment of the effect of contrast agents on stem cell function and their therapeutic potential must be more thoroughly studied from a long-term aspect. A future goal for the development of contrast agent labeling that goes beyond simply cell localization is the monitoring of functional cell status, in which the contrast agent becomes detectable by intrinsic activation mechanisms due to particular enzyme expression [84] or via upregulation by the activation of the corresponding promotor in transgenic cells [85,86].

In the case of large lesions, however, cell transplantation alone is not sufficient for tissue regeneration, and thus it is necessary to bridge the lesion site with a permissive environment that fills the cavity, reduces the glial scar and enables axonal ingrowth. In addition to various types of biomaterials, nanofibers, especially self-assembling nanofibers, are suitable implantation materials whose chemical and structural properties can be tailored to promote cell adhesion, growth and differentiation, or that can be coupled with various drugs, growth or other factors improving regeneration.

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Executive summary

Cell therapy in the CNS

- Adult mesenchymal stem cells have promising therapeutic potential in human medicine for CNS cell therapy and are already used for autologous transplantation in clinical trials.

Magnetic nanoparticles for labeling stem cells

- To monitor cells *in vivo*, superparamagnetic iron-oxide (SPIO) nanoparticles, as contrast agents visualized by MRI, are used for cell labeling.

In vivo tracking of stem cells in brain & spinal cord lesions

- MRI detection as well as histological analysis demonstrated that grafted adult as well as embryonic stem cells labeled with SPIO nanoparticles migrate into a lesion site in the brain as well as in the spinal cord, where they then elicit functional improvement.

Nanofibers as scaffolds in spinal cord repair

- Spinal cord injury is accompanied by tissue damage and the formation of physical and biochemical barriers that prevent axons from regenerating. Biologically compatible nanofiber or hydrogel scaffolds can serve as a permissive bridge for axonal regeneration and the re-establishment of damaged connections.

Electrospun nanofibers

- Electrospun nanofibrous scaffolds with oriented fibers can serve as suitable guidance conduits for nervous tissue repair.

Self-assembling peptide nanofibers

- Self-assembling nanofibers with incorporated specific functional ligands promote the growth of cultured neural cells and improve regeneration after their injection into a spinal cord lesion.

Conclusion

- Stem cells have been shown to selectively target injured brain and spinal cord tissue and to improve functional recovery.
- SPIO labeling together with a noninvasive MRI technique enables the fate of transplanted cells to be followed in the host organism and establishes optimal conditions for transplantation.
- Biologically compatible nanofiber or hydrogel scaffolds can serve as a permissive bridge for axonal regeneration and the re-establishment of damaged connections.

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