Review article

A review of nanotechnology strategies for neuron regeneration after spinal cord injury

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Abstract: It was estimated that 2.5 million people have spinal cord injury, which more than 130,000 new injuries reported each year. These patients have serious complications. To date, there are not any available definite and reliable clinical treatments for spinal cord injury to restores the injury-induced loss of function to reach a degree that an independent life can be guaranteed. In the last decade, with the emerging of nanotechnology, nanomaterials such as nanowires, nanofibers, nanoparticles, liposomes, and carbon-based nanomaterials were offered for effective treatments of spinal cord injury. The use of nanotechnology offers promising future perspectives for spinal cord injury treatment. This article reviews the recent applications of the most widely used nanomaterials such as nanowire, nanofiber, graphene and nanotube for SCI treatment.

Keyword: Spinal Cord Injury; Treatment; Nanotechnology; Graphene; Nanotube; Nanofiber; Nanowire

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1. Introduction

The normal architecture of the human spinal cord can be radically disrupted by injury that interferes with the signals transduction (1, 2). The main reason associated with treatment failure of spinal cord injury (SCI) is damage of axons that can't spontaneously regenerate, and prevent recovery of axonal circuits involved in the function after injury (3). SCI leads to many serious complications such as infections in the bladder, kidneys, bowel problems, and cardiac and respiratory dysfunctions (1, 4, 5). All of these problems have a strong impact on the quality of life (6). In the worldwide, was estimated that 2.5 million people lives with SCI, that more than 130,000 new injuries reported each year (2). Due to the considerable personal and social impacts of SCI and its high prevalence, it is a medical emergency so that, should be explored therapeutic strategies for target the SCI (1). Available treatments of SCI have involved medication, surgery, stem cell transplantation, molecular therapy and tissue engineering. To date, there isn't any available definite and reliable clinical treatments for patients with SCI that restores the injury-induced loss of function to reach a degree that an independent life can be guaranteed (7).

During the last decade, nanotechnology has raised as a required technology for producing of great scientific developments in medicine and health care. Nanomaterials including nanowires, nanofibers, nanoparticles, and carbon-based nanomaterials have been used to provide more effective treatments for SCI. Although the use of new techniques based on nanotechnology is still very young and have not yet led to a definitive cure but reveal possible treatment revolution.

Many researchers are working on the SCI treatment based on nanotechnology, and analyzing of all of them is more than the scope of this article. In this review, for enhancing the future researches quality, after a review of available treatment methods, we've focused on the most widely used nanomaterials including nanowire, nanofiber, graphene and nanotube for treatment of SCI. In this article, our purpose is, finding of the missing parts and taking a step to facilitate future researches.

2. SCI treatment

2.1. Conventional therapies

For treatment of SCI, there are several conventional methods with benefits and drawbacks (Table 1). Typically, the physicians stabilize and decompress spinal

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Table 1: Conventional methods for treatment of SCI

Method	Advantages	Disadvantages
Surgery	Remove fragments of bones and foreign objects, avoid the pain and spinal deformities in the future.	Surgery cannot reverse damage to the nerve cell and spinal cord.
Medications: Intravenous methylprednisolone	Reducing damage to nerve cells. Decreasing inflammation near the site of injury.	Immune suppression followed by increased susceptibility to infections (pneumonia, infection, etc.). Increasing the risk of gastrointestinal abnormalities (ulcers, bleeding and bowel obstruction), hyperglycemia, adult respiratory distress syndrome, deep vein thrombosis and pulmonary embolism.
Stem cell	Self renewality. Ability to become any cell in the body.	Ethical concerns. Human neural stem cell tendency to generate more glial cells, especially astrocytes, than neurons in natural states.
Molecular therapy	Control axonal growth using activator and inhibitor molecules and moderate the inflammatory responses.	Uses of these treatment methods are usually not sufficient alone, and in combination with other methods are used.
Tissue engineering: hydrogels	Bridging the spinal cord injury and reconstructing the damaged connections.	Natural hydrogels biodegradation rate is hard to control. The most used synthetic hydrogels are non-degradable. Hydrogels may elicit immune reactions from the host where they will be implanted and heterogeneity between batches may also be observed.

cord by surgery that associated with high dose methylprednisolone (MP) therapy (8-10). There are much controversy in the use of surgical procedure and MP because there is no consensus about the real beneficial effects of these two methods (1). Surgery is recommended for many reasons such as removing bone fragments, foreign bodies, blood clots, broken vertebrae, herniated discs, spinal tumors and etc. (8). If the vertebrae of the spine appear unstable, the doctor may do a spinal fusion. Spinal fusion may be done by metal plates, screws, wires and / or metal rods and sometimes small pieces of bone from other parts of the body (usually hip or knee) or from a cadaver (bone bank). Surgery cannot reverse damage to the spinal cord but is often needed for stabilizing of the spine to prevent future pain or deformity (8).

MP with anti-inflammatory properties has been used for treatment of acute SCI. When the MP is administered within 8 hours after spinal cord injury, some sensory and motor improvement have been reported (9). In recent years the use of MP has been challenged. Some evidence showed that harmful effects of MP, including immune suppression followed by increasing of susceptibility to infections (pneumonia, infection, etc.), increasing the risk of gastrointestinal abnormalities (ulcers, bleeding and bowel obstruction), hyperglycemia, adult respiratory distress syndrome, deep vein thrombosis and pulmonary embolism (11-15).

Self-renewality and ability to be any cell in an organism made stem cell an attractive candidate to contribute to SCI (7). Aguayo's and his colleagues in the late 1970s were pioneer that showed peripheral nerve grafts promoted regeneration of CNS axons in the cell therapy (16) and Reier's group showed that grafted fetal spinal cord supported regrowth of host axons (8). In 2010, a Swiss agency has sponsored phase I/II clinical trial on treatment of chronic spinal cord injury using stem cells. The company has used human neural stem cells (hNSCs) and the patient immune system has suppressed for 9 months after transplantation. Interim analysis of clinical data in May 2014 has shown that the significant post-transplant was gained in sensory function first reported in two patients that now have been observed in two additional patients (17). Transplantation of Different stem cells was used by researchers for SCI treatment such as mesenchymal stem cells (18-22), induced pluripotent stem cells (iPSCs) (23-26), Schwann cells (27-32), olfactory ensheathing glia (33, 34), neurotrophin-expressing

fibroblasts (35-37), activated macrophage (38-40) and Embryonic stem cells (41-44).

Promising results have been obtained from cell therapy of an in-vivo model of SCI. Despite the hopes of repairing SCI using stem cell transplantation, is an issue that need to be resolved, including ethical concerns (7) and human neural stem cells tendency to generate more glial cells, especially astrocytes, than neurons in natural states, even though neurons are largely considered to be more critical than glial cells for the treatment of SCI (45).

In recent years, molecular therapy represent significant advances towards the better understanding of the mechanisms involved in the SCI, control of axonal growth using activator and inhibitor molecules and moderates the inflammatory responses. It is hoped that with the development of this science, we can develop therapeutic strategies to promote axonal regeneration through the injured spinal cord and reduce the damage caused by SCI (46, 47). Anti-inflammatory factors such as interleukin-10 (47), minocycline (48-50), non-steroidal anti-inflammatory drugs (NSAIDs) (51-56), erythropoietin (55, 57), riluzole (58-60), have been used to treat SCI by molecular therapy. Different researchers worked on many axonal promoting factors such as synthetic glycolipid (61) and Neurotrophic factors (62, 63). Using of these treatment methods is not sufficient alone, and usually are used in combination with other methods.

In recent years, studies have begun to explore the possibility of using tissue engineering technology to repair SCIs. Tissue engineering is an emerging area in biomaterial research that possesses great therapeutic potential (64, 65). One of the primary goals of SCI treatment is to bridge the SCI and reconstruct the damaged connections. Hydrogels are biocompatible implants used in tissue engineering in SCI patients. They built a permissive environment and bridge the lesion cavities and they act as a scaffold for the regeneration of neurons and their axons, glia and other tissue elements.

The important specifications of scaffold are a simpler design, easier to transplant and suitable for various types of injury. Collagen (66, 67), Alginate (47, 68, 69), Poly (α-hydroxy acids (Poly (alpha-hydroxy acids), Methacrylate-based hydrogels (19), chitosan (70-72), synthetic hydrogels (19, 73), Polyethylene glycol (74, 75), Fibrin (76, 77), Fibronectin (78), Agarose (79, 80), Silk fibroin (81-83) have been used as a scaffold for treatment of SCI injury. Although usage of hydrogels are the most promising tools for treatment of the spinal cord (12, 84), there are also some challenges: the natural hydrogels degradation rate is hard to control and the most used synthetic hydrogels are non-degradable (85). Hydrogels may elicit immune reactions from the host where they will be implanted and then heterogeneity between batches may also be observed (19).

2.2. New Medical approaches based on Nanotechnology

Although many studies in recent years have promised for the future, and many groups are working on development of treatments that address the SCI injury, but to date, almost all therapies have failed to be effective and it is urgent to develop new therapeutic strategies to treat SCI patients (6).

Nanotechnology contain an enormous number of nanostructures with the size of 1 to 100 nm (86). The result of their small size have different structural and functional properties from bulk materials such as chemical reactivity, electrical conductance, optical effects, magnetism, and physical strength (87, 88). The use of Nanotechnology offers promising future perspective for treatment of incurable diseases such as SCI. In the following, we reviewed nano-materials with more attention in SCI treatment.

2.2.1 Graphene

A two-dimensional sheet of carbon atoms arranged in a hexagonal grid, that makes for the thinnest, most electrically and thermally conductive material in the world while still being flexible, transparent and super strong (Figure 1) (89).

Graphene's photo-conversion efficiency, biocompatibility and flexibility in size, coupled with mechanical strength, are beneficial for making composite bio-materials. Its electrical conductivity can be applied to organs with properties, like nerve tissues and spinal elements (89).

Graphene has been used for two goals in SCI treatment: scaffold for neural cell growth and directing hNSCs to neurons than glial cells (90).

Park and colleagues in 2011 showed neuronal differentiation of hNSCs enhance on grapheme (91). They found graphene worked as an excellent cell-adhesion layer during the long-term differentiation process and induced the differentiation of hNSCs more toward neurons to glial cells. They also found good electrical conductivity of graphene helps the differentiation of neurons. Their results suggested that graphene can be used as an excellent nanostructure scaffolds for promoting neuron stem cell adhesion (91).

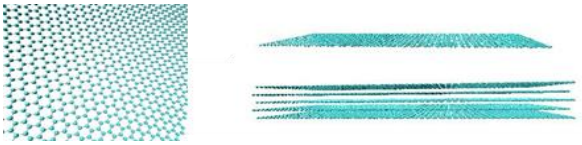

Figure 1: A visual depiction of the structure of a microscopic segment of graphene, one layer graphene and multi layered graphene.

In 2014, Serrano reported that novel free-standing, porous and flexible scaffolds of 3D graphene oxide with potential for neural tissue regeneration. Embryonic neural progenitor cell adhesion, morphology, viability, and neuronal/glial differentiation were investigating (92). Highly viable neural networks were formed on these 3D scaffolds, contributed from both neurons and glial cells and full of axons, dendrites, and synaptic connections (92).

Biocompatibility of graphene for interface with cortical neurons of rat was investigated by Sahni and colleagues in 2013. In their experiment, lactate dehydrogenase levels were measured as cytotoxicity markers. There was little difference in cell viability between surface coated with graphene and uncoated surfaces (93). They indicated that graphene was not more cytotoxic than the bare control surface. Phase contrast microscopy showed the attachment of neurons to the graphene-coated surface and their ability to extend longer. Neurotic processes showed that neuron had normal morphology and metabolism (93).

Graphene foam (a three-dimensional porous structure), as a novel scaffold for neuronal stem cell was used for the first time by Li and colleagues in 2013 (87). It was revealed that three-dimensional graphene foams not only can support neuron stem cell growth, but also in comparison between the two dimensional graphene films, keep cell at an active proliferation state than that of two-dimensional graphene films.

Phenotypic analysis showed that three-dimensional graphenes can increase the neuron stem cell differentiation towards astrocytes and in particular neurons. In addition, good electrical coupling of three-dimensional graphene, with differentiated neuron stem cells for efficient electrical stimulation was observed (94).

2.2.2 Nanotube

A carbon nanotube (CNT) is a one-atom thick sheet of graphene rolled up into a seamless cylinder with diameter of the order of nanometer (Figure 2) (95).

Since the beginning of the 2000s, CNTs have been introduced in pharmacy and medicine in therapeutics (96). CNTs have attracted a great deal of attention due to their unique superior strength, flexibility, electrical conductivity, and availability of chemical functionalization (96, 97). CNT is a good candidate for scaffold and drug carriers in neuronal tissue because:

- CNTs structural characteristics and dimensions are similar to the neural machinery elements (cytoskeletal elements in neurons, ion channels and signalling proteins).
- CNTs can show the electrophysiological activity of nerve cells. The electrical properties of CNT can be designed in a way that is proportional to the charge transport features of neuro-electrical neural interfacing.
- The CNT mechanical and chemical properties are suitable for prolonged implantation in neuronal tissue.
- CNT biocompatibility and biodegradability in the neural tissue (98).
- The conductivity of the CNT is stable in biological environments and will not degrade when it oxidizes in aqueous solution (99).
- Easy functionalization of the insoluble pristine CNT that enhance its aqueous dispersibility and has licensed their application in physiological environments including the nervous system (100).

The first use of carbon nanotubes in neuroscience investigation was reported in 2000 by Mattson et al. (97) who grow rat-brain's neurons on multiwall carbon nanotubes (MWCNTs). Only one or two neurite extended on the uncoated nanotubes, but when MWCNTs were covered with biomolecules 4-hydroxynonenal, the neurons exhibit extensive branching. The findings of this study showed nanotubes can be used as a suitable substrate for the growth of nerve cells. Chemical functionalized CNTs were used for this purpose by researchers. Jose A. Roman and colleagues in 2011 showed that single-walled carbon nanotubes coated with polyethylene glycol (SWNT-PEG) increased the length of selected neurites in vitro. They found that after SCI administration of SWNT-PEG, neurofilament-positive fibers and corticospinal tract fibers in the lesion increased, and reactive gliosis did not increase. Therefore the size of the lesion reduced. In addition, SWNT-PEG caused recovery in hind limb locomotor without causing hyperalgesia (101).

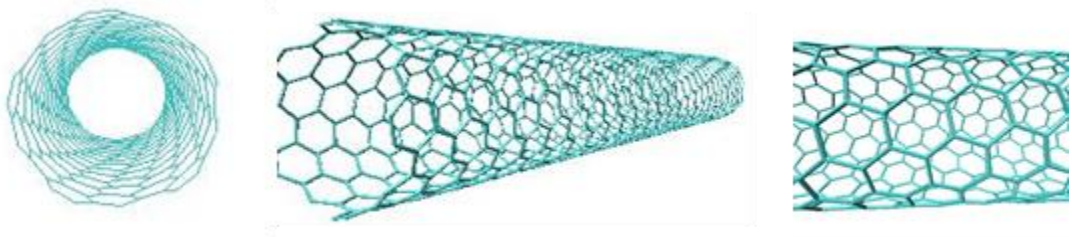

Figure 2: Schematic figure of the structure of nanotube.

Ding and colleagues in 2015 evaluated the neuroprotective role of functionalized MWCNTs carrying brain derived neurotrophic factor (BNDF), nogo-66 receptor (NgR) and Ras homolog gene family member A (RhoA) in SCI. Results showed that in the therapeutic group BDNF level was clearly increased while the expression of RhoA and NgR was decreased in the cerebral cortex. Treatment with functionalized MWCNTs diminished the spinal cord injury and promoted the functional recovery in SCI mice (102). Nanotube in combination with other substances as nanocomposite was used for the restoration of damaged neural tissue. For example, after experimental SCI, administration of the CNT/Nafion nanocomposite in a rat model, promote axon's regeneration into the lesion cavity and functional recovery of the hind limbs. CNT/Nafion nanocomposite caused a decrease in lesion volume, increase in neurofilament-positive fibers and corticospinal fibers in the lesion, and no increase in reactive gliosis. Additionally, after SCI, administration of CNT/Nafion nanocomposite induced a modest improvement in hind limb locomotor recovery without inducing hyperalgesia (103).

Gupta and colleagues in 2015 studied (104) the challenge of directional neuron growth on the MWCNTs / chitosan composite. Uniform distribution and alignment of MWCNTs in the chitosan matrix and good interfacial bonding, of carbon nanotubes (CNTs) improve the strength by 21.9%, elastic modulus by 12.7% and tensile strength by11.2%, as compared with the random MWCNT/chitosan scaffold. 50-60% of neurons are found to be aligned in the direction of MWCNT alignment in the scaffold (104). Chi-Shuo Chen in 2011 used CNT-silk composite scaffolds for improvement of neuron differentiation efficiency from human embryonic stem cells. In addition, axonal lengths for evaluating the progress of neuronal development were measured. Increasing of β-III tubulin and nestin expression on silk-CNT scaffolds, suggested augmented neuronal differentiation. In addition, longer axons with higher density were found to associate with silk-CNT scaffolds. The silk-CNT composite scaffolds can serve as a promising opportunity for nerve repair for SCI patients (105).

CNT direction in composite is an important factor in neural growth. Alignment of MWCNTs introduces highly anisotropic electrical conductivity (100,000 times higher) along its orientation, as compared to the transverse direction of the scaffold. This is the ideal requirement for a neural scaffold to guide cells in the appropriate direction (104).

The surface charge of CNTs can be used to control the neurite outgrowth. As Hu and colleagues showed in 2004, that more numerous growth cones, longer average length of neurite, and elaborate neurite branching are on the positively charged CNTs compare to neutral or negatively charged ones (101).

In interfacing nanotube with neural circuits, nanotube substrates in a narrow range of conductivity promote the outgrowth of neurites with a decrease in the number of growth cones as well as an increase in cell body area, while at higher conductance these effects disappear (99).

Nanotube has several disadvantages including: lack of solubility in aqueous media, and the biodistribution and pharmacokinetics of CNTs that was affected by many physicochemical characteristics such as shape, size, chemical composition, aggregation, solubility surface, and fictionalization (106).

2.2.3 Nanowire

Nanowires are extreme slender structures with a diameter on the order of nanometer scale in two dimensions, and their length is thousand times longer

This structure causes the electrons and photons within nanowire experience quantum confinement effects and also distinctive electrical and optical properties (107-110). Nanowire exists in many forms made of metals, semiconductors, insulators and organic compounds (107, 111). The composition can be altered to create heterostructures, make it possible to tailor the band structure and electronic properties of the wire (112). Nanowires are being studied for using in electronics, energy conversion, optics, chemical sensing and biological systems (107, 111). The morphology and high aspect ratio makes them interesting for applications as a substrate for neuron growth.

A gallium phosphide (GaP) nanowire that is biocompatible, III/V semiconductor with well-characterized electrical and optical properties was used by Hallstrom and colleagues in 2007 for neuron attachment and extension (112). Substrate covered by nanowires with 2.5 μm long and 50 nm wide, supported axonal growth. Cell survival was better on nanowire substrates than on planar control substrates (113). Then they investigated the neuron growth on the rows of GaP nanowires (114). The axons are prevented from crossing the rows and aligned in parallel in close to each other. The ability of cells to form distinct adhesion to individual nanowires is one of the mechanisms promotes axonal guidance (114).

Fredrik Johansson in 2008 attempted to induce guidance of nerve cells using magnetic Ni-nanowires. Dorsal root ganglia neurons from mice were cultured on the nanowire. Regenerated axons also displayed contact guidance on the wires. There were no overt signs of toxicity caused by the Ni-nanowires (115).

Bechara and colleagues in 2012 have developed polymeric scaffolds with nanowire surfaces that were biofunctionalized with an electro-conductive polymer that able to provide physiological levels of electrical stimulation to NSCs. The presented results showed that these nanowire surfaces enhance NSC adhesion, proliferation

and differentiation for up to 7 days of culture (116).

2.2.4 Nanofiber

The nanofibrous porous network structure highly resembles to the native extracellular matrix, and the high aspect ratio has been shown to promote the adhesion, proliferation and differentiation of various cells (117).

According to numerous in vitro studies, nanofibrous scaffolds, can serve as excellent guidance conduits for cell therapy and nerve tissue repair (117). Yang et al. in 2004, have studied the influence of the poly (l-lactic acid) (PLLA)-based electrospun nanofibrous scaffolds on neural stem cells (NSCs). Their results indicated that nanofibers oriented randomly (150–350 nm) in addition to stem cell adhesion, will made their differentiation as well (118, 119). Xua and colleagues found NSCs grown on Polyhydroxyalkanoates (PHA) nanofiber with copolymer 3-hydroxybutyrate /3-hydroxyhex-anoate (PHBHHx) is useful for repairing SCI injury. Compared to the 2D films, 3D nanofiber matrixes appeared to be more suitable for NSC attachment, synaptic outgrowth and synaptogenesis (120). Studies have shown the nanofiber diameter effects on the neuron growth. Christopherson in 2008 cultured rat hippocampus-derived adult NSCs on laminin-coated electrospun polyethersulfone nanofiber meshes with different diameters and demonstrated that fiber diameter significantly influences NSC differentiation and proliferation. NSCs showed a 40% increase in oligodendrocyte differentiation on 283-nm fibers and 20% increase in neuronal differentiation on the 749-nm fibers, in comparison to tissue culture polystyrene surface. As the fiber diameter decreased, higher degree of proliferation and cell spreading and lower degree of cell aggregation was observed (121).

According to studies, the use of nanofibers as a substrate increases the growth of neurons. The question is whether the structural order of nanofibers has any effect in the growth of neurons? Yang et al in another study tried to understand the role of aligned nanofibers in neural tissue engineering. The results demonstrated that NSCs elongated and their neurites outgrew along the direction of the fiber orientation of the aligned nanofibers. Further, it was observed increased rate of NSCs differentiation on aligned nanofibers (119). Also, Corey and colleagues in 2007 examined growth of neurites from dorsal root ganglia explants on electrospun poly- L -lactate nanofibers of high, intermediate, and random alignment. Neurites on highly aligned substrates were 20 and 16% longer than neurites on random and intermediate fibers, respectively (122). In 2009, Xie and colleagues understand the unique patterns of neurite outgrowth from primary dorsal root ganglia (DRG) cultured on scaffolds of electrospun nanofibers having different orders, structures, and surface properties. They demonstrated that the neurites when cultured on randomly oriented nanofibers extended radially outward from the DRG main body without specific directionality. In contrast, the neurites cultured on a parallel array of aligned nanofibers preferentially extended along the long axis of fiber. When seeded at the border between regions of aligned and random nanofibers, the same DRG in response to the underlying nanofibers simultaneously expressed aligned and random neurite fields. When cultured on a double-layered scaffold where the each layer nanofibers were aligned along a different direction, the neurites were found to be dependent on the fiber density in both layers. This biaxial pattern clearly indicates that neurite outgrowth can be influenced by nanofibers in different layers of a scaffold, rather than the top layer only (123).

Nanofiber diameter and orientation effects on the neuron growth and in the application of nanofibers, finding its suitable physical properties are challengeable. After becoming clear that neuron growth on the nanofiber has a large impact on neuron regeneration, effects of nanofiber surface modification on neuron growth were investigated. When polymers are convert to nanofibers, many of their properties, including antibacterial properties, biodegradability, cell adhesion and proliferation, and mechanical properties changed and need to be regulated by controlling the structure and properties of the nanofibers or compositing with other synthetic polymers (124).

Li and colleagues in 2007 used collagen-modified nanofibers (from polymer with different amount of carboxyl groups) for neural stem cell culture, and unmodified nanofibers were used as a control. Results indicated that the modification of collagen could increase the attachment and viability of the cultured neural stem cells (125). Yiqian Zhu in 2010, have shown that aligned Poly (L-lactide) /Poly (D, L-lactide-co-glycolide) nanofibers immobilized with anti- inflammatory factor Rolipram can promote axon growth from the dorsal root ganglion tissue and reduce the population of astrocytes and chondroitin sulfate proteoglycans in the lesion (126). Aligned Laminin-functionalized polycaprolactone (PCL) nanofibers embedded to three-dimensional hyaluronic acid hydrogels used by McMurtrey to support neuronal cell cultures. Aligned nanofibers were shown to enable considerable control over the direction of neurite outgrowth in (3D) neuronal cultures. Specifically, the average length of neurites per cell in 3D HA constructs with laminin-functionalized nanofibers compared to the same laminin fibers on 2D laminin surfaces increased by 66%, increased by 59% compared to 2D surface coated by laminin without fibers, and increased by 1052% compared to HA construct without fibers. Laminin functionalization of fibers also was doubled in average neurite length over plain PCL fibers in

Table 2: Advantage and disadvantage of the most used nanoparticles in neuron growth

Nanomaterial	Advantages	Disadvantages
Graphene	Differentiation of hNSCs more toward neurons to glial cells. Excellent cell-adhesion layer Biocompatible. Efficient electrical stimulation of neuron.	One-atom-thick graphene is so thin that it can slice directly into the cell
Nanotube	Similarity to the neural machinery elements. The electrical properties of CNT can be designed in a way that is proportional to the charge transport features of neuro-electrical neural interfacing. Suitable mechanical and chemical properties for prolonged implantation in neuronal tissue. Biocompatible and biodegradable in the neural tissue. Stable conductivity in biological environments. Easy functionalization of insoluble pristine CNT that enhance its aqueous dispersibility and has licensed their application in physiological environments including the nervous system. Surface charge of CNTs can be used to control the neurites outgrowth.	Lack of solubility in aqueous media The biodistribution and pharmacokinetics of CNTs are affected by many physicochemical characteristics such as shape, size, chemical composition, aggregation, solubility surface, and fictionalization
Nanowire	Nanowire helps axonal outgrowth. Cell survival was better on nanowire substrates than on planar control substrates. There were no overt signs of toxicity caused by tested nanowires.	
Nanofiber	The nanofibrous network porous structure highly resembles to the native extracellular matrix, and the high aspect ratio has been shown to promote the adhesion, proliferation and differentiation of various cells. Randomly oriented nanofibers (150–350 nm) in addition to cause stem cell adhesion, will cause their differentiation as well. 3D nanofiber matrices appeared to be more suitable for NSC attachment, synaptic outgrowth and synaptogenesis. As the fiber diameter decreased, higher degree of proliferation and cell spreading and lower degree of cell aggregation were observed. Neural stem cells elongated and their neurites outgrew along the direction of the fiber orientation of the aligned nanofibers. Increased rate of Neural stem cells differentiation on aligned nanofibers than random fibers. Nanofiber made of conductive polymers could be a good candidate for neuronal tissue scaffolds exhibited 40–90% more neurite formation and 40–50% longer neuritis.	Such toxic solvents that used during the synthesis of nanofiber might affect the structural conformation of several biopolymers, proteins and result in undesired cellular response. A critical need exists to replace these toxic organic solvents with aqueous based or less toxic solvents during the synthesis. Polymers are prepared to nanofibers, many of their properties, including antibacterial properties, haemostatic properties, biodegradability, cell adhesion and proliferation, and mechanical properties, need to be regulated by controlling the structure and properties of the nanofibers or compositing with other synthetic polymers Nanofiber diameter and orientation effects on the neuron growth and finding suitable diameter of nanofiber is challengeable.

the same 3D HA constructs (127).

Electrical stimulation of neurons in the absence of topographical features also has been shown to guide axonal extension. Currently, electroactive nanofibers have often been fabricated as scaffolds to induce electrical stimulation for neural tissue engineering. Polymers with electrons in their backbones such as Polyaniline (PANI), Polypyrrole (PPY), and poly (3,4-ethylenedioxythiophene) (PEDOT) known as conductive polymers and could be a good candidate for use as a substrate for synthesis of nanofiber and the growth of neurons (128, 129). despite these polymers having suitable characteristics for use in the body, have features such as PPy fragility and insolubility after synthesis or PANI low process ability, flexibility and biodegradability that limit their use alone (128). To compensate the deficiencies of conductive polymers, they must be used in combination with other component. Lee and colleagues in 2009 examined the combined effect of nanofiber structures and

electrical stimulation. Conductive meshes of PPy on random and aligned electrospun poly (lactic-co-glycolic acid) (PLGA) nanofibers maked PPy–PLGA scaffold that supported the growth and differentiation of rat hippocampal neurons comparable to non-coated PLGA control meshes. This experiment suggested that PPy– PLGA is suitable as conductive nanofibers for neuronal tissue scaffolds. Electrical stimulation studies exhibited 40–90% more neurite formation and 40–50% longer neurites compared to unstimulated cells on the same scaffolds. In addition, stimulation of the cells on aligned PPy–PLGA fibers resulted in longer neurites and more neurite-bearing cells than stimulation on random fibers of PPy–PLGA, suggesting a combined effect of electrical stimulation and topographical guidance (128). Xie in 2009 prepared conductive core–sheath nanofibers. Poly (e-caprolactone) (PCL) and poly (L -lactide) (PLA) used as templates to produce uniform sheaths with in-situ polymerization. These conductive core–sheath nanofibers suggest a unique system to study the synergistic effect of different cues on neurite outgrowth in vitro. It was demonstrated that explanted dorsal root ganglia (DRG) cohere well to the conductive core–sheath nanofibers and produce neurites across the surface when there is a nerve growth factor in the medium. Furthermore, the neuritis can be oriented along one direction and enhanced by 82% in terms of maximum length when uniaxially aligned conductive core–sheath nanofibers are compared with their random counterparts. Electrical stimulation, when applied via the conductive core–sheath nanofibers, was shown to further increase the maximum length of neurites for random and aligned samples by 83% and 47%, respectively, relative to the controls without electrical stimulation. Together these, results indicate the potential use of the conductive core–sheath nanofibers as scaffolds in applications such as neural tissue engineering (123). Abidian in 2009 reported an application of PEDOT traces within agarose gel for axonal regeneration. PEDOT-modified agarose conduits support superior neural regeneration as compared to the plain agarose conduits (130).

A challenge for use of nanofibers in SCI treatment is such toxic solvents used during the synthesis pathway of nanofiber that might affect the structural conformation of several biopolymers and proteins and result in undesired cellular response. A critical need exists to replace these toxic organic solvents with aqueous based or less toxic solvents during the synthesis (131).

3. Conclusion:

In this review we investigated the nanotechnology based methods for neuron regeneration and treatment of SCI. Now, the nano-structures contain carbon nanotube, graphene; nanowire and nano-fiber have been used for this purpose more than others. Researchers showed graphene has not cytotoxic effect and can be used as an excellent nanostructure scaffolds for promoting neuron stem cell adhesion and directing hNSCs to neurons than glial cells. Also functionalized CNT scaffolds can serve as promising opportunity for nerve repair of SCI. Of course, the direction and charges of CNT in composite are two important factors in neural growth. In addition to these two carbon nanostructures, the morphology and high aspect ratio of nanowire makes them interesting for applications as a substrate for neuron growth. At the end of the list are nanofibers that numerous studies have been done on them and have more potential for neuron regeneration and SCI treatment. According to the studies, nanofibers scaffolds especially electro active nanofibers, can serve as excellent guidance conduits for nervous tissue repair.

As mentioned above, the proposed new therapies in SCIs, such as cell therapy, do not have full efficacy in improving symptoms after injury and therefore need to be used for combination therapy to improve the conventional therapies. Based on in vitro studies, animal models and a few available clinical trials seem to use nanoparticles as a complementary therapy can be helpful in this area. Finally, we summarized advantage and disadvantage of the most used nanoparticles in neuron growth in table 2. Further studies and move them to the clinical phase are needed to reach a general conclusion.

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5. Conflict of interest

No conflict of interest was declared.

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None.

7. Author contribution

All authors passed four criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editor.

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