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Carbon nanotubes in neuroregeneration and repair ☆

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ABSTRACT

In the last decade, we have experienced an increasing interest and an improved understanding of the application of nanotechnology to the nervous system. The aim of such studies is that of developing future strategies for tissue repair to promote functional recovery after brain damage. In this framework, carbon nanotube based technologies are emerging as particularly innovative tools due to the outstanding physical properties of these nanomaterials together with their recently documented ability to interface neuronal circuits, synapses and membranes. This review will discuss the state of the art in carbon nanotube technology applied to the development of devices able to drive nerve tissue repair; we will highlight the most exciting findings addressing the impact of carbon nanotubes in nerve tissue engineering, focusing in particular on neuronal differentiation, growth and network reconstruction.

Contents

Abbreviations: CNS, central nervous system; DRG, dorsal root ganglia; ECM, extracellular matrix; EN, ethylenediamine; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; fwCNT, few-walled CNT; KCC2, potassium chloride cotransporter 2; LBL, layer-by-layer; MAP2, microtubule-associated protein 2; MEA, multi-electrode array; MSC, mesenchymal stem cells; MWCNT, multi-walled carbon nanotubes; NCAM, neural cell adhesion molecule; NGF, nerve growth factor; NSC, neural stem cells; PAA, poly(acrylic acid); PABS, poly-m-aminobenzene sulfonic acid; PEG, polyethylene glycol; PEI, polyethyleneimine; PLCL, poly(L-lactic acid-co-caprolactone); PLO, polyornithine; SEM, scanning electron microscope; siRNA, small interfering RNA; SMI-32, antibody recognizing non-phosphorylated neurofilaments; SWCNT, single-walled carbon nanotubes.

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1. Introduction

A decade of advances in nanotechnology has disclosed exciting perspectives and innovative approaches for tissue regeneration and, more recently, for nerve tissue repair [1,2]. Although still limited, the application of nanotechnology-based platforms to neuroscience witnessed an impressive growth, with an increasing amount of studies proposing nanomaterial based scaffolds as novel tools able to tune nerve cell behaviour. In this scenario, carbon nanotubes are placed as timely and promising players. Such materials are at the leading edge of nanotechnology, due to carbon nanotube well documented electrical, thermal and mechanical properties [3]. Carbon nanotube cylindrical morphology is reminiscent of that of distal neuronal dendrites [4], small cellular processes crucially involved in the ability of neurons to express complex computational skills. This similarity, together with carbon nanotube topographic features, physical properties, as conductivity, and surface-to-volume ratio [5], sets the stage for carbon nanotube exploitation in devices able to interface neuronal physiology. This review will highlight the most exciting findings that challenge carbon nanotubes in the neuroscience arena.

2. Nanomaterials: supports for neuro-reconstruction

The development of neuro(prosthetic)-implants to favour the survival of damaged neurons, or axonal regrowth, and neuronal synaptic signal transmission, holds the promise to contrast the functional impairment that follows neuronal loss or degeneration. In this perspective, the search for new materials able to support these processes appears critical. Since a decade, nanotechnology is providing a significant contribution to this field, increasingly attracting the attention of the clinical neuroscience community [6].

Ideally, any strategy developed to repair the damaged central nervous system (CNS) should address the regrowth of injured axons, the plastic remodelling of neuronal circuitry [7] and/or the generation of new neurons by the use of the high-potential stem cell transplantation [8–11]. Each of these objectives requires governing several complex processes: axons regrowth requires overcoming an unfavourable, and inhibitory environment, documented, for example, in the injured area after spinal cord lesions [12], and requires proper axonal spatial organization, target recognition and the reconstruction of functional synapses. Stem cells successful transplantation requires cell survival and appropriate differentiation toward the neuronal lineage. The design of effective strategies for neuroregeneration-supporting scaffolds has to take into account all of these steps: while some of them have been achieved in in vitro experimental systems, their successful translation in the in vivo condition is still sparsely reported.

In this scenario, neuro-repair strategies and tissue engineering are strictly interconnected. Cells in general, and neurons in particular, are able to self-organize in complex structures when residing in an appropriate physico-chemical environment, and large efforts are currently made to develop synthetic material-based implants to be applied as scaffolding structures to provide a biocompatible and bioactive support for the promotion of cellular reorganization toward a functional neuronal assembly [13–16]. The ideal material should meet several requirements. First, it should be biocompatible, non-immunogenic and it has to avoid gliotic reactions and scar formation; second, it has to favour neuronal differentiation (in the case of stem cell-based strategies) and axons extension, it has to support plastic re-arrangements of resident neuronal networks and endogenous extracellular matrix (ECM) deposition, while potentiating the residual ability of CNS neurons to regenerate [14,17]. In this respect, synthetic nanomaterials appear as promising candidates: they can be produced with more control and reproducibility than their natural counterparts (thus strongly limiting the problems of biosafety and immunogenicity) and they can be engineered as biocompatible platforms able to promote neuronal regeneration across injured areas, and to synergistically contribute to the controlled and localized delivery of regeneration-supporting drugs (e.g. trophic factors) [14,18].

2.1. Reasons why we should apply nanomaterial in tissue repair

The use of nanomaterials in the design of tissue scaffolds in the CNS is primarily due to their abilities to favour neuronal adhesion, to re-create an ECM-like microenvironment and to interact with neuronal membranes at the nanoscale [2]. In fact, a fundamental step, in any strategies aimed at improving CNS regenerative ability, is the manufacturing of scaffolds which are able to control (and to selectively tune) cellular adhesion [19], to govern axonal regrowth and neuronal physiology [20–22].

Growth substrates with a nanostructure similar to the finest neuronal processes of axons and dendrites allow an unprecedented control in the interactions between neuronal membranes and the nanomaterial in itself. Although, in principle, both micro and nanometer scale topographies are able to impact on cellular morphology and proliferation, likely via the bio-mimicry of environmental cues [2], the interaction of neurons with their growing substrate is governed by mechanisms which mainly occur at the nanoscale level. In growing neurons, sensing the extracellular environment is accomplished by various adhesion structures (e.g. neural cell adhesion molecule — NCAM, N-cadherin and integrins [20–22]), which are extremely sensitive to (and modulated by) the substrate features, such as nanotopography or physico-chemical properties. In particular, membrane contacts with the growing environment largely rely on the presence of adhesion sites (focal adhesions) in the 5–200 nm range that, accordingly, are sharply affected by substrate nanotopographical features [23,24]. Furthermore, neuronal adhesion is favoured when the roughness of the growth substrate matches the size of neuronal processes, and neurons show a marked preference for nanorough surfaces (like e.g. carbon nanotube films [25]), able to guide neurite extension (see below). The enrichment of current materials, traditionally used to improve regeneration in lesioned tissues (namely collagen, polysaccharides, self-assembling peptides, ECM-like materials [18]) with synthetic nanomaterials holds the concrete potential of improving neuroregenerative processes. This could be achieved also via the controlled presentation of tissue specific instructions in nanostructured platforms, able to support neuronal differentiation and to direct (re)growth [14,24,26–28].

Polymeric materials employed in the design of neuroregeneration-promoting platforms, although able to provide a biomimetic environment, are deprived of any electrical conductivity. Recently, it has been suggested that non-conductive biomaterials currently used in scaffold design may limit the engineering of electrically propagating tissue [2]. This observation hints at the use of tailored composite scaffolds obtained by blending conductive nanomaterials with traditional bio-materials. Carbon nanotubes possess high electrical conductivity and several nanotube-based CNS applications are being developed, such as neural prosthesis for monitoring neural activity. Recently, carbon nanotubes have attracted tremendous attention for the development of nano-bio hybrid systems able to govern cell specific behaviours in cultured neuronal networks [29–35].

3. Carbon nanotubes as nerve tissue reconstructing platforms

Carbon nanotubes are cylindrical nanostructures made up of graphene sheets wrapped onto themselves [36]. In neuroscience applications, the mostly used geometries are single-walled carbon nanotubes (SWCNT), made up of a single graphene sheet rolled-up and closed at its ends by hemispheric fullerene caps, and multi-walled carbon nanotubes (MWCNT), made up of several concentric graphene cylinders. Currently, carbon nanotube-based applications in neuroscience include: electrical interfaces for neuronal stimulation and recording (that drastically improve the electrode performance, both in vitro and in vivo [5,29,30,37,38]) as well as platforms to promote neuronal survival, differentiation, growth and performance [31–35,39–49]. Starting more