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Review Article

Recent progresses in neural tissue engineering using topographic scaffolds

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Abstract: Neural tissue engineering as alternatives to recover damaged tissues and organs is getting more and more attention due to the lack of regeneration ability of natural tissue nervous system after injury. Particularly, topographic scaffolds are one of the critical elements to guide nerve orientation and reconnection with characteristics of mimic the natural extracellular matrix. This review focuses on scaffolds preparation technologies, topographical features, scaffolds-based encapsulations delivery strategies for neural tissue regeneration, biological functions on nerve cell guidance and regeneration, and applications of topographic scaffolds in vivo and in vitro. Here, the recent developments in topographic scaffolds for neural tissue engineering by simulating neural cell topographic orientation and differentiation are presented. We also explore the challenges and future perspectives of topographical scaffolds in clinical trials and practical applications.

Keywords: Neural tissue engineering, topographic scaffolds, topographic orientation, nerve cell guidance, nerve regeneration

Introduction

The nervous system integrates organ systems by the central nervous system (CNS) and peripheral nervous system (PNS) supported communication pathways to maintain homeostasis in the body [1]. Aging and nervous system injuries lead to serious individual morbidity and have significant socio-economic burdens on society. Since nervous systems lacks of regenerative ability under normal conditions, there are no effective clinical strategies for treatment of nervous system injuries. Autografts transplantation has been commonly considered as the gold standard for nerve repair. However, this method faces donor secondary complications of wound healing defects and neuropathic pain and immunology problems [2]. To meet these challenges, neural tissue engineering as an emerging alternative to regenerative medicine has been committed to using new biomaterial design methods and engineering strategies to repair the function of the nervous system [3, 4].

Neural tissue engineering has significant advantages in the nervous system regeneration by providing necessary physical scaffolds for cell components and initiating key intercellular communication. By manufacturing extracellular matrix (ECM)-mimicking scaffolds with controllable topographical features, physiochemical properties and surface microenvironment, it is possible to regulate the biological behavior and function of nerve cells, promote the growth of neurons and axon regeneration, and recover injured nervous system diseases [5]. So far, many studies have clarified the effects of different topographical orientations and the biological characteristics of scaffold morphology on nerve tissue regeneration. Recent researches demonstrate that the morphological characteristics and orientation effect of scaffolds play a crucial role in the regulation of cell morphology, migration, proliferation and differentiation [6].

Here, we review the design, various manufacturing methods and biological characteristics of topographical scaffolds for neural tissue engineering. Furthermore, we also summarize the

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latest progress of topographical oriented scaffolds in the research of nerve tissue regeneration, and prospect its application perspectives in neural tissue engineering, especially in neural stem cells-based regenerative medicine.

Fabrication strategies of scaffolds for neural tissue engineering

Neural tissue engineering is committed to fabricate ideal scaffolds to adjust the physicochemical and mechanical properties of biological tissues in a targeted manner. To achieve this goal, the fabrication of neural constructs has gone through a long process. Several engineered technologies of scaffolds construction mimic the physicochemical and biological characteristics of natural ECM have been developed to repair and regenerate damaged nerve tissues, including electrospinning, photolithography and 3 dimensional (3D) bioprinting. Here, we will introduce representative technologies used to fabricate scaffolds for neural tissue engineering.

Electrospinning

Electrospinning is widely used in neural tissue engineering as a low-cost and simple technique for fabricating scaffolds with topographic characteristics. Taking advantage of the controllable porosity, high surface volume ratio, and appropriate mechanical properties of electrospinned products, fibers with different orientations and layered structures can be manufactured [7]. Importantly, the scaffolds constructed with these electrospun-fibers can mimic the ECM structures of natural issues. Previous research results indicated that the morphology of electrospun fibers was regulated by factors such as voltage, polymer concentration, flow rate, solution viscosity, and porosity, etc. [8-10]. Various polymers with good biocompatibility from natural and synthetic sources have been developed for processing into electrospun fibers such as gelatin [11], collagen [12], silk fibroin [13], poly(lactic-co-glycolic acid) (PLGA) [14] and polycaprolactone [15], etc.

More and more studies reported that since electrospinning fibers-based scaffolds required appropriate mechanical strength and topographical cures, it is common to select polymers from natural or synthetic sources to prepare composite scaffolds for neural tissue engineering. For example, Asghari and colleagues reported a composite nanofiber scaffold and explored its application in nerve tissue repair and regeneration [16]. They fabricated nanofibers with polyvinyl alcohol, gelatin and crocin by electrospinning technology, and investigated the effects of crocin concentration on proliferation and differentiation of mesenchymal stem cells (MSCs). The obtained scaffold had the ability to promote differentiation of MSCs into neural cells through mechanisms of promoting MAP-2 and NETIN expression. In addition, electrospun fibers served as ECM-mimicking topographical cues to provide contact guidance for cultured cells, allowing them to elongate and align in a directional manner along the fiber axis. A study on polyhydroxyalkanoate blend fiber scaffold presented that controlling the diameter of fibers by changing electrospinning parameters could induce the biological behaviors of nerve cells [17]. The directionally aligned electrospinned fiber scaffolds supported the growth and guided the unidirectional distribution of SCs.

Photolithography and soft lithography

Lithography is a rapid prototyping technique for manufacturing ECM-mimetic topographical structures based on a variety of materials. Representative lithography methods contain of scanning beam lithography, immersion pen lithography, electron beam lithography, nanoimprint lithography, capillary lithography, photolithography and soft lithography [18]. Among these, photolithography and soft lithography technologies have significant advantages in creating 2D or 3D scaffold structures ranging from 5 to 500 nanometers, and are widely used in neural tissue engineering [19]. However, the photolithography method has shortcomings include high cost, low compatibility, poor surface controllability, and limited selection of polymer materials in biology and scaffold-based construction technologies. In contrast, the design and development of soft lithography technology can overcome the above limitations. Importantly, as a simple micromachining method, soft lithography is gradually replacing photolithography to be the main scaffold manufacturing method by using stamps, molds and templates for replicating the morphology of the substrates [20]. Specifically, after pouring a prepolymer such as polydimethylsiloxane onto

the mold, the pattern forming and scaffold preparation processes are completed through the molding and embossing stages [21]. One such study investigated the directional migration and extension behaviors of cells along PLGA nanopatterned patches constructed based on PDMS sheets [22]. Babaliari and colleagues have been successfully replicated continuous and discontinuous microchannel patterns on PLGA substrates through soft lithography technology, and concluded that the micropatterned scaffold acquired the capabilities of enhancing adhesion, differentiation and directional extension of SCs in vitro [23]. Another recent study grafted pectin into methacrylate polymer by ultraviolet assisted lithography technology to generate micropatterns and construct 3D cell culture systems [24]. This observation confirmed that the obtained micropattered substrates accompanied by improved cell diffusion characteristics, and could promote stem cell differentiation and neuronal maturation.

3D bioprinting

3D bioprinting acts as the most famous rapid prototyping technology and uses 3D printing technology for depositing biological ink on a substrate to create the required scaffold structures [25, 26]. The development of 3D bioprinting strategies has promoted the advancement of neural tissue regeneration and personalized treatment methods. Currently, inkjet printing, extrusion-based methods and laser assisted printing are popular 3D bioprinting techniques for neural tissue engineering [27]. By reviewing the current literatures, 3D bioprinting has been found to mimic the complex ECM structure of the nervous system and build neural tissue models with controllable accuracy [28]. The obtained 3D bioprinting scaffolds with excellent cell compatibility, easy adhesion and suitable viscoelastic properties show outstanding advantages in cell adhesion, differentiation and directional growth [29]. For example, Restan et al. developed MSC-laden neural scaffold based on bioink composed of fibrin using microfluidic RX1 bioprinter [30]. The constructed 3D bioprinting scaffold could promote the differentiation of stem cells into dopaminergic neurons by the aid of bioactive growth factors. It was important to mention here that printing conditions were a key variable limiting scaffold performance. On the one hand, Li and colleagues changed the composition ratio of sodium alginate and gelatin to find the optimal composition of biological ink. On the other hand, they investigated the effects of neural scaffold printing parameters such as temperature, speed and pressure of encapsulated stem cells on the survival, proliferation and differentiation abilities [31]. Subsequent experiments were carried out in the optimal conditions for cell encapsulation in biological ink to explore the mechanism of cell-laden bioprinting scaffold in nerve tissue regeneration and intercellular communication. Moreover, to improve the conductivity of scaffolds and exert the synergistic effect of electrical stimulation on nerve cells, electrically conductive biological materials are often introduced into bioinks. A study has shown that addition of graphene oxide improved the cellular biocompatibility of traditional biomaterials by providing more bioactive functional groups for 3D printing scaffolds. Compared to the original graphene oxide-free bioink based scaffold, the neural scaffold constructed with 3D printable graphene oxide bioink exhibited higher cell viability in terms of stem cell adhesion and cell function improvement [32]. In summary, although the rapid development of 3D bioprinting technology has opened a new chapter in neural structures printing and nerve injuries treatment, it is still necessary to further explore the potential mechanisms of printed scaffolds for promoting nerve regeneration and achieve the transformation of clinical applications.

Topographic features of scaffolds on nerve cell guidance and regeneration

The nerve growth in an unguided or inappropriate direction leads to low efficiency in the construction and connection of meaningful neural networks. Topography guides nerve cells to respond to biomaterials with excellent biocompatibility, biodegradability and mechanical properties, which is the key factor of neuroregenerative medicine. Researchers regulate cell growth behaviors and biological processes by fabricating different topographical features of scaffolds. To achieve effective guidance of nerve growth, scaffolds with orderly structures or surface functional modifications can be used according to actual requirements. Researchers also pointed out that physical properties of bio-

materials, such as topography, porosity, and roughness, can influence cellular behaviors. The impact of topographical features of scaffolds on nerve cell guidance and regeneration are discussed.

Grooved scaffolds

Most cells respond to parallel grooves by clearly extending and orientating along the grooves, while on flat substrate surface cells usually present random orientation with more rounded morphologies. Grooves are the key determinants of neural cell alignment and orientation. As Hsu and coworkers reported that human pluripotent stem cells (hPSCs) could response to the groove topography, and cell alignment, stiffness, and nerve differentiation are regulated by grooves depth (**Figure 1A**) [33]. These features could be quantitatively analyzed by means of optical microscope, scanning ion conductivity microscope and atomic force microscope. Specifically, under differentiation conditions, neuronal markers β III-tubulin positive cells (neurons) increased within deeper grooves, while the proliferation markers of Ki67 and Nestin decreased. Compared with 3 μm depth grooves, 10 μm depth grooves had more significant effects on cell morphology and alignment, such as direction control and cell elongation. These findings indicated that the deeper groove could enhance the neuronal differentiation, neurite extension and cell orientation of hPSCs. The reason for nerve directional growth was that 10 μM depth grooves act as barriers to constrained cells inside where cell cytoskeletons could not bend across because of high stiffness, and the contact guidance between cells and substrate produced cell tension and caused the increase of cell elongation. In another breakthrough study, the authors integrated materials and microstructures to design a biomimetic scaffold based on graphene oxide, which regulated the activity of neurons through clues guided by topography, electricity and chemistry (**Figure 1B**) [34]. Nerve growth factor-incorporated Fe_3O_4-graphene nanoparticles (GFPNs) deposited on the patterned substrates, composed of reduced graphene oxide, polyethylenemine, and iron oxide, promote the adhesion of PC12 cells, enhance neuronal differentiation and axon orientation. They used GFPN complexes composed of reduced graphene oxide, polythylene, and iron oxide deposit on the patterned substrates to explore the regulatory effect of the constructed bionic scaffold on PC12 topographical and biological. It was demonstrated that this grooved substrate could promote the adhesion of PC12 cells, enhance neuronal differentiation and axon orientation. Besides, cells obtained directional axon elongation along the grooved pattern to form cell connections and construct neural transmission networks when applied of additional electrical stimulation on the GFPN-deposited scaffold.

In a typical experiment, researchers investigated the moderation effect of scaffold surface topography to human NSC (hNSC) differentiation (**Figure 1C**) [35]. Study results have shown that topographical structures have a great influence on cell alignment, growth direction and neuronal differentiation. And researchers found that the shapes and dimensions were crucial for the differentiation of neurons and astrocytes. More specifically, cell culture on groove nanopatterned surfaces provided better ECM-mimicking topographical cues with promoted focal adhesion and enhanced axonal orientation. The results illustrated Tuj1-(neurons) and GFAP-positive cells (astrocytes) decreased as the size of grooves ranged from 300 to 1500 nm. Zhang et al. have also studied the biological behaviors of the grooved micropatterns (**Figure 1D**) [36]. They presented a based on poly(d,l-lactide-co-caprolactone) (PLCL) patterned film by introducing graphene oxide nanosheets for Schwann cells (SCs) culture. In this study, SCs directionally migrated along the linear grooved micropatterns to promote the performance of the axonal elongation and neuron differentiation. It was demonstrated that the graphene oxide modified grooved surface could enhance cell contact area and promote the cell-substrate interaction to produce a better cell adhesion for directed migration of SCs. These findings suggested that the resultant grooved scaffold has significant application prospects for nerve regeneration and functional repair of damaged tissue.

Porous scaffolds

Porous scaffolds provide higher surface area for cell attachment and growth, which has significant advantages in nerve tissue engineering applications. The interconnected porous struc-

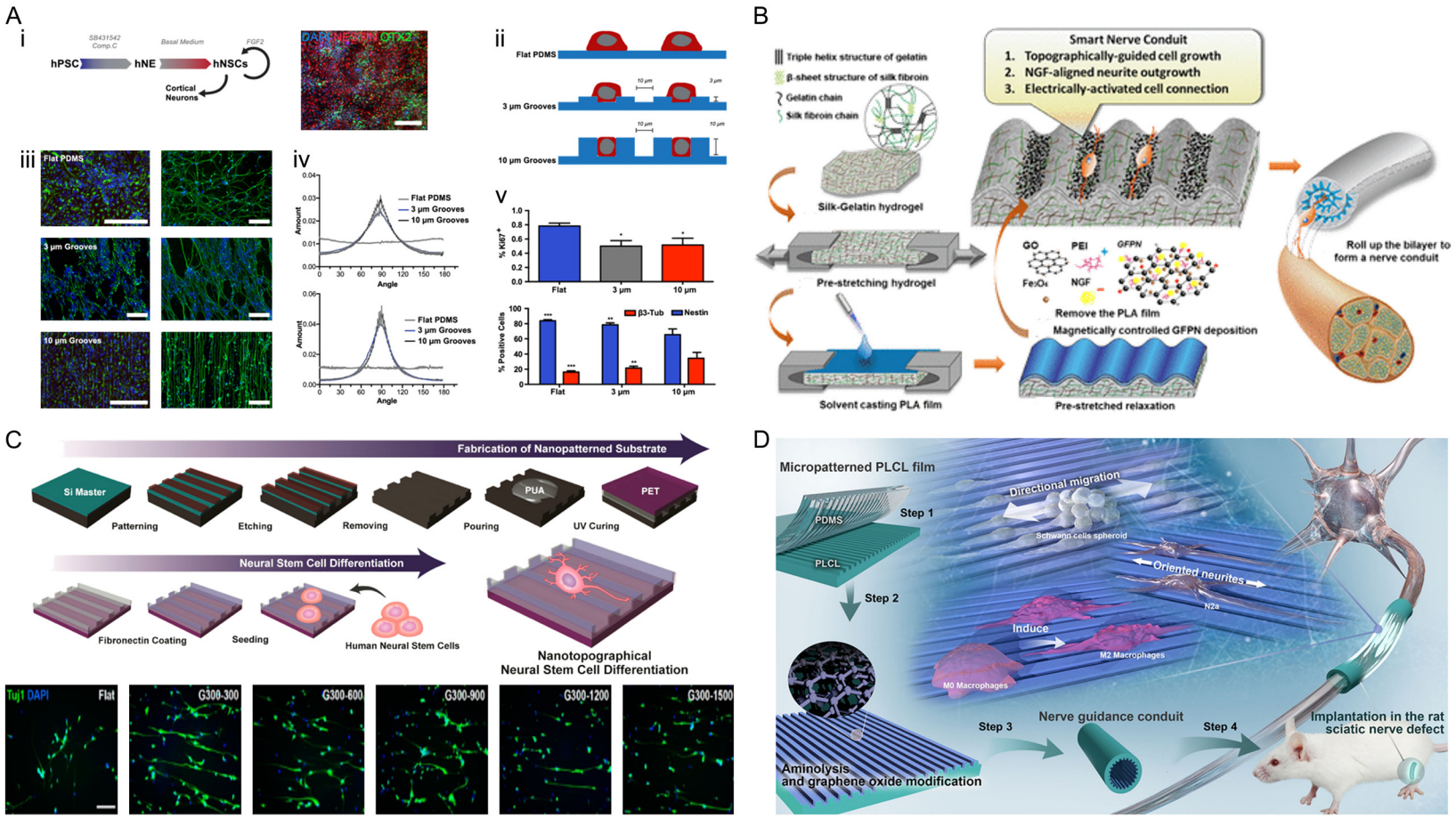

Figure 1. Schematic design of grooved structure scaffold mediating nerve directional conduction. A. The differentiation and alignment of hNSCs on PDMS grooved substrates. (i) Schematic process of neuron differentiation. (ii) Schematic diagram of cell attachment on flat, 3 μM and 10 μM grooved PDMS substrates. (iii) Cell alignment of NSCs and neurons cultured on flat, 3 μm grooved, and 10 μm grooved PDMS substrates. (iv) Cell alignment quantification of NSCs and neurons. (v) Differential expression analysis of cell proliferation and neuronal differentiation. Reprinted with permission from Hsu et al. [33] Copyright 2022 American Chemical Society. B. Schematic illustration of the construction steps of GFPN-deposited scaffold and growth behavior of PC12 cells on grooved pattern. Reprinted with permission from Lin et al. [34] Copyright 2020 American Chemical Society. C. Schematic illustration of fabrication topographical nanopatterns and hNSCs differentiation induced on grooved substrates. Reprinted with permission from Yang et al. [35] Copyright 2013 American Chemical Society. D. Schematic illustration of fabrication grapheme oxide nanosheets introduced PLCL film and its application in axonal elongation and nerve regeneration. Reprinted with permission from Zhang et al. [36] Copyright 2020 American Chemical Society.

ture allows the material transportation and information transmission between cell-cell and cell-matrix to promote cell growth and migration. Previous studies have shown that pore size is a key factor affecting the biological behaviors of cell proliferation and differentiation. When cells are constrained within the scaffold with small pore size and contacted early, thus inhibiting proliferation and initiating differentiation.

Embryonic neural progenitor cells (ENPCs) exhibited more effective differentiation behavior in 3D reduced graphene oxide porous scaffolds (**Figure 2A**) [37]. On the one hand, the authors adjusted the sizes of graphene oxide nanosheets to change the biocompatibility of the scaffolds and the adhesion ability of ENPCs. On the other hand, alteration the pore sizes and mechanical integrity of the scaffolds to match the microenvironment of ENPCs and enhance the directional differentiation to neurons. The results demonstrated that ENPCs could form synaptic connections through pores to promote the formation of interconnected neuronal circuits in vitro after being inoculated into this 3D porous scaffolds, achieving the goal of building a biologically active neural microenvironment and repairing damaged tissues. To increase the growth of neurons and guide the connection of neurites for nerve repair and therapy, Fan et al. designed a 3D porous microtubule array with directional arrangement using advanced femtosecond laser direct writing technique (**Figure 2B**) [38]. The constructed 3D porous microtubule scaffolds ensured the directional connection and conduction of neural circuits by controlling the array direction, and played a directional role in guiding the growth of primary embryonic hippocampal neurons and neurites. It was demonstrated that the inner diameter, wall thickness and tube length parameters of microtubules played a key role in neural growth and maturation. It is worth mentioning that when the inner diameter, wall thickness and tube length of microtubules were 6 μm, 1 μm and 155 μM respectively, achieving the best effect of accelerating neurite growth.

Another novel research used biocompatible resin prepared by methacrylate to produce laser-fabricated 3D porous scaffolds (**Figure 2C**) [39]. After inoculating neural stem cells (NSCs) derived from hPSCs on the scaffolds, neurons with electrical activity could be differentiated by topographical guidance of NSCs. The resulted 3D culture system induced formation of functional synaptic circuits and neural networks in vitro. This work provided ideas in developing of cell functional development and biomimicking ECM structure of some specific tissues in vitro. Conductive scaffolds were developed by integrating the advantages of graphene with high surface area, porous structures and operability (**Figure 2D**) [40]. Benefiting from the ripples and wrinkles on the surface of graphene skeleton, it could induce neurons to arrange in a directional direction along the 3D-ordered porous graphene scaffold. A significant improvement in axon outgrowth, dendrites complexity and neural network were seen on 3D scaffolds surface compared to 2D graphene films. Further, the cross-linked complex neural network possessed higher electrical activity, and could carry out long-distance functional signal transmission and intensive connectivity along the graphene skeleton.

Fiber scaffolds

Among various biomimicking scaffolds, fiber scaffolds mimic the ECM ultrastructure acquire the ability of orienting neurons and axons for nerve tissue engineering. By encouraging directional growth, it occurred spontaneously without the guidance of external conditions. Current studies demonstrated that aligned fibers can guide nerve cells growth and extension along their arrangement. For example, You et al. fabricated multichannel silk fibroin nanofiber scaffold functionalized with laminin through electrospinning technology (**Figure 3A**) [41]. The composite fibers had the ability of guiding directional arrangement of neurons and axons regeneration. Additionally, the resulted biofunctionalized nanofiber scaffold provided a favorable topographical microenvironment for the directional migration and axonal extension of PC12 cells, suggesting the promising application potential for spinal cord injury (SCI) repair and regeneration.

Another study focused on the preparation of aligned conductive fiber scaffold by co-doping of pyrrole (PPy) with poly L-lactic acid (PLLA) based on the method of chemical oxidation polymerization (**Figure 3B**) [42]. The obtained PPy-PLLA electrospun nanofiber scaffold could

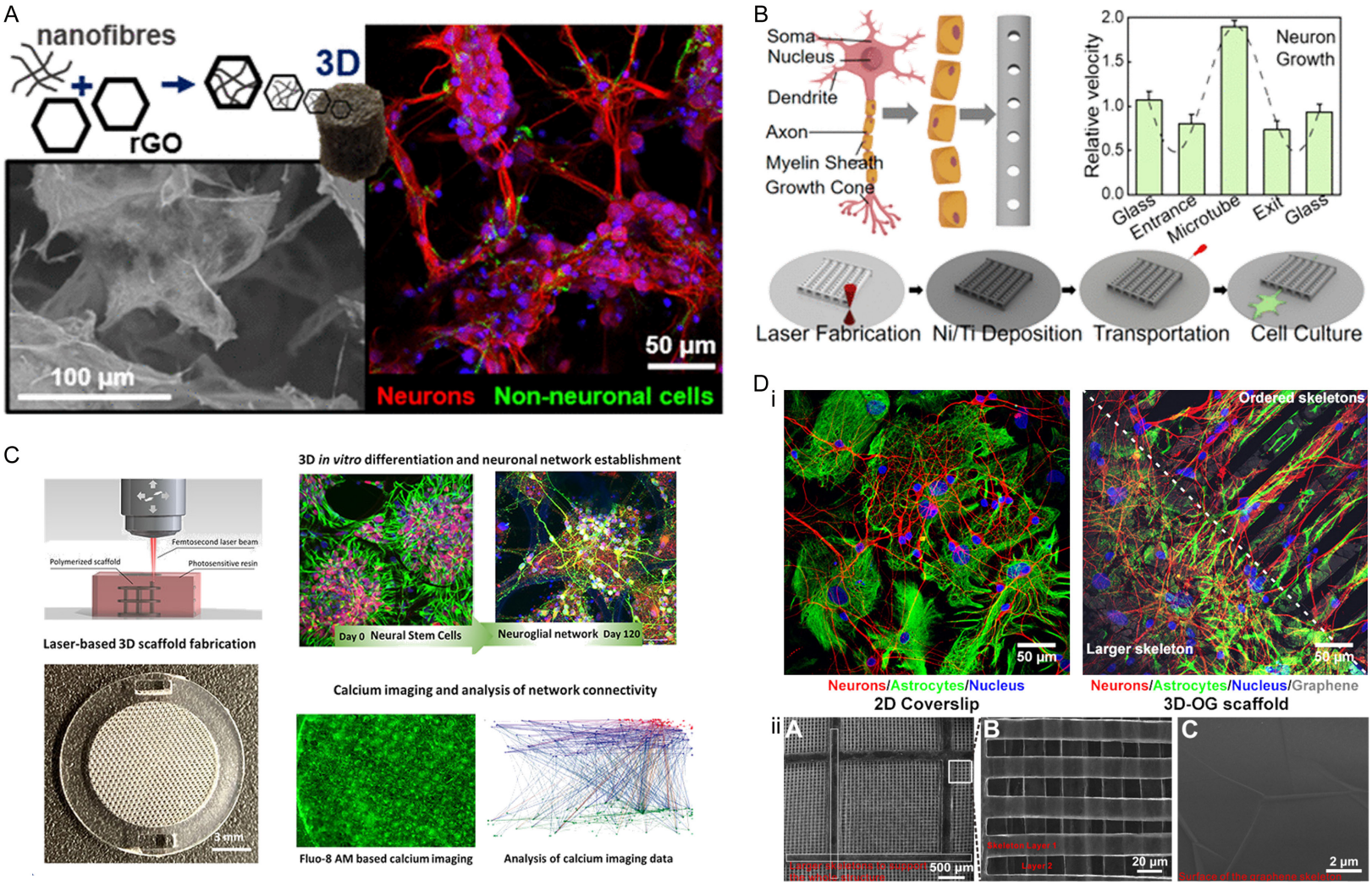

Figure 2. The diagram of porous scaffold promoting neural interaction. A. Schematic illustration of fabrication 3D-porous reduced graphene oxide scaffolds and ENPCs differentiation studies on this porous scaffold. Reprinted with permission from Girao et al. [37] Copyright 2020 American Chemical Society. B. Design and manufacture of 3D porous microtubules for accelerating neurites growth and directional neuronal connections. Reprinted with permission from Fan et al. [38] Copyright 2022 American Chemical Society. C. Generation of NSCs 3D culture systems in vitro for differentiation and neural network establishment. Reprinted with permission from Koroleva et al. [39] Copyright 2021 American Chemical Society. D. Design of graphene-based 3D porous scaffold for neural growth and network formation. (i) Immunofluorescence images of cortical cells culture on 3D ordered graphene scaffold vs 2D substrate. (ii) Morphology and structure of 3D ordered scaffolds. Reprinted with permission from Xiao et al. [40] Copyright 2020 American Chemical Society.

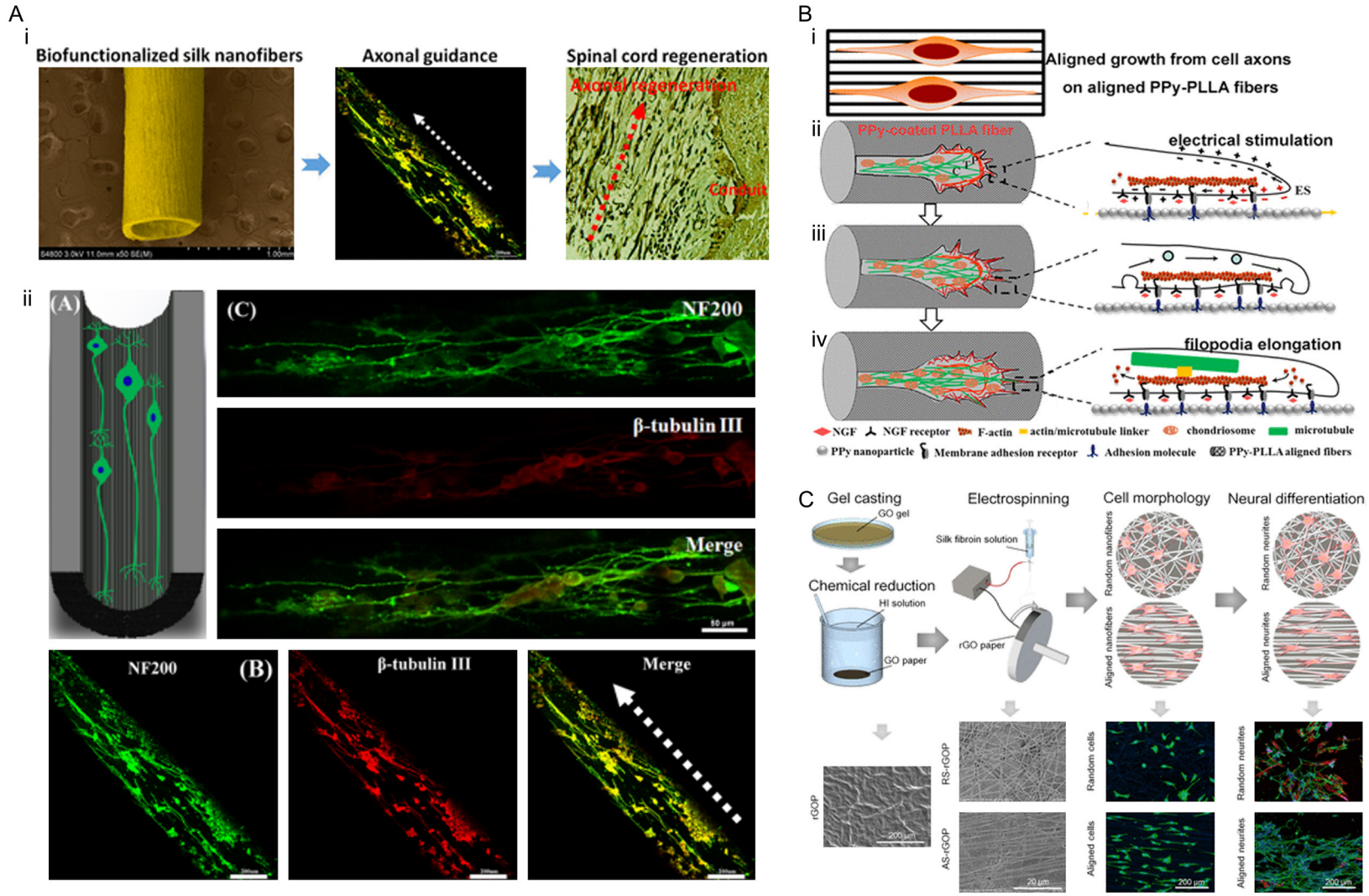

Figure 3. Schematic diagram of fiber scaffold orientation promoting axon elongation. A. Fabricated multichannel silk nanofiber scaffold for axon regeneration and repair injured spinal cord. (i) Illustration of fabricated biofunctionalized silk nanofiber scaffold for axonal guidance and SCI regeneration. (ii) Schematic diagram and fluorescence images of PC12 cells culture on scaffold surface for 10 days. Reprinted with permission from You et al. [41] Copyright 2020 American Chemical Society. B. Schematic illustration of PC12 cells axon elongation on PPy-PLLA scaffold surface. (i) After differentiation. (ii-iv) Alteration of growth cone. Reprinted with permission from Zou et al. [42] Copyright 2016 American Chemical Society. C. Schematic fabrication process of heterostructured silk nanofiber scaffold and biological behavior research of SH-SY5Y cells on scaffold surface. Reprinted with permission from Qing et al. [43] Copyright 2018 American Chemical Society.

guide 68% of PC12 cells to extend along the fiber axis. Meanwhile, Zou et al. also analyzed the mechanism of neurite directional extension under external guidance of electrical stimulation on conductive PPy-PLLA scaffold surface. The results showed that the combined stimulation factors, including the topographical cues of PPy alignment and fiber surface roughness, and external electrical stimulation guidance, played a pivotal role in promoting neurite elongation and nerve repair. Other a significant investigation about heterostructure composite scaffold with ordered arrangement was developed to regulate neural stem cells fate (**Figure 3C**) [43]. For the fabrication process, aligned silk nanofibers and reduced graphene paper as composition to improve conductivity and biocompatibility of the resulted composite fiber scaffold. The experimental results demonstrated that SH-SY5Y cells showed directional growth and neuronal differentiation across on the fiber arrangement direction. Besides, authors illustrated the fabricated novel fiber scaffold using electrospinning technology could improve the efficiency of SH-SY5Y cells differentiating into neurons, which indicated a broad application prospect in the treatment of nervous system diseases and injuries.

Multichannel conduit scaffolds

Due to the limited regenerative therapies that can be used to treat nerve injury in clinic, the development and manufacture of nerve conduits have attracted the attention of researchers in recent years. The nerve conduits provide ECM-like environment, topographic orientations and appropriate mechanical properties to support axon regeneration and achieve the purpose of reconnecting severed or damaged axons. Many improvements in conduit constructions have been made through novel materials selection and scaffolds design to promote neuron regeneration and functional repair of nerve injury sites.

A recent study on dopamine functionalized nerve guide conduits devoted to establishing synaptic connections at nerve injury sites (**Figure 4A**) [44]. Among this, dopamine acted as a neurotrophic factor and promoted axonal regeneration of injured nerve tissue by supporting the survival, growth and differentiation of neurons. This novel nanocomposite scaffold constructed by authors were composed of dopamine, carbon nanofibers and polycaprolactone. In vitro experimental studies demonstrated that the aligned multichannel conduits provided support for the adhesion, migration and differentiation of nerve cells. In contrast, when there were no directional topographical cues on substrate surface, cells would grow in random directions. The findings showed that this newly developed conduit scaffold provided a suitable microenvironment for neuronal migration to promote nerve growth and connection. Specifically, the scaffold component of dopamine was used to stimulate nerve tissue to expand new connections after neuronal migration, and carbon nanofibers were used to repair damaged nerve circuits to mediate communication. Another study introduced quercetin into the nanofibers and fabricated a 3D functionalized patterned scaffold for neural tissue engineering (**Figure 4B**) [45]. The obtained circular conduit scaffold had the abilities of guiding neural cells directional differentiation and growth. Scaffold design mimicked the ECM of the nerve tissue accompanied with physiological similarity, and were promising to be combined with some biological stimuli to achieve the synergistic effect of inducing nerve tissue regeneration.

Conductive scaffolds as promising tool have been fabricated to affect growth, directional arrangement and differentiation of nerve cells for neural tissue engineering. Benefiting from the directional guidance of nerve conduits to nerve cells, various studies have introduced the scaffold structure based on conduits into nerve tissue engineering to repair injured nerves. Addition of a polypyrrole coating to the scaffold composed of poly(l-lactide-co-ε-caprolactone) and poly(l-lactide-co-glycolide) improved biocompatibility, thus enhancing the abilities of SCs cells attachment and proliferation, ultimately replacing autologous graft to repair of peripheral nerve injury (**Figure 4C**) [46]. Another important research made a multichannel nerve conduit stent based on poly(lateral-co-trimethylene carbonate), in which the fibers arranged directionally on the inner wall act as topographical cues (**Figure 4D**) [47]. Inspired by the concept of shape memory, they obtained the nanofiber planar shape through high-temperature molding process and achieved the uniform distribution of cells. While

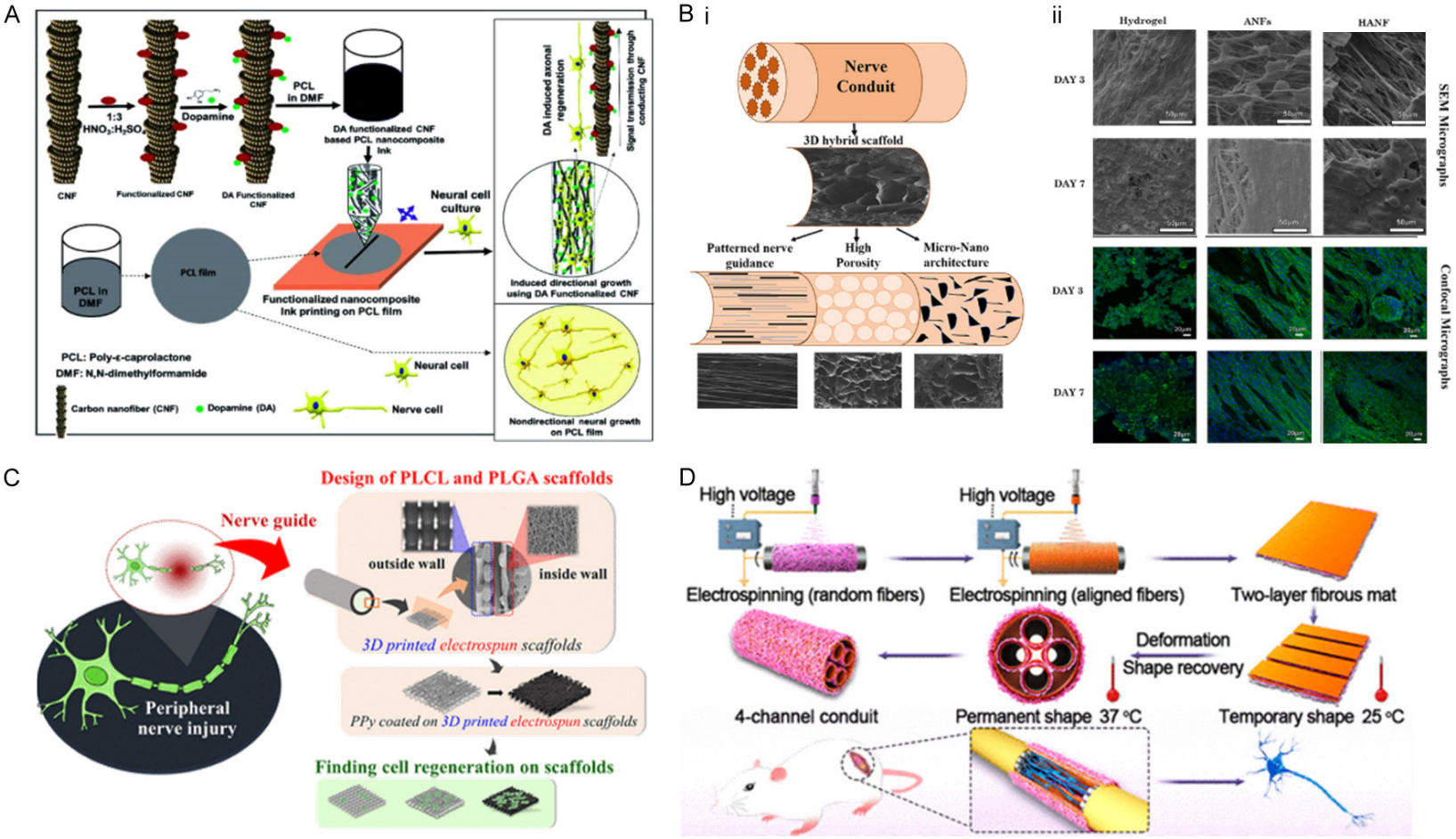

Figure 4. The diagram of multi-channel conduit scaffold supporting directional arrangement and regeneration of neurons. A. Schematic illustration of dopamine functionalized conduit scaffold for axonal regeneration. Reprinted with permission from Houshyar et al. [44] Copyright 2020 The Royal Society of Chemistry. B. Fabrication of quercetin-functionalized conduit scaffold for neural cells directional differentiation and growth. (i) Schematic illustration process of fabrication 3D circular conduit scaffold for nerve guidance. (ii) Scanning electron microscope and confocal micrographs of SH-SY5Y cells growth on substrates. Reprinted with permission from Vashisth et al. [45] Copyright 2020 American Chemical Society. C. Schematic illustration of polypyrrole-coated 3D conduit scaffold fabrication process for nerve regeneration. Reprinted with permission from Namhongsa et al. [46] Copyright 2022 American Chemical Society. D. Schematic fabrication process of shape memory multichannel conduits scaffold for recovering rat sciatic nerve injury. Reprinted with permission from Wang et al. [47] Copyright 2020 American Chemical Society.

at 37°C, the scaffold was triggered to automatically recover its tubular shape and formed the resulted multichannel conduits. This study concluded that the multichannel conduit scaffold showed better performance in promoting cell growth and recovering rat sciatic nerve injury.

Hydrogel scaffolds

Compared to traditional 2D cell culture, 3D cell culture systems support more complex interactions between cells and matrix by providing ECM-like environment. Hydrogels as excellent scaffold biomaterials with porous and soft properties are often developed for nerve tissue engineering. Recently, various researches have been devoted to promoting the survival and differentiation of nerve cells through material designs and changing the porosity of hydrogels. For example, Fan et al. developed a strategy to fabricate a 3D biomimetic scaffold by mixing gelatin methacrylate hydrogel and NSCs derived from induced pluripotent stem cells (**Figure 5A**) [48]. In vitro studies showed that NSCs cultured in this resulted hydrogel displayed robust neurite growths and multiple neuronal differentiations. For evaluation experiment in vivo, the hydrogel scaffold was filled in the pathological part of spinal cord of mice to investigate its effect on neuronal regeneration at 6 weeks. It was demonstrated that the constructed 3D hydrogel scaffold could be used for repairing SCI by reducing inflammatory reaction, inhibiting glial scar formation and promoting axon regeneration. Hamrangsekachaee et al. embedded a 3D hydrogel by subcutaneous preconditioning to improve nerve cells survive in an inflammatory environment for treatment of SCI (**Figure 5B**) [49]. Studying the mechanism of subcutaneous preconditioning by measuring neurogenic markers and several cytokines. On the 28th day after embedding hydrogel subcutaneously, SCI regeneration effect was found and regeneration cytokine interferon-γ was detected with the abilities of enhancing NSCs survival and promoting neurons regeneration, indicating a broad application prospect in stem cells-based regenerative medicine.

Another study applied Ti3C2Tx MXene hydrogel scaffold to target spiral ganglion neurons (SGNs) constructing of 3D hydrogel culture system (**Figure 5C**) [50]. The conductive matrigel of Ti_3C_2Tx MXene provide topographical premise for the introduction of electrical stimulation. These key factors of conductive matrigel and electrical stimulation had synergistic effect on SGNs growth, growth cone development and intercellular signaling. This work presented potential value in improving and optimizing the hearing effect after cochlear implantation. Chang et al. developed a 3D nanofibrillar cellulose hydrogel scaffold for improving the cell survival rate in stem cell therapy (**Figure 5D**) [51]. The goal of this work is to promote the survival of embryonic stem cells (hESCs) after transplantation. The purpose of producing functional auditory neurons was achieved by regulating the survival and differentiation ability of hESCs-derived otic neuronal progenitors (ONPs) in vivo and in vitro. It was demonstrated that the survival rate of ONPs spheroids in 3D nanofibrillar cellulose hydrogel scaffold was significantly higher than previous studies of inner ear stem cell transplantation without using immunosuppressive drugs.

Scaffolds as carriers for neural tissue regeneration

A major challenge for nerve regeneration and functional recovery is to provide various nutrients and growth factors needed for cell adhesion, growth and adhesion. Scaffolds of nerve tissue engineering can be used as a carrier to effectively deliver encapsulations to the injured sites. Then, it can adjust the phenotype of nerve cells by releasing encapsulations into ECM and integrate with injured tissues to promote cell migration to enhance tissue repair and regeneration. In recent years, researchers have developed various encapsulations implantation techniques to recover the function after nerve injury with gratifying clinical trial results. Herein, we describe that the biological scaffolds as extracellular vesicles, growth factors, cells or drugs carriers to provide the nutrition and nutrient factors needed for survival and regeneration of the injured cells to promote cell proliferation, survival and differentiation.

Extracellular vesicles

The main challenge of tissue regeneration and functional recovery is to provide cells with nutrients and growth factors necessary for growth, migration and differentiation. Extracellular vesicles (EVs) are nanoscale particles (30-1000

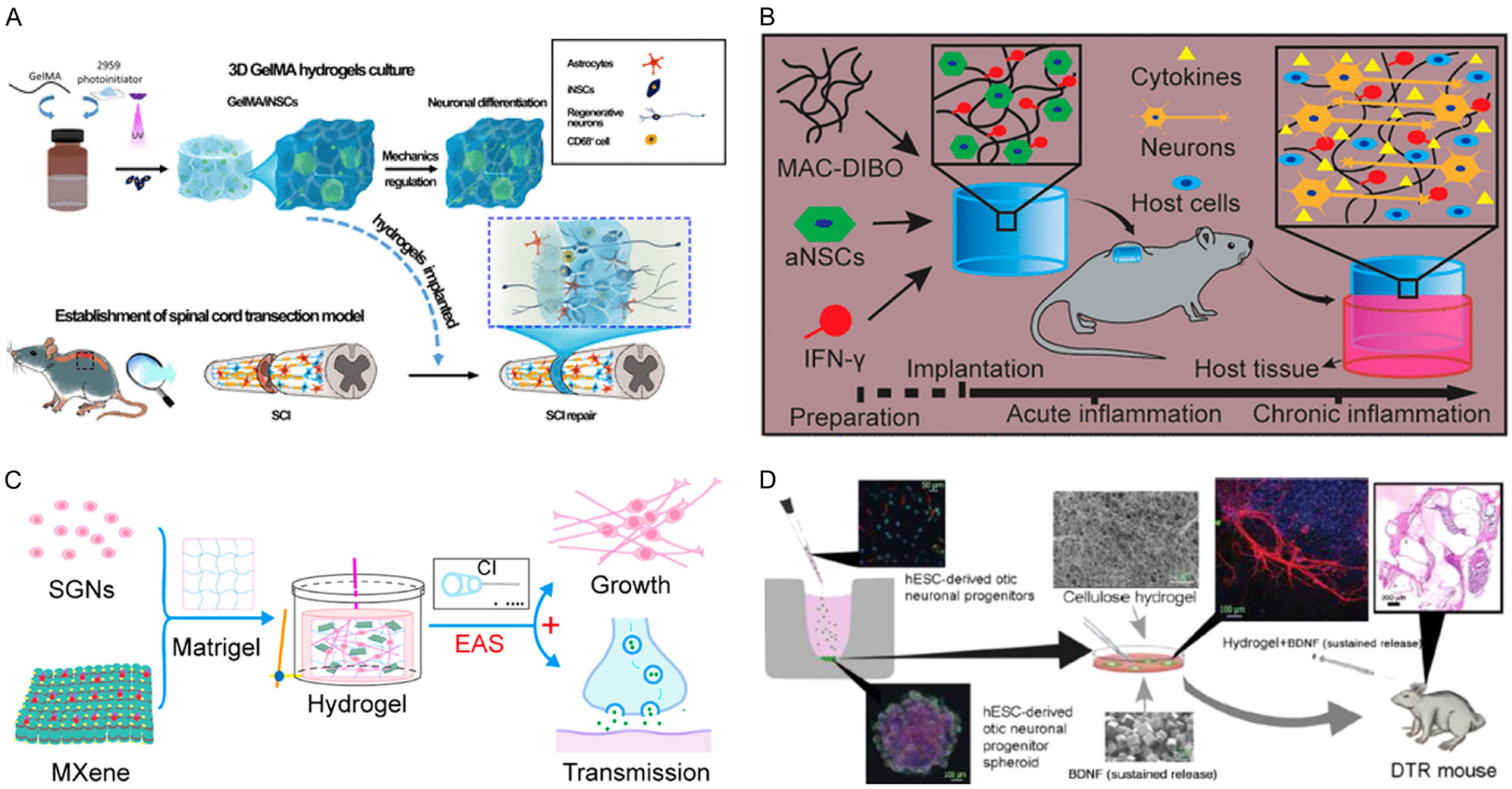

Figure 5. The diagram of interaction between nerve cells and microenvironment promoted by hydrogel scaffold. A. Schematic illustration of 3D hydrogel scaffold fabrication process and its application in animal experiment. Reprinted with permission from Fan et al. [48] Copyright 2018 The Royal Society of Chemistry. B. Schematic illustration of subcutaneously embedded hydrogel scaffold for SCI repair. Reprinted with permission from Hamrangsekachaee et al. [49] Copyright 2022 American Chemical Society. C. Schematic illustration of 3D Ti3C2Tx MXene hydrogel scaffold fabrication process and its application in SGNs growth and cells signal transmission. Reprinted with permission from Liao et al. [50] Copyright 2022 American Chemical Society. D. Schematic fabrication process of 3D nanofibrillar cellulose hydrogel scaffold for functional auditory neurons regeneration. Reprinted with permission from Chang et al. [51] Copyright 2020 Elsevier.

nm) with structure of lipid bilayer secreted by cells, including nucleic acids, proteins, etc. As a natural delivery carrier, it mediates nerve transmission, cell growth, transfer, differentiation and signal communications between cells by transporting its contents to target cells. Compared with other scaffold implants, EVs have gained wide attention in the application of nerve tissue engineering due to its advantages of avoiding being swallowed and degraded by macrophages, long internal circulation time, targeting and overcoming biological barriers. Recently, many studies were performed to investigate the effects of EVs implantation techniques in delivery signals between cells and matrix for neural tissue engineering.

Hypoxic preconditioned derived EVs played a key role in the treatment of traumatic spinal cord injury (**Figure 6A**) [52]. This EVs implantation strategy promoted angiogenesis of MSCs and repaired of nerve tissue of SCI. Immobilization of the EVs "signal box" within 3D scaffolds to transmit signals between cells and matrix was a well-established approach that allowed the specific targeted delivery of molecules and the continuous transmission of biomolecular signals. In one study, an innovative alginate scaffold with immobilization of MSCs-EVs for therapy of pain induced by nerve injury (**Figure 6B**) [53]. In vitro studies showed that the resulted EVs implantation scaffold presented biological activities of promoting neurite growth, neuroprotection and anti-inflammatory on PC12 cells. And in vivo study used the right L5/6 spinal nerve ligation pain model of rats to explore the effects of scaffold in anti-inflammatory and neurotrophic. In detail, the authors wrapped the constructed 3D EVs-implanted alginate scaffold onto the ligated L5/6 spinal nerves for injury treatment. This study demonstrated that the pain caused by nerve injury could be effectively relieved by significantly increasing myelin basic protein and IL-10 expressions in axons at 21 days after scaffold implantation.

For another study, Li et al. structured a peptide-modified 3D adhesive hydrogel scaffold by EVs implantation technique (Exo-pGel) to treat and relieve central nervous system diseases (**Figure 6C**) [54]. The long-term circulation and continuous release of implanted EVs in the host nerve cells was a necessary prerequisite for the success of this strategy. Results of this study revealed that local implantation of Exo-pGel provided EVs-coated extracellular matrix to establish of enhanced microenvironment for the survival of injured nerve tissue cells. The explored mechanism proved that Exo-pGel could promote the recovery and regeneration of injured nerve tissues by effectively reducing inflammation and oxidation. Researchers immobilized MSC-EVs in a collagen scaffold to promote NSCs migration and SCI repair (**Figure 6D**) [55]. The scaffold could effectively retain EVs and recruit endogenous NSCs into the injured sites to induce the generation of neurons. By virtual of enhancing nerve regeneration and decreasing scar deposition, this EVs-implanted collagen scaffold presented excellent performance in nerve tissue regenerations and motor function reconstructions after SCI. Much improvements have been made to develop more promising EVs-implanted biological scaffolds and accelerate the clinical transformation of scaffolds in the treatment of nerve injury.

Growth factors

Growth factors (GFs) play an important role in regulating the biological behavior of stem cells and promoting the information transmission between cell-cell and cell-matrix [56]. Nerve cells need the support of neurotrophic factors to maintain survival, development and connection with target cells. Unfortunately, there were lack of endogenous neurotrophic factors in nerve injured tissues and the targeted deliveries of exogenous neurotrophic factors are facing challenges. It is worth noting that immobilization of GFs in scaffold materials can not only provide physical and nutritional support for nerve injury repair, but also achieve targeted on-demand release [57, 58]. In the past decades, researchers have designed various of GFs-implanted neural tissue engineering scaffolds for safely delivering these key neurotrophic factors to the injured site to promote cell proliferation, differentiation and migration. Recently, satisfactory research results have been acquired and the goal of nerve repair and functional recovery has been approached.

According to a report, the half-life of neurotrophic factor 3 (NT3)-implanted chitosan scaffold was long enough to support the release of

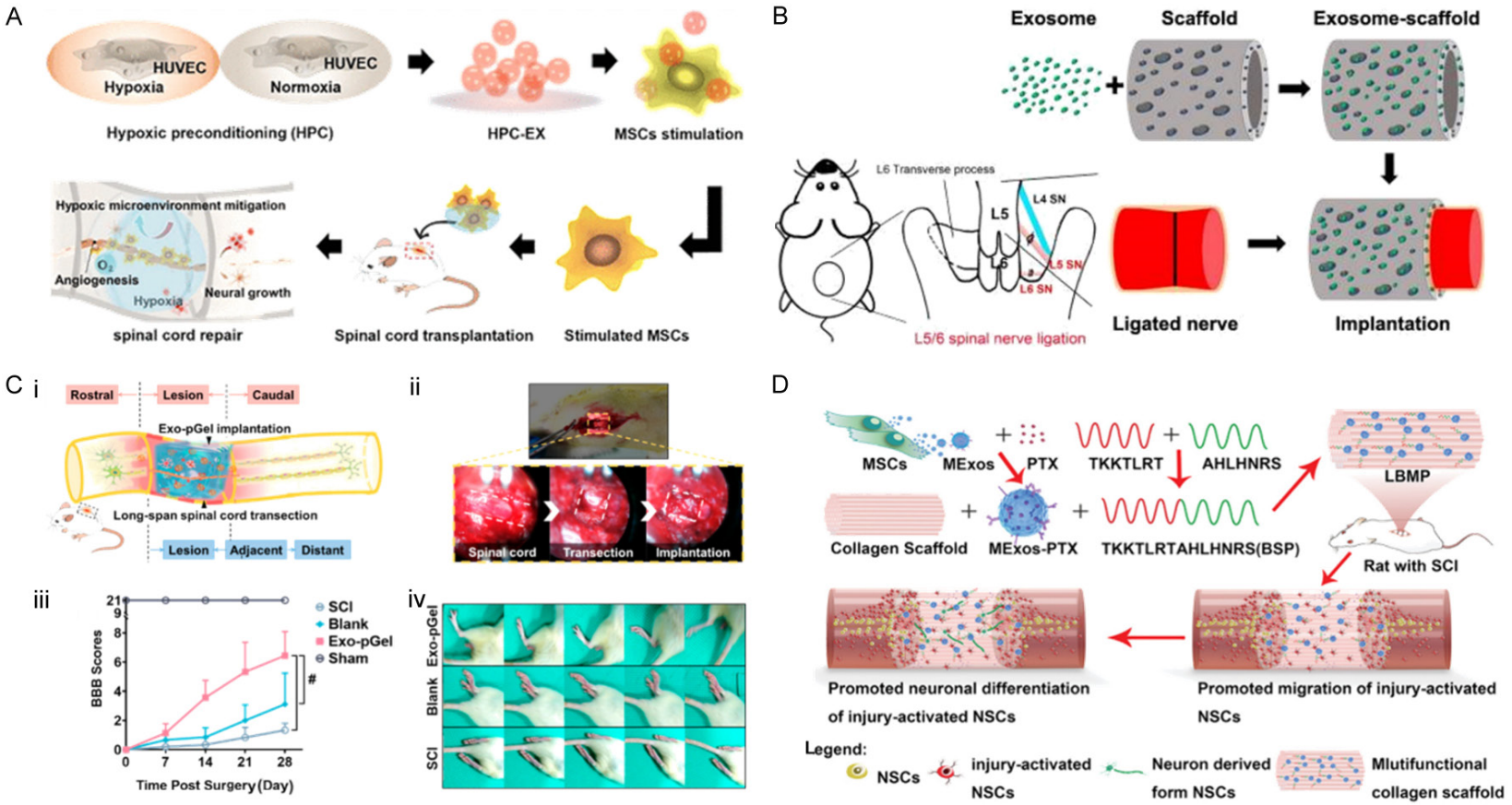

Figure 6. The diagram of extracellular vesicle implanted biological scaffold promoting neuronal survival and repair. A. Schematic illustration of hypoxic preconditioned derived EVs in SCI therapy. Reprinted with permission from Li et al. [52] Copyright 2022 American Chemical Society. B. Schematic illustration of fabrication and implantation of EVs-immobilized alginate scaffold onto the ligated L5/6 spinal nerves for injury treatment. Reprinted with permission from Hsu et al. [53] Copyright 2020 Dove Medical Press. C. Schematic illustration of Exo-pGel implantation strategy for SCI therapy and functional recovery of nerve tissue. Reprinted with permission from Li et al. [54] Copyright 2020 American Chemical Society. D. Schematic fabrication process of EVs-collagen scaffold and its application in SCI motor function recovery. Reprinted with permission from Zhang et al. [55] Copyright 2021 John Wiley & Sons.

NT3 under physiological conditions for 14 weeks. When the NT3-chitosan scaffold was implanted into the spinal cord injury site of rhesus monkey, it would cause the regeneration of motor axons, growth of cells in the lesion area and connection of the distal spinal cord. In this work, the author proved that NT3-chitosan scaffold had the ability of nerve regeneration and promoting the recovery of motor and sensory functions [59]. To study the effect and mechanism of silk fibroin/chitosan scaffold (SFCS) loaded with brain-derived neurotrophic factor (BDNF) and NT3 in the neuroprotection of SCI, Ji et al. selected Sprague-Dawley rats as SCI research model. After implantation of the resulted SFCS, the pathological symptoms of injured tissue were obviously relieved, and presented a state of smooth spinal cord, reduced scar and inflammatory activity [60]. Li et al. designed a laminin-coated gellan gum hydrogel scaffold (LN-TGG) with immobilization of nerve growth factor (NGF) to promote the adhesion, proliferation and differentiation of neural stem cells. With the help of immunofluorescence, q-PCR and western blot analysis, the researchers proved that the LN-TGG 3D culture system loaded with NGF could provide a favorable microenvironment for the adhesion and growth of neuronal stem cells [61]. In addition, GFs were also found to be the target of clinical transformation candidate drugs. Among these, insulin-like growth factor-1 had broad application potential in the treatment of peripheral nerve injuries [62].

Cells

Cells-based therapies deliver stem cells or function-specific cells to target organs to promote the functional regeneration of target cells and the secretion of bioactive substances for neural tissue engineering. The conventional stem cell delivery strategy of direct injection present poor therapeutic effect due to the immune clearance of host immune system. More attractively, cells-encapsulated scaffold technology has been reported to have excellent performance in improving the survival of loaded cells and overcoming the immune clearance after implantation. In addition, the use of cell-encapsulated scaffold can enhance the signal transduction between cell-cell and cell-ECM, which has potential value in nerve regeneration and functional recovery after injury [63-65].

According to previous studies, SCs encapsulated conduit scaffold composed of chitosan and collagen had the abilities of inducing peripheral nerve regeneration and axonal extension [66]. Biodegradable materials were used in nerve tissue engineering due to controllable cell orientation and neuron extension. Wen et al. developed a simple strategy for in-situ preparation of biodegradable starch foam scaffolds loaded with SCs by using starch gelatinization property [67]. Specifically, the bubble size and mechanical behavior of scaffold could be adjusted to regulate the diffusion of nutrients through controlling the content of starch. When serum was introduced into the culture system, the degradation of starch foam scaffold was induced and SCs cells were released. It was demonstrated that the resulted 3D tissue construct played a key role in regulating stem cells survival and axon extension, indicating the application in nerve tissue engineering. In another study, acid-soluble chitosan and alginate were spun on the arranged electrospun fiber bundles to investigate the effect of cells implantation on cell growth and neurite extension [68]. The researchers proved that the primary neurons encapsulated in the fiber scaffold survived well and the internal neurites were prolonged. Importantly, NSCs were also often encapsulated in biological scaffolds to promote the directional differentiation of neurons. Song et al. constructed a polyphenol-doped 3D scaffold for providing microenvironment for growth, proliferation and differentiation of encapsulated NSCs [69]. Mogas et al. used laminin functionalized Hemopatch™ as scaffold to promote the growth of NSCs and deliver them to the injured site of SCI for nerve function regeneration [70]. As an excellent implantation carrier, 3D porous collagen scaffold maintained the bioactivity of NSCs and promoted cells extension with excellent biocompatibility [71]. It was demonstrated that collagen scaffold construct encapsulated with NSCs could improve the hind limb motor function in T8 completely transected rat model by promoting nerve regeneration.

Drug

Drug delivery strategies include diffusion of encapsulated drugs and covalent bond based chemical immobilization to ensure sufficient drug concentration and sustained drug release

time at the lesion sites [72]. Among them, the delivery strategy of directly encapsulating drugs with scaffold using diffusion-based method is more common, and the topographic properties of scaffold regulates drug release. Meanwhile, to avoid harmful side effects associated with systemic drug delivery, researchers have focused on the development of scaffold based local drug delivery systems in recent years [73]. Benefits from its excellent biocompatibility, it has been used in neural tissue engineering to provide structural support and topographical guidance for nerve regeneration.

Early work showed polytetrafluoroethylene conduit, poly(lactide-co-caprolactone) film, fibrin gel and PLGA microspheres scaffold encapsulated small molecule drug of tacrolimus have been used to promote local nerve regeneration [74-77]. A conductive hydrogel scaffold composed of chitosan-aniline oligomers and agarose as local delivery system was developed to enhance the growth and proliferation of nerve cells by applying electrical stimulation to control drug release [78]. Mahumane and colleagues designed a drug delivery scaffold for nerve tissue regeneration [79]. It was demonstrated that this poly(lactic-co-glycolic acid) electrospun scaffold composite encapsulated with N-Acetylcysteine acquired the ability to enhance the survival and proliferation of PC12 and human glioblastoma multiform cells. One study encapsulated the neuromodular drugs of fingolimod in PLGA nanoparticles to construct PuraMatrix hydrogel 3D culture system for combining local delivery and neural tissue repair [80]. In vitro results showed that fingolimod encapsulated scaffold increased the survival rate of NSCs and promotes their fate of differentiating into neurons. An extension of this work investigated the effects of fingolimod encapsulated PuraMatrix scaffold in mouse model of compressive spinal cord injury [81]. After transplantation of the resulted scaffold for 7 days, increased survivability and migration ability of nerve cells at the lesion site were achieved leading to eventual recovery of neural function. Another approach to induce differentiation of NSCs using spinal cord-like scaffold [82]. In this work, the behavioral effects and molecular mechanisms of encapsulated O-GlcNAc transferase inhibitor of OSMI-4 on NSCs has been explored for the first time.

Biological effects of scaffolds with different topographic orientations

In the field of neural tissue engineering, topography of scaffolds reflects the spatial characteristics of the material surface [83-92]. The vitro and in vivo effects of topological oriented scaffolds have confirmed that the ordered scaffold structure affects the growth characteristics of cells. The connection and communication between neurons are necessary to maintain the normal function of nerve cells. Topographic scaffolds mimicking the native ECM microenvironment can effectively guide the growth of neurites under a desired way in response to topographical cues, devoting to nerve injury repair through neural reconnection and neural network construction. This section summarizes the common cellular biological behavioral responses to topographical orientation of scaffolds.

Morphology and alignment of cells

Imitating the ECM structure of natural tissues and promoting cell ECM interactions is a key part of neural scaffold construction. The function of nerve cell signal transduction and transmission of biochemical signals to distant tissues requires accurate cell orientation and axonal alignment [93]. Therefore, the production of scaffolds with a directional arrangement structure to guide the growth and alignment of nerve cells in a certain direction is crucial for the development and functional regeneration of neural tissue [94]. According to previous studies, many topologically structured neural scaffolds have been designed to regulate the morphology and growth characteristics of nerve cells [95]. In general, cells seeded on randomly oriented scaffolds present irregular shapes with a random orientation, while cells seeded on oriented scaffolds exhibit elongated shapes with alignment direction along the scaffold axis. This may be due to the fact that aligned scaffold facilitates the growth of neural processes and unidirectional signal pathway guidance [96]. According to the report, cells tend to be oriented along pits or similar topographic structures on the surface of the scaffold [97]. Sun and colleagues designed a micropatterned silk fibroin scaffold and clarified that the alignment of neuronal cells along the topographical cues of the scaffold was ben-

eficial to the increase in the percentage of neurons and the length of the neurites [98]. Another study found that on the aligned pyrrole plasma-coated nanofibers scaffold, the biological behavior of cells presented high neurite protrusions and cell viability, and the filamentous pseudopodium extended to the entire plasma membrane surface [99]. It has also been reported that the mechanical strength and elastic modulus of the stent affect the alignment and extension of neurons [100]. Furthermore, the construction of magnetically orienting nerve scaffold provided a new idea for cells to provide internal orientation guidance and simple preparation of magnetically oriented injectable hydrogel for nerve injury therapy [101]. In addition, the impact of scaffold topographical guidance on cell morphology and alignment has been verified on various cells, such as MSCs, NSCs, neuronal cells, oligodendrocyte promoter cells, and SCs [102], etc. So far, researchers have focused on the mechanism of how scaffold topography influences the biological activity of cells and proposed that cells can recognize the biophysical properties of the substrate to convert external mechanical signals into intracellular biochemical signals. They concluded that cells exhibited different morphologies and orientations due to contact guidance of scaffolds from in vitro and in vivo studies [97, 103].

Adhesion and proliferation

The adhesion and proliferation ability of cells on scaffold surface is a fundamental prerequisite for performing specific neural functions. It is worth to mention here that ECM biochemical interaction between cells and topographical cues is crucial for cell function and fate. In recent years, the construction of biomimicking scaffolds for neural tissue engineering has provided mechanical and biochemical stimulation to nerve cells. The initial adhesion of nerve cells to the scaffold surface supports the survival and proliferation of surrounding tissues [104]. It has been determined that the adhesion of NSCs on the surface of composite gel composed of carboxymethyl chitosan and gelatin was significantly improved with the incorporation of poly(3,4-ethylenedioxythiophene) nanoparticles, which might be attributed to the high porosity, excellent water absorption and large ECM-mimicking surface area of the hydrogel composite scaffold [105]. Studies by Que and colleagues have shown that integrin binding epitopes affected the adhesion of stem cells to the microenvironment, thereby affecting the proliferation process of stem cells [106]. According to previous reports, endothelial cells and SCs exhibited enhanced adhesion and growth of the dorsal root ganglion (DRG) on chitosans and artemisia sphaerocephala composite scaffolds modified with dopamine as compared to randomly oriented cover slides. The morphology and surface roughness of the scaffold acted as topographical cues to provide a suitable environment for cells growth behaviors [89]. Another study altered the swelling capacity and pore size of composite scaffolds by adjusting the mixing ratio of gelatin and polyvinylpyrrolidone to produce integrated structures for supporting cell adhesion and prolonged proliferation [107]. Considering the influence of the ultrastructure of the scaffold on the interaction between nerve cells and the surrounding environment, the hybrid scaffold of oxidized polyvinyl alcohol and chitosan sponges has been designed for neural tissue engineering [108]. In general, there was no clear conclusion about the effect of scaffold morphology on the adhesion and proliferation of different types of cells. By reviewing the current literatures, it has been found that the adhesion and proliferation of nerve cells were comprehensively affected by a variety of factors, such as scaffold morphologies, biomaterial types, cell types, mechanical stiffness of the ECM-like scaffolds and molecular epitopes of adhesion proteins [106, 109], etc.

Migration

Cell migration plays an important role in reconstructing and maintaining the biological characteristics and functions of neural tissue by sending signals to host cells to stimulate and guide the growth process of host axons. Generally speaking, stem cells have the ability to homing to host cells or tissues, leading them can directly migrate onto the injured sites for tissue repair induction. In neural tissue engineering, scaffolds can provide structural support and chemical clues for nerve cells to promote nerve cells or neurons migration into host cells for tissue regeneration after injury. The process of cell migration to host tissues involves the assembly and reconstruction of focal receptors, which

promote directional migration to the sites of the host nerve cell by regulating of adhesion between cells and ECM-like scaffolds. It is worth mentioning that the migration trajectory of cells exhibits an oriented movement along the topological axis. According to recent reports, cell migration as a complex biological process in cell life activities is induced by many factors, including the mechanical properties of scaffolds and chemical factors of neural microenvironment. In recent years, scientists have been committed to developing and designing scaffolds with different topographical morphologies and exploring their roles in cell migration.

Zhu et al. developed an aligned ECM-implanted scaffold for directional regeneration after tissue injury. They found that compared to random orientation scaffolds, cells grow longitudinally across ECM-like scaffolds with aligned microchannels and the directionally induced orientation increases with the increment of culture days [92]. To verify the impact of the size of microchannels on the SCs migration, Liu et al. designed a PDMS-based scaffold with micropattered channels for nerve regeneration after injury. Consistent with previous research results, axonal extension decreased with increasing channel sizes and exhibited large DRG axon penetration depth and fast SCs migration speed on small size scaffold with 50 μm microchannels [110]. Another study used chitosan conduit integrated PLGA scaffold for SCs culture and investigation on microenvironment regeneration [111]. Results showed that SCs migrated along PLGA scaffolds and guided axonal regeneration in a targeted manner. In addition, regulating the ratio of PLGA to chitosan could alleviate inflammatory reactions during autologous transplantation. To further promote cell migration by adjusting the physical properties of the scaffold, Koppes et al. applied the thermal drawing process to produce fiber scaffolds with different geometric shapes [112]. They compared DRG growth on neural scaffolds with circular, square and grooved channels. It was observed that a significant increase of neurite growth and extension length in both rectangular and grooved channels as compared to simple circular fiber scaffolds. However, all aligned geometric scaffolds resulted in enhanced migration of SCs and an increase in total axonal growth length compared with randomly guided scaffold. In general, the topographical cues of scaffolds regulated cell migration through various pathways, such as morphology, orientation, size and roughness [113], etc.

Application of scaffolds in neural tissue engineering

Benefiting from the topological guidance effect of scaffolds on nerve cells, various studies have been explored for repairing and regenerating nerve damages, suggesting the application prospects in the field of neural tissue regeneration. It is worth mentioning that neural scaffolds have also shown significant advantages in the field of hearing, such as reducing immune rejection, facilitating the combination of bioactive agents, alleviating the pressure of insufficient supply of donor organ transplants and generating the possibility of clinical conversion [114]. Scaffolds for auditory regeneration are usually implanted with cells and growth factors to response signals in the microenvironment and induce tissue regeneration to restore damaged organ functions. All these components provide suitable spatial structure for cell survival and complex interactions with the surrounding microenvironment [115]. Because the implementation of mammalian auditory function mainly depends on the transcription of the sound input to the inner ear hair cell into the cochlear nucleus by the spiritual ganglion neurons (SGNs), maintaining the development of SGNs function and promoting the regeneration of SGNs are essential to rescue hearing. So far, various scaffolds have been developed for auditory function recovery or regeneration, such as matrigels, electrospun fibers, hydrogels and 3D printing-based biomaterials scaffolds.

For example, Wille et al. constructed a biodegradable scaffold consisting of polyglycolide and poly-ε-caprolactone was accompanied by coating heparan sulfate (HS) to guide neuronal growth [116]. The obtained scaffold could provide support for SGNs and promote their survival in vitro by delivering BDNF to exert neuroprotective effects. The results showed that the synergetic effect of HS and BDNF on inner ear therapy could enhance the growth of SGNs neurite to establish neuronal guidance. Yan et al. utilized a 3D-matrigel scaffold to ensure in

vitro cultivation of SGNs for up to 6 months [117]. They found that this 3D culture system could promote the SGNs growth neurites by increasing the area of growth cones and enhancing synaptic density. In another study, IKVAV peptide was assembled on PuraMatrix hydrogel through biological functional modification to develop an injectable scaffold [118]. Coating laminin protein significantly improved the biocompatibility of the scaffold to support SGNs neurites adhesion. It was demonstrated that the constructed peptide-based biofunctional hydrogel scaffold could promote the adhesion and growth of SGN neurites for hearing regeneration and auditory function recovery in patients with sensorineural hearing loss and deafness. Additionally, researchers prepared a novel nanofiber scaffold containing aligned PLLA and polycaprorolactone electrospun nanofibers with the mixing ratio of 4:1 [119]. The composite nanofibers were coated with Matrigel and implanted into the guinea pig model through cochlear duct surgery. Importantly, the introduction of nanofibers provided contact guidance and established synaptic connections for the directed growth of neurons. This approach utilized the directional guidance of topographical cues to induce intracellular signal transduction and the electrical activity of scaffold to promote integration with cochlear implants, providing a more optimized nerve prosthesis design strategy for hearing loss treatment. Hackelberg et al. pointed out that aligned composite nanofiber scaffolds had better performance in promoting the growth of neurites and guiding neuronal differentiation than random nanofibers for auditory nerve regeneration. Based on the above examples, as an emerging therapy with immature research, neural scaffold has unpredictable potential in the restoration or regeneration of auditory function.

Next, researchers further explore the regulatory mechanism of neural stem cells mediated by scaffolds. Research has shown that scaffolds can influence the biological behavior of neural cells through Topographic contact guidance and the interaction between cell surface ligands and receptors [119]. The proliferation, adhesion and migration of nerve cells lay the foundation for nerve regeneration and repair [120, 121]. To achieve precise regulation of nerve regeneration, researches in recent years have continuously deepened the exploration of the signal mechanism of scaffold mediated nerve cell proliferation, adhesion and migration. On the one hand, cells respond to the topographical characteristics of the scaffolds through mechanical sensing, thereby regulating the biological behaviors of nerve cells. On the other hand, neural cells seeded on scaffolds respond to topographical cues by secreting specific chemical factors that affect intracellular signaling pathways and establish feedback mechanisms with host cells [122]. According to reports, Multiple signaling pathways such as Wnt/β-catenin, MAPK and PI3K-Akt play important roles in the regulation of biological behaviors of nerve cells mediated by ECM-like scaffolds [90, 91, 123].

Rey et al. used 3D Nicholid scaffolds to provide an ECM-like microenvironment for stem cell proliferation and differentiation for neural tissue engineering [124]. Specifically, after implantation of Nicoid in a traumatic SCI mouse model, the transcriptome changes of neural precursors stem cells (NPCs) were investigated by analyzing the biochemical signals that were transformed from scaffold based external mechanical stimuli into NPCs. The results indicate that PI3K-Akt-mTOR, focal adhesion and integrated mediated cell adhesion pathways played an important role in the adhesion and cytoskeletal construction of NPCs. Additionally, Nicoid scaffold promoted the proliferation of NPCs through p53 and Wnt signaling pathways. Moreover, MAPK, RAP1, FoxO and Hippo signaling pathways mediated nerve regeneration and repair after SCI injury which hold great potential in stem cell therapy-based regenerative medicine. For induction of MSCs differentiation, a methacrylated gelatin scaffold coated with graphene oxide was designed [125]. This 3D construct promoted the secretion of endogenous bone morphogenetic protein (BMP) by MSCs, establishing a positive feedback mechanism for MSCs differentiation through BMP-SMAD1/5 signal transduction pathway. Although some research results have been achieved in the field of scaffold surface morphology affecting neural cell growth through mechanical transduction mechanisms, further exploration of other signaling pathways involved in scaffold mechanical strength and biocompatibility were still needed.

Conclusion and future perspectives

Nervous system injury and neurodegenerative diseases seriously affect the life quality of patients and socio-economic development. To overcome these challenges, neural tissue engineering comprehensively applies cells, materials and engineering methods for nerve regeneration and functional recovery after nerve injury. With the continuous development of materials science and engineering methods, new scaffolds designed specifically to mimic neural tissue structures and functional regeneration of the nervous system have received widespread attention. Utilizing topographical cures and mechanical transduction on the surface of the scaffold to repair damaged nerve tissue and reduce the inflammatory response of the transplant, overcoming issues related to donor limitations and complications at the injured sites. This article reviews the manufacturing strategies and related applications of scaffolds for neural tissue engineering.

Although various porous and nano-patterned scaffold structures have been designed and constructed for nerve regeneration, there is still a gap between the current experimental research stage and clinical application, which attribute to the complexity of the human nervous system and the safety of long-term implantation of scaffolds. In addition, many patients implanted with scaffolds are often plagued by transplant and nerve compression, leading to a challenge in the degradation and metabolism of scaffolds in the body that needs to be addressed urgently. Therefore, there is still a long way to go before various nerve scaffolds developed by researchers in recent years can be transformed into clinical applications. Developing scaffolds that can highly mimic the ECM microenvironment and convert mechanical cues into chemical signals to directionally restore the function of the nervous system is the way out of the current dilemma.

Numerous previously reported studies help us understand the relationship between scaffold morphology and cell growth behavior. Future researches should focus on improving scaffold manufacturing processes and searching for biomaterials that highly simulate ECM to achieve long-term stability in vivo and large-scale production to meet clinical needs. In addition, to explore the impact of customized scaffold topologies for certain tissue regeneration on cell behavior, it is necessary to further understand the specific molecular mechanisms underlying the long-term effects of implanted scaffolds. Importantly, the combination of scaffold manufacturing strategies and encapsulations loading has significant implications for axonal growth and nerve regeneration. By encapsulating growth factors, cells and EVs into scaffolds, the embedded encapsulations are effectively transported to the injured sites. Most importantly, EVs can induce neuronal growth in vitro and promote neural regeneration in vivo by releasing encapsulated substances into ECM to enhance tissue repair and regeneration with satisfactory results. It can be expected that with the continuous improvement of scaffold manufacturing processes and the emergence of more suitable biomaterials, precise regulation of neural cell behaviors can be achieved at the mechanism level, contributing to the targeted repair of certain nerve tissues. In summary, the design and development of neural scaffolds are of great significance to the proposal of personalized and accurate treatment plans for patients with central and peripheral nervous system injuries through continuous improvement and optimization of scaffolds.

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Disclosure of conflict of interest

None.

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