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REVIEW

Advanced strategies for 3D-printed neural scaffolds: materials, structure, and nerve remodeling

Jian He[1] · **Liang Qiao**[2] · **Jiuhong Li**[5] · **Junlin Lu**[6] · **Zhouping Fu**[3] · **Jiafang Chen**[1] · **Xiangchun Zhang**[3] · **Xulin Hu**[4]

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Abstract

Nerve regeneration holds significant potential in the treatment of various skeletal and neurological disorders to restore lost sensory and motor functions. The potential of nerve regeneration in ameliorating neurological diseases and injuries is critical to human health. Three-dimensional (3D) printing offers versatility and precision in the fabrication of neural scaffolds. Complex neural structures such as neural tubes and scaffolds can be fabricated via 3D printing. This review comprehensively analyzes the current state of 3D-printed neural scaffolds and explores strategies to enhance their design. It highlights therapeutic strategies and structural design involving neural materials and stem cells. First, nerve regeneration materials and their fabrication techniques are outlined. The applications of conductive materials in neural scaffolds are reviewed, and their potential to facilitate neural signal transmission and regeneration is highlighted. Second, the progress in 3D-printed neural scaffolds applied to the peripheral and central nerves is comprehensively evaluated, and their potential to restore neural function and promote the recovery of different nervous systems is emphasized. In addition, various applications of 3D-printed neural scaffolds in peripheral and neurological diseases, as well as the design strategies of multifunctional biomimetic scaffolds, are discussed.

✉ Liang Qiao
qiaoliangjoy@gmail.com

✉ Xiangchun Zhang
zhangxc@tricaas.com

✉ Xulin Hu
huxulin1993@163.com

[1] College of Basic Medicine and Forensic Medicine, Henan University of Science and Technology, Luoyang 471023, China

[2] The First Affiliated Hospital, College of Clinical Medicine of Henan University of Science and Technology, Luoyang 471003, China

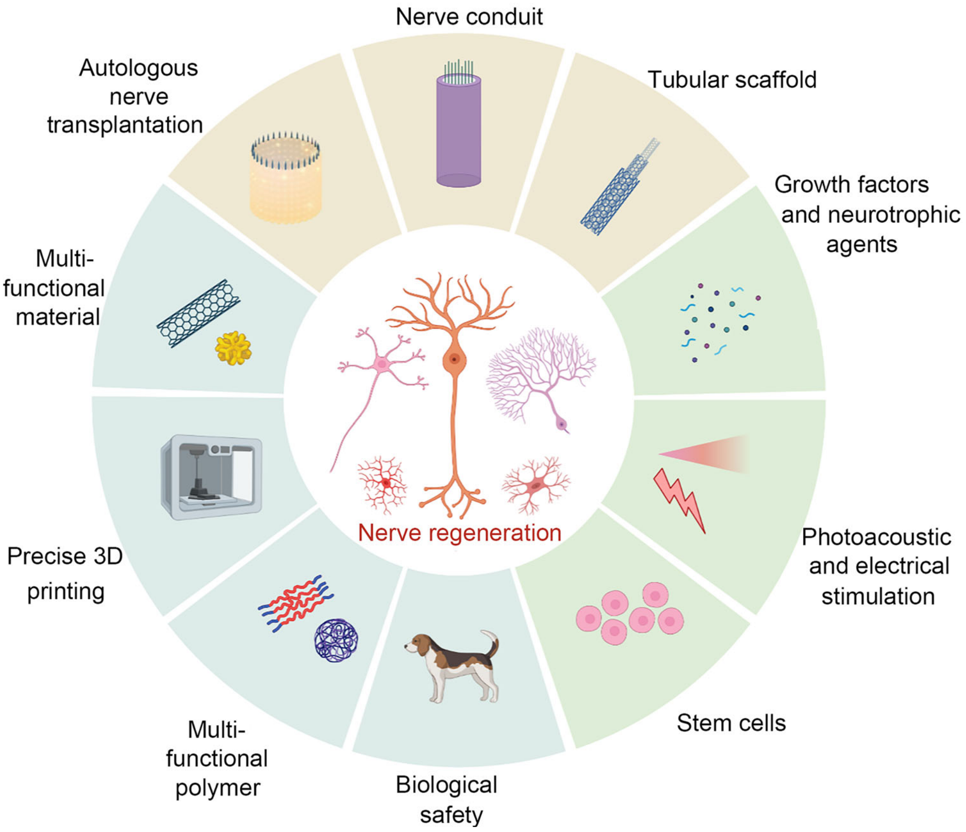
[3] Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

[4] Clinical Medical College, Affiliated Hospital of Chengdu University, Chengdu University, Chengdu 610081, China

[5] Department of Cardiovascular Surgery, Department of Biotherapy, Cancer Center and State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, China

[6] Department of Neurosurgery, West China Hospital, Sichuan University, Chengdu 610041, China

Graphic abstract



Keywords Nerve regeneration · 3D printing based neural scaffolds · Biomaterials · Nervous system · Design strategies

Introduction

Nerve regeneration is the process of tissue regeneration and function restoration to repair nerve tissues damaged by trauma or disease. It can help restore damaged nerve function in the treatment of neurological diseases and injuries [1]. Neural regeneration is mainly employed in the following aspects: (1) brain trauma (neural regeneration can help treat brain trauma, such as stroke and brain or spinal cord injury); (2) neurodegenerative diseases (nerve regeneration plays a role in addressing neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases); (3) peripheral nerve injury (nerve regeneration can help treat peripheral nerve injuries, such as the amputation of arms and legs) [2–4]. The human nervous system comprises two main divisions: the central nervous system (CNS) and the peripheral nervous system. The CNS encompasses the brain and spinal cord [5]. It primarily controls and regulates various physiological and psychological activities, such as thinking, feeling, action, and emotion. Peripheral nerves refer to neural tissues outside the brain and spinal cord, comprising all neurons and nerve fibers, including afferent, efferent, and mixed nerves [6]. Peripheral nerves are mainly responsible for transmitting sensory and motor information and controlling limb activities and reactions. Their damage interrupts the transmission of nerve signals, affecting the ability to feel and move a limb. Failure to promptly repair these nerves can result in muscle loss, paresthesia, and pain. Therefore, repairing and regenerating peripheral nerves are employed in clinical applications, including rehabilitation treatment after limb stumps and trauma. Recent studies conducted on peripheral nerve repair mainly involved the following aspects: three-dimensional (3D) printing technology, electrical nerve stimulation and neurotrophic agents, neural stem cell transplantation, and nerve regeneration materials [7–9].

In contrast, repairing the CNS is relatively challenging. Current clinical treatments for central nerve injury mainly employ methods to protect nerve cells and facilitate nerve regeneration, such as the use of neuroprotective and neurotrophic agents [10]. Researchers have uncovered a degree of self-healing capability in the brain and spinal cord wherein the nervous system adapts to injury and restores function via reorganization and reconnection [11]. The regeneration and reconnection of nerve cells are limited after CNS injury owing to the complexity of the CNS, hindering their original function restoration. Research on CNS repair has recently shown continuous progress, including cell transplantation therapy, gene therapy, and artificial intelligence technology [12–14]. Unfortunately, numerous challenges need to be overcome to realize nerve regeneration in practical applications. The unique structure and function of nerve tissues inherently complicate their regeneration. The process of nerve regeneration is complex, involving the interactions of multiple cell types and signaling pathways, and much remains to be investigated in neurogenesis. Clinical neuroregenerative treatments mainly include surgical and medical treatments, but they offer limited therapeutic effects. Therefore, performing in-depth research on nerve regeneration is essential. Moreover, the clinical translation of research findings is overly complex.

Three-dimensional printing technology is an emerging technology with promising applications in nerve regeneration [15]. It can manufacture intricate neural structures, including neural conduit and scaffolds. Neural tubes are hollow, tubular structures that guide nerve regeneration, whereas neural scaffolds are 3D structures designed to support and facilitate the growth of nerve cells [16]. Three-dimensional printing offers flexibility for the fabrication of neural tubes with various sizes and shapes, and even complex neural scaffolds to accommodate different nerve regeneration needs. These 3D neural structures have the potential to address various injuries and diseases [17, 18]. In addition, biomimetic 3D models of the nervous system can be employed to investigate the pathogenesis and treatment of diverse neurological diseases.

This overview summarizes the research progress on 3D-printed neural scaffolds for repairing central and peripheral nervous systems (Fig. 1). The critical issues that hinder the regeneration of the nervous system are highlighted. Our focus centers on the development of therapeutic strategies for nerve regeneration, including the promotion of diverse neural materials and the improvement of structural design strategies. Main abbreviations mentioned in the paper are listed in Table 1. In addition, various fabrication techniques for constructing neural scaffolds are described. Extrinsic and intrinsic mechanisms activated by different neural injuries are considered for triggering neuronal differentiation and axon formation. Moreover, a brief overview of the potential applications of 3D-printed neural scaffolds in addressing medical disease is provided. Furthermore, the challenges and perspectives that may apply to various tissue nerve injuries are discussed.

Neural scaffold construction techniques

Electrospinning

Electrospinning is a promising technique for the production of nanofibers with various applications in biomedicine and materials science [19]. Electrospun nanofibers are prepared by layering polymer solutions or melting jets using electrostatic force, resulting in structures with a high surface area and a porous structure. This method is cost-effective and enables the customization of fiber properties by adjusting spinning parameters (Fig. 2) [20]. Neural scaffold design requires mimicking the microenvironment of the injured system. Natural repair is limited in a damaged nervous system, where neurons require a suitable microenvironment for proliferation. For instance, Ghorbani et al. developed conductive PLA–MWCNT neural scaffolds via wet electrospinning. The scaffold surface incorporated alginate, gelatin, and MWCNTs, which can induce the transdifferentiation of mesenchymal stem cells (MSCs) [21]. Similarly, conductive PCL–CS scaffolds loaded with soluble gold ions were prepared for nerve regeneration. Compared to the PLA–MWCNT neural scaffolds, the conductive PCL–CS scaffolds enhanced the attachment and proliferation of fibroblasts [22].

The electrospun fibrous scaffold closely mimics the extracellular matrix, facilitating the growth of regenerated axons and promoting nerve regeneration. Its fiber arrangement, nanomorphology, and diameter can be precisely engineered during preparation. In addition, drugs, nucleic acids, or proteins can be loaded into these scaffolds, stimulating glial cell phenotype transformation and supporting more effective nerve regeneration [23, 24]. Functional electrospun conduits offer structural guidance at the bone defect site, promoting nerve regeneration in the immune microenvironment with improved vascular regulation. Dong et al. developed an oriented nanofibrous scaffold containing deferoxamine (DFO), promoting cellular bridge formation and immune microenvironment modulation. The directionally arranged nanofibers of the scaffold can modulate macrophage polarization and reduce inflammatory factor expression. Controlled DFO release further promotes human umbilical vein endothelial cell (HUVEC) migration and tube formation by upregulating hypoxia-inducible factor-1α (HIF1-α), VEGF, and stromal cell-derived factor-1α (SDF-1α) expression [25]. In another study, PLA–SF sponge scaffolds were found to promote stem cell proliferation and significantly enhance the repair of peripheral nerve function in rats with 10-mm-long sciatic nerve defects. Furthermore, electrospinning can be used to construct biomaterial-free neural scaffolds, reducing immunogenicity and other complications [26].

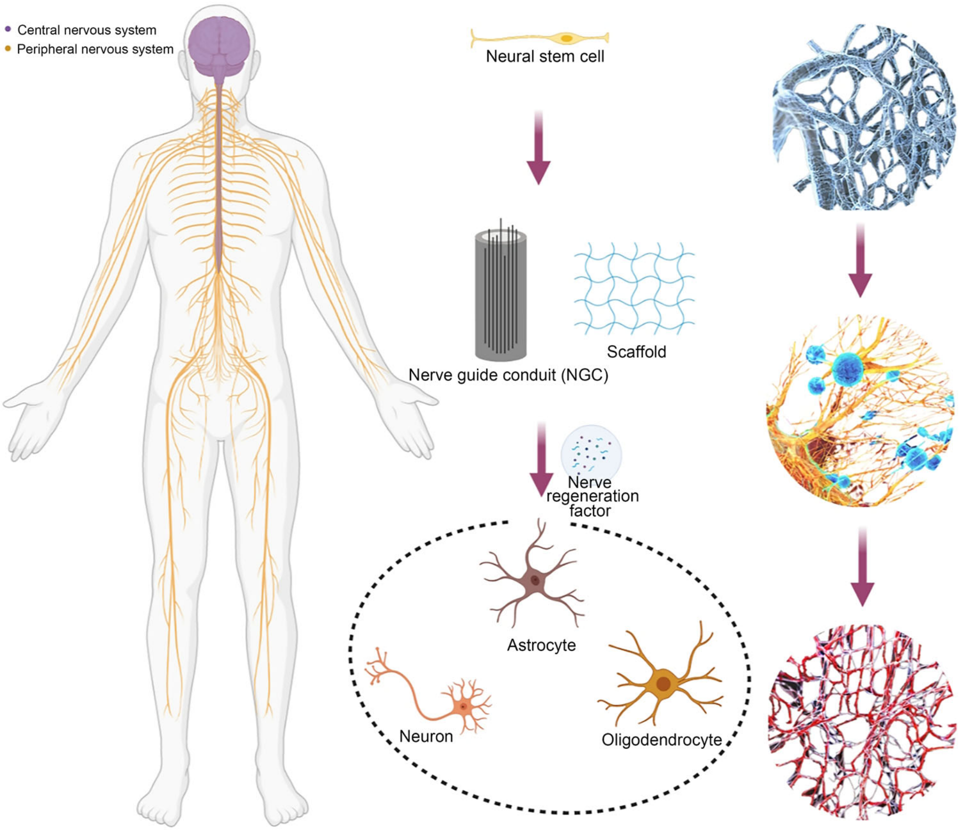


Fig. 1 Functional scaffold promoting central and peripheral nerve regeneration

3D printing

With the assistance of imaging and computer technology, 3D printing seamlessly integrates materials and components with diverse characteristics, significantly expediting the development of new medical products (Fig. 3) [27]. In theory, 3D printing allows the combination of various materials, enabling the construction of multifunctional scaffolds (Table S1 in Supplementary Information) [28].

Inkjet printing

Three-dimensional inkjet printing constructs neural scaffolds by precisely depositing bioinks or materials, such as GG, SA, and gelatin layer by layer. Based on sol–gel and 3D printing technology, Zhang et al. produced a GG–ST scaffold biocompatible with L929 fibroblasts and RSC96 SCs [29]. Neural differentiation is a prerequisite for nerve regeneration. Li et al. constructed a new sodium alginate (SA) hydrogel to promote the efficiency of EMSC growth and neuronal differentiation [30]. However, the electrical insulation of the scaffolds hinders the transmission of electrical signals during nerve regeneration, limiting the in vivo efficacy of the 3D-printed neural tissue scaffolds. To address this challenge, Song et al. prepared an electrically conductive hydrogel (ECH) scaffold with modified PEDOT. The modified component comprised chondroitin sulfate and tannic acid, which can effectively improve the water solubility and electrical properties of the ECH. NSCs can efficiently survive (>90%) on this scaffold and tend to differentiate into neurons with elongated axons [31].

Stereolithography (SLA) and digital light processing (DLP)

SLA and DLP can produce intricate scaffold structures with fine details that mimic the complex architecture of neural tissues, facilitating guided axonal growth. These methods allow for the direct integration of bioactive components (e.g., growth factors, CNTs, and extracellular matrix proteins) into scaffold materials, thereby enhancing their capacity to support nerve regeneration and axonal growth [32]. Lee et al. employed SLA to fabricate MWCNT–hydrogel neural scaffolds with adjustable porous structures. Combining amine-functionalized MWCNTs with polymers enhances the electrical conductivity and modulates the surface morphology of their scaffolds. Most importantly, it regulates NSC growth and neurite outgrowth [33]. However, SLA can be expensive and has a low printing speed. Careful material selection is essential for SLA, and postprocessing steps such as cleaning or surface modification may be required.

Table 1 Some abbreviations mentioned in this article

Material	Abbreviation	Material	Abbreviation
Arg-Gly-Asp	RGD	Bacterial cellulose	BC
Bone marrow mesenchymal stem cells	BMSCs	Carbon nanotubes	CNTs
Cellulose nanofibrils	CNFs	Chitosan	CS
Collagen–chitosan	C–C	Collagen–heparin sulfate	C–H
Conditioned medium	CM	Conductive polymer hydrogels	CPHs
Copolymerizing polyaniline	PANI	Decellularized extracellular matrix	dECM
Deferoxamine	DFO	Dopamine	DA
Ectomesenchymal stem cells	EMSCs	Electrical stimulation	ES
Endothelial cell	EC	Gelatin methacrylate	GelMA
Gellan gum	GG	Gellan gum–starch	GG–ST
Graphene	G	Graphene foams	GF
Graphene oxide	GO	Human umbilical cord blood MSCs	HUCMSCs
Ile-Lys-Val-Ala-Val	IKVAV	Induced pluripotent stem cells	iPSCs
Low-level light therapy	LLLT	Magnetic 3D bioprinting	M3DB
Magnetic nanoparticles	MNPs	Matrigel	MA
Mesenchymal stem cell condition medium	MSC-CM	Multiwall carbon nanotubes	MWCNTs
Neural stem cells	NSCs	Human dental pulp stem cells	hDPSCs
Poly(ethylene glycol) diacrylate	PEG-DA	Poly(2-hydroxyethyl methacrylate)	pHEMA
Poly(3,4-ethylenedioxythiophene)	PEDOT	Poly(L-lactic acid-co-caprolactone)	PLCL
Poly[3(S)-methyl-morpholine-2,5-dione-co-lactic]	P(MMD-co-LA)	Polyacrylamide	PAM
Polycaprolactone	PCL	Polydopamine	PDA
Polylactic acid	PLA	Polylactic-co-glycolic acid	PLGA
Poly-L-ornithine	PLO	Polypyrrole	PPy
Polyvinylidene fluoride	PVDF	Salivary gland	SG
Schwann cell	SC	Silicified collagen scaffolds	SCSs
Silk fibroin	SF	Silk-hydroxyapatite	Silk-HAP
Sodium alginate	SA	3D-printed polylactic acid scaffolds	3DP-PLASs
Vascular endothelial growth factor	VEGF	3D-printed collagen–chitosan-secretome	3D-CC-ST

Fused deposition manufacturing (FDM)

FDM precisely controls layer-by-layer material deposition by employing mechanically supportive and biodegradable polymers, such as PCL and PLGA. However, compared to 3D printing methods such as SLA, FDM exhibits limited resolution, which may impact the fine details of the scaffold structure and hinder axonal growth guidance. In addition, achieving biodegradation rates matching the regeneration rates of nerve tissue in FDM-printed materials may be challenging, often requiring additional modifications or coatings.

Electrohydrodynamic (EHD) jet printing

To enhance scaffold compatibility with the implantation microenvironment, EHD jet printing has been employed to fabricate intricate and finely detailed neural scaffold structures. Notably, EHD jet printing allows for the precise dispersion of living cells within the scaffold during printing. This capability allows the fabrication of nerve conduits that offer an optimal microenvironment for severed peripheral nerves. However, EHD jet printing can be technically complex and may require subsequent postprocessing steps.

Challenges of neural scaffold construction technologies

Although electrospinning and 3D printing technologies have been extensively studied, they still exhibit certain drawbacks. Electrospinning is a relatively slow process and is limited in terms of large-scale and uniform fiber production. In addition, it often involves volatile solvents, potentially causing health and environmental concerns. Furthermore, the use

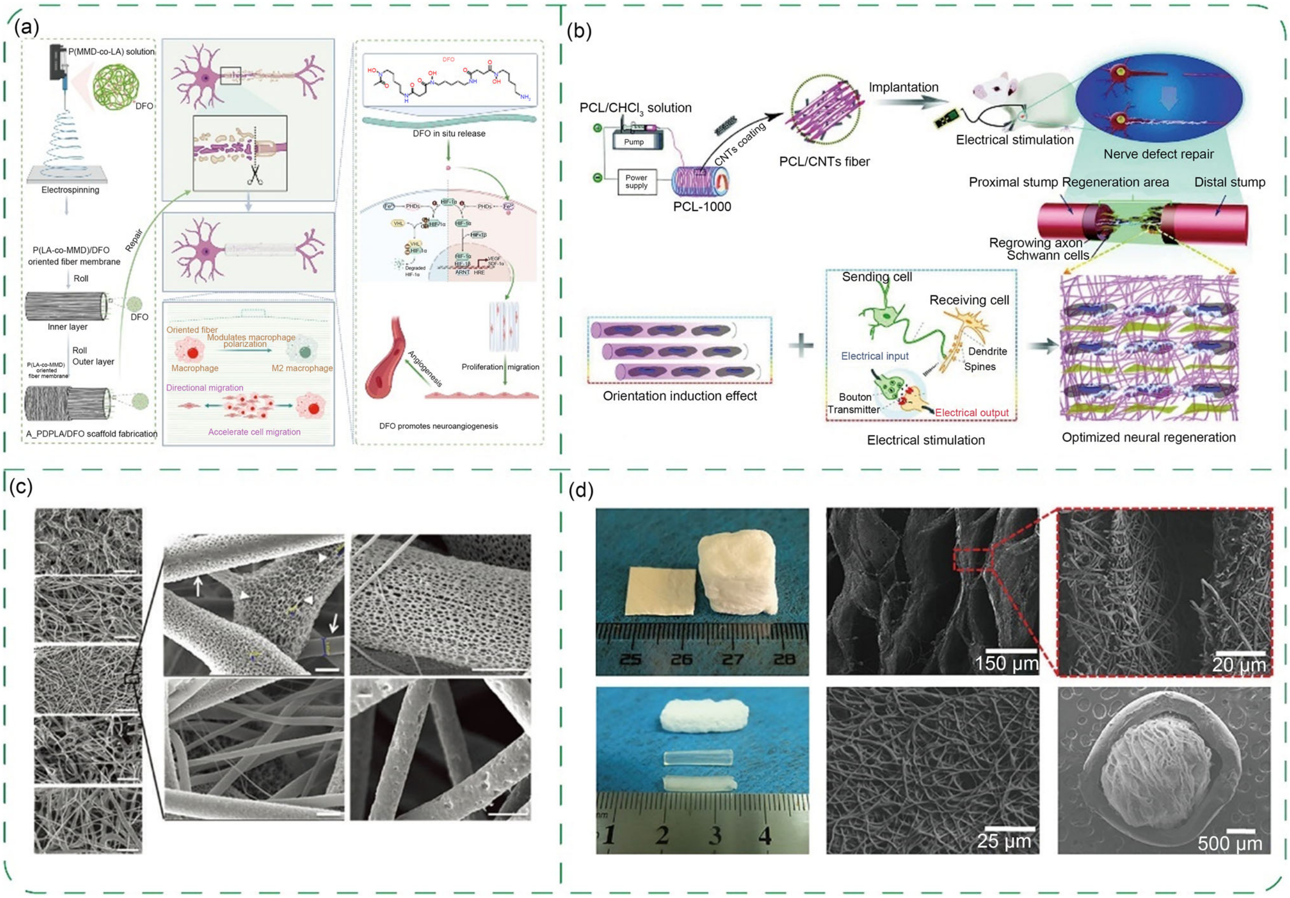


Fig. 2 Design and development of electrospinning-based scaffolds for nerve regeneration: **a** DFO-loaded P(MMD-co-LA) scaffolds promoting nerve regeneration and angiogenesis (reproduced from Ref. [25], Copyright 2021, with permission from Elsevier Ltd.); **b** PCL and CNT conductive composite fibers (reproduced from Ref. [47], Copyright 2020, with permission from Wiley–VCH GmbH); **c** PLA nanofiber scaffolds coated with MWCNTs (reproduced from Ref. [21], Copyright 2018, with permission from SAGE Publications); **d** PLA–silk sponge (reproduced from Ref. [26], Copyright 2019, with permission from the authors, licensed under the terms of the Creative Commons Attribution License). P(MMD-co-LA): poly[3(S)-methyl-morpholine-2,5-dione-co-lactic]; DFO: deferoxamine; PCL: polycaprolactone; CNT: carbon nanotube; PLA: polylactic acid; MWCNTs: multiwall carbon nanotubes

of high-voltage equipment in electrospinning poses safety risks and noise-related issues. A comprehensive assessment of environmental and health risks associated with solvents and chemicals used in electrospinning is essential. Moreover, electrospun fibers tend to exhibit poor mechanical properties and high brittleness. Similarly, neural scaffolds fabricated using 3D printing technology must be improved in terms of mechanical properties. While 3D printing offers speed advantages, achieving the necessary pore size for nerve repair can be inconsistent. In addition, the selection of suitable printing materials and the use of high-resolution devices can significantly increase costs.

However, challenges remain for deploying neural scaffolds in clinical applications. Although the materials chosen to construct neural scaffolds are biocompatible, whether they can avoid triggering immune responses or causing tissue damage in living organisms, integrate seamlessly with host tissues, and promote tissue regeneration must be investigated using animal models and human trials. Therefore, the biodegradation cycle and products of scaffold materials, as well as the scalability and reproducibility of neural scaffolds, are the key features of preclinical application supervision.

Neural scaffold materials

Materials used for nerve regeneration include natural, synthetic, and composite materials. Natural materials offer excellent biocompatibility and degradability, promoting nerve regeneration and tissue repair [34]. In contrast, synthetic materials offer enhanced controllability and stability, enabling precise structure fabrication. Meanwhile, composites combine the advantages of the two material types,

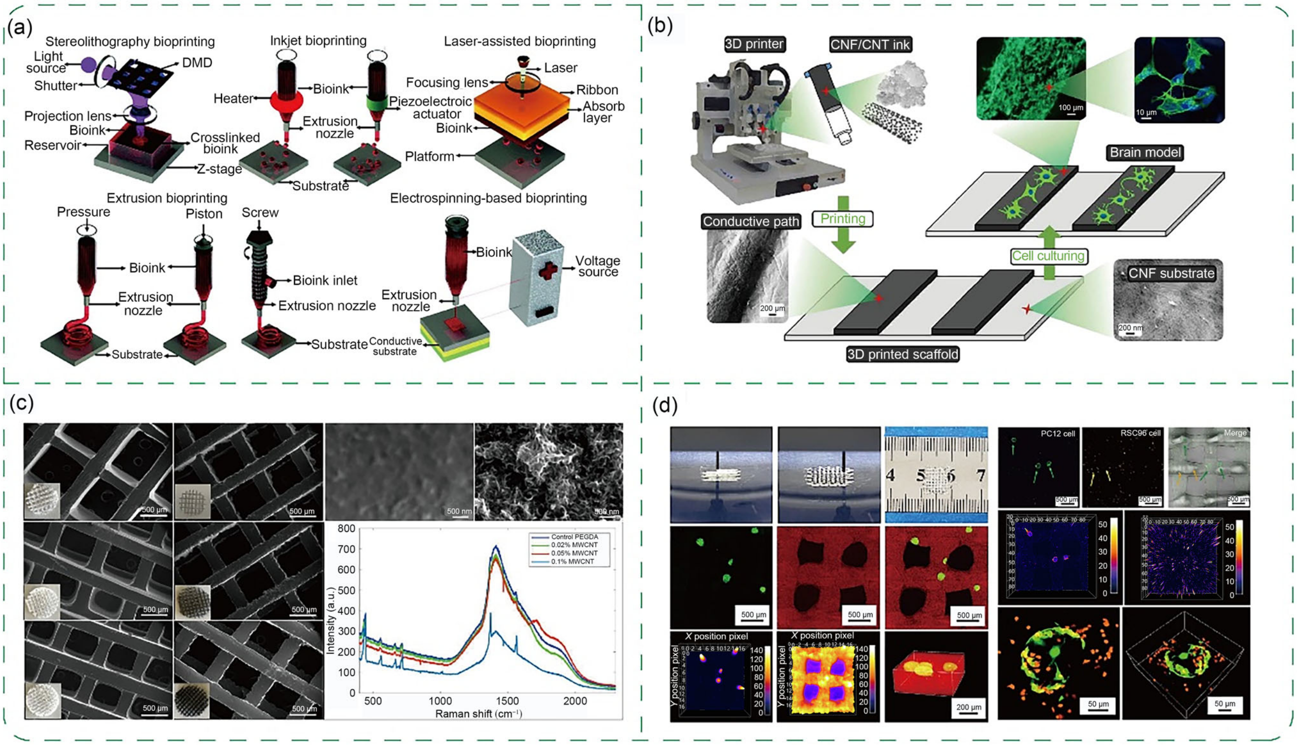


Fig. 3 Design and development of a 3D printing-based scaffold for nerve regeneration: **a** different types of 3D bioprinting techniques (reproduced from Ref. [8], Copyright 2022, with permission from the authors, licensed under the terms of the Creative Commons Attribution License); **b** 3D-printed neural scaffolds based on a conductive nanocellulose ink (reproduced from Ref. [57], Copyright 2018, with permission from Elsevier Ltd.); **c** MWCNT–hydrogel composite neural scaffold fabricated using a stereolithography 3D printer (reproduced from Ref. [33], Copyright 2018, with permission from IOP Publishing Ltd.); **d** 3D composite hydrogel scaffold comprising microspheres (reproduced from Ref. [41], Copyright 2020, with permission from Elsevier Inc.). MWCNT: multiwall carbon nanotube

seeking a balance between biocompatibility and controllability. In this section, we introduce biomaterials for constructing neural scaffolds and discuss the challenges associated with developing materials for biomedical and tissue repair applications (Table S2 in Supplementary Information).

Natural materials

Natural materials such as collagen, gelatin, alginate, chitosan, and other natural polymers have been employed in neural scaffold construction owing to their biocompatibility and biodegradability (Fig. 4).

Collagen

Collagen is a protein that constitutes the extracellular matrix in nerve tissues and is readily broken down by natural enzymes [35]. Sun et al. developed a 3D-printed C–C scaffold to modulate the microenvironment for axonal regeneration. This innovative scaffold not only reduced the formation of scars and cavities but also enhanced nerve fiber regeneration and functional recovery [36]. To enhance scaffold cytocompatibility and provide a porous 3D structure, Liu et al. constructed 3D-CC-ST at low temperatures (20 °C). In a canine traumatic brain injury (TBI) model, 3D-CC-ST not only reduced the cavity size at the implantation site but also promoted revascularization and nerve fiber repair. Most intriguingly, the scaffold promoted neuronal differentiation and synapse formation by modulating the levels of TBI-related inflammatory factors, resulting in a significant reduction in apoptosis [37].

However, the insufficient mechanical properties of collagen limit its widespread medical applications. Jiang et al. developed a C–H scaffold with suitable physical properties, degradation rates, and biocompatibility. The C–H scaffold promoted blood vessel and nerve regeneration and effectively repaired structural defects in the cerebral cortex and corticospinal tract in the rat model [38]. The crosslinking of heparan sulfate with collagen improved the mechanical properties of the C–H scaffold. Furthermore, the scaffold improved the pathological process of damage repair and increased the number of neurofilament-positive cells [39].

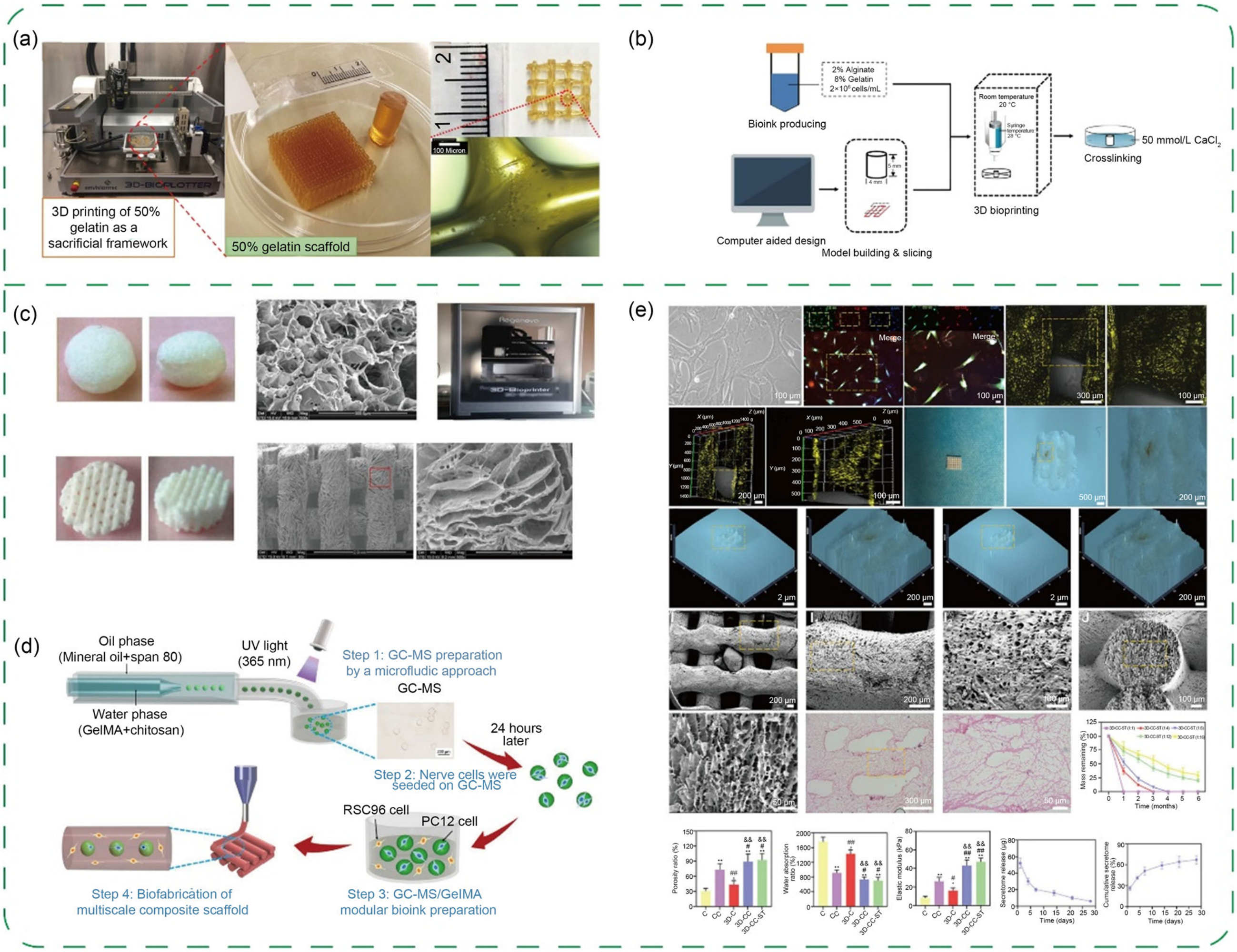


Fig. 4 Design and development of 3D-printed nerve regeneration scaffolds based on natural polymers: **a** alginate scaffold (reproduced from Ref. [42], Copyright 2019, with permission from Elsevier Ltd.); **b** gelatin–alginate neural hydrogel scaffold (reproduced from Ref. [43], Copyright 2019, with permission from Elsevier B.V.); **c** collagen–heparin sulfate scaffold (reproduced from Ref. [39], Copyright 2017, with permission from Wiley Periodicals, Inc.); **d** GelMA–chitosan scaffold (reproduced from Ref. [41], Copyright 2020, with permission from Elsevier Inc.); **e** collagen–chitosan scaffold (reproduced from Ref. [37], Copyright 2022, with permission from the authors, licensed under the terms of the Creative Commons Attribution License). GelMA: gelatin methacrylate

Gelatin

Collagen is susceptible to environmental denaturation, whereas gelatin derived from collagen is easily storable and moldable into various shapes. GelMA structures that involved RGD and matrix metalloproteinase have been used. Zhou et al. synthesized a 3D-printed dopamine-modified gelatin methacrylate (GelMA-DA) scaffold to promote NSC growth. The interconnected structures of the scaffold accelerated the formation of neural networks by enhancing the expression of neuronal genes (*TUJ1* and *MAP2*), although the *Nestin* gene expression was downregulated [40]. In another study, Chen et al. constructed a multisize composite scaffold comprising GelMA–CS microspheres (GC–MS) and GelMA hydrogel, simulating both the micro- and macroenvironments of the neural network structure. This scaffold offers a suitable environment for SC proliferation and neuron regeneration, effectively promoting neurite growth [41].

Alginate

Brown algae extract alginate is a natural polysaccharide that gels in the presence of calcium ions, and its degradation products are nontoxic. Naghieh et al. employed indirect bioprinting to construct 3D scaffolds with different concentrations (0.5%, 1.5%, and 3%) [42]. Although lower alginate concentrations could be used to adjust the biocompatibility and conductivity of the scaffolds, they lacked sufficient biological functionality. Therefore, Wu et al. constructed a hydrogel neural scaffold comprising gelatin and alginate, providing a suitable microenvironment that enhanced the adhesion and survival of SCs by improving the expression of cytokines [43].

Chitosan

Chitosan is a natural polymer derived from chitin and possesses excellent biocompatibility and antibacterial properties. Li et al. synthesized a functionalized CS–collagen composite scaffold that can simultaneously release IKVAV and VEGF. This scaffold effectively supported the proliferation of ECs and SCs and accelerated nerve regeneration and vascularization by upregulating the expression levels of multiple genes and proteins. Moreover, the scaffold showed excellent angiogenesis induction in embryos and effectively promoted myelination in rat models [44].

Synthetic materials

Polymer synthetic materials have been widely used to construct neural scaffolds, including PLGA, PPy, PCL, and pHEMA (Fig. 5).

PCL

Owing to the degradability of PCL, the neural scaffold prepared using PCL can load and control the release of neurotrophic factors, continuously activate the neurofactor signaling cascade, and increase the survival of primary neurons [45]. However, PCL must be combined with other technologies to endow PCL-based neural scaffolds with electrical conductivity. To address the poor conductivity of PCL-based neural scaffolds, Wang et al. fabricated a flexible conductive scaffold (Au-PCL) by combining a PCL-based microgrid with a sputter-coated gold nanolayer via melt electrowriting. This gold nanocoating significantly enhanced neural differentiation and improved neurite outgrowth compared to uncoated PCL scaffolds [46]. However, although the gold coating can provide high electrical conductivity to the scaffolds, it may not achieve ES in situ. Therefore, nanomaterials that can facilitate in-situ electrical conduction have been used. Zhang et al. prepared PCL–CNT composite conductive fibers with various orientation degrees by adjusting the electrospinning speed. The unique electrical conductivity and arrangement of the scaffold enhanced the nerve repair effect and promoted the directional growth of nerve cells. In addition, the PCL–CNT composite fibers significantly enhanced myelination and axon regeneration under ES, effectively repairing sciatic nerve damage [47].

PPy

Compared to materials such as PCL and PLGA, PPy has superior electrical conductivity, making it a promising choice for neural scaffold construction. Zhao et al. fabricated a stable PPy–SF conductive composite scaffold that performed well under ES and exhibited excellent toughness. The micropatterned structure of the scaffold effectively promoted SC proliferation and migration while regulating the expression of neurotrophic factors under ES. Most importantly, it activated the mitogen-activated protein kinase (MAPK) signal transduction pathway in a rat model, enhancing in vivo axon growth [48]. Vijayavenkataraman et al. used the PPy-b-PCL block copolymer to construct 3D porous nerve guide conduits (NGCs) using a novel electrojet 3D printing process. The mechanical properties of the scaffold matched the human body’s natural peripheral nerves (about 6.5 MPa). An increased PPy-b-PCL ratio significantly enhanced the scaffold conductivity (0.28–1.15 mS/cm), enabling human embryonic stem cells to attach to the scaffold surface and differentiate into peripheral neurons. However, excess PPy-b-PCL remarkably decreased the mechanical properties of the scaffold [49].

Other synthetic materials

Current neural scaffold materials are primarily synthesized from degradable polymers, but nondegradable synthetic polymers can also be integrated into clinical applications. Although they cannot be naturally degraded, their mechanical properties meet the implantation requirements and maintain the structural stability of their scaffolds. Cheng et al. manufactured a porous PVDF–PCL composite neural scaffold, where PVDF demonstrated the capacity to promote SC differentiation and proliferation through transient ES. In rat sciatic nerve defects, PVDF–PCL scaffolds effectively facilitated electrophysiological, morphological, and functional nerve recovery [50].

Similar to SA, pHEMA forms highly stable hydrogels in water. Badea et al. developed pHEMA-based 3D hydrogel scaffolds using direct ink writing and performed surface modification using poly-D-lysine (PDL) containing the RGD peptide (RGD–PDL). The scaffold significantly influenced the growth of NIH–3T3 murine fibroblasts and MC3T3-E1 cells, guiding the formation of primary hippocampal neuronal networks in rats [51].

Nonetheless, varying stress patterns may be formed during nerve regeneration in injured tissues, leading to scaffold deformation. Thus, scaffold materials must possess both toughness and durability. Dong et al. developed a CPH using PANI and PAM as raw materials, which exhibited excellent conductivity and biocompatibility. PANI can transmit

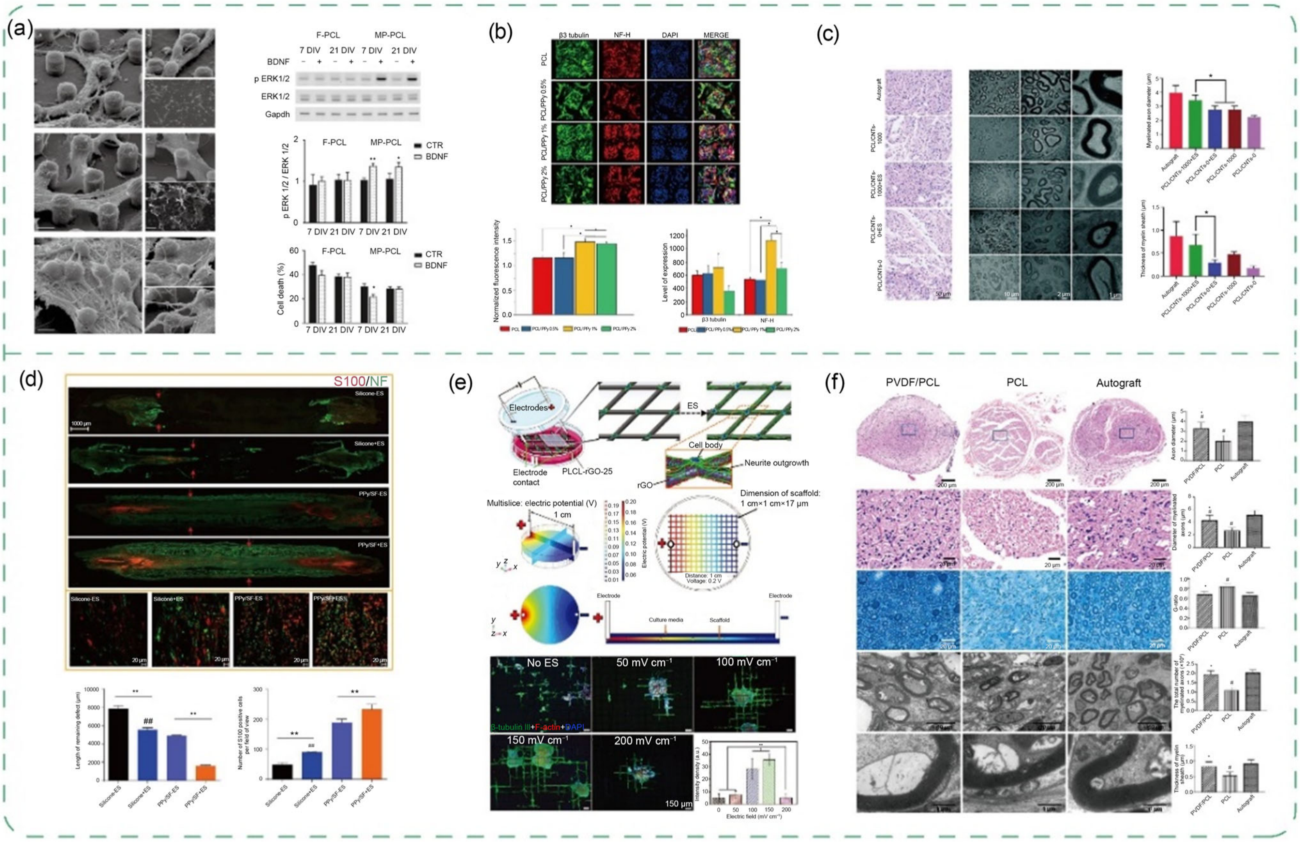


Fig. 5 3D-printed neural scaffolds using synthetic polymers: **a** controlled release of BDNF using a PCL scaffold (reproduced from Ref. [45], Copyright 2018, with permission from Springer Science + Business Media, LLC, part of Springer Nature); **b** conductive PCL–PPy scaffold (reproduced from Ref. [49], Copyright 2019, with permission from the authors, licensed under the terms of the Creative Commons Attribution License); **c** conductive PCL–CNT composite fiber scaffolds (reproduced from Ref. [47], Copyright 2020, with permission from Wiley–VCH GmbH); **d** conductive PPy–SF composite scaffold (reproduced from Ref. [48], Copyright 2020, with permission from Elsevier Ltd.); **e** conductive PLCL–rGO scaffold (reproduced from Ref. [56], Copyright 2020, with permission from Wiley–VCH GmbH); **f** ES-stimulated PVDF–PCL scaffolds (reproduced from Ref. [50], Copyright 2019, with permission from Elsevier Ltd.). BDNF: brain-derived neurotrophic factor; PCL: polycaprolactone; PPy: polypyrrole; CNT: carbon nanotube; SF: silk fibroin; PLCL: poly(L-lactic acid-co-caprolactone); rGO: reduced graphene oxide; ES: electrical stimulation; PVDF: polyvinylidene fluoride

electrical signals when subjected to near-infrared light, facilitating the repair and replacement of damaged peripheral nerves. The mechanical properties of CPH were comparable to those of neural tissues, and its electrical conductivity remained unaffected even when CPH was stretched, ensuring that the scaffold's performance was maintained during physical activity [52].

Nanoparticles

The precise spatial arrangements of carbon atoms into two-dimensional sheets and one-dimensional tubes form graphene and CNTs, respectively, with robust electrical conductivity [53]. Gasparotto et al. developed neural scaffolds based on graphene@PLA, featuring specific morphologies (lines and ridges) that encouraged the alignment of myoblasts and fibroblasts. The scaffolds effectively supported the proliferation and differentiation of various cells, including iPSCs, fibroblasts, neurons, and myoblasts. The presence of graphene enhanced iPSCs' capability to merge neuroectoderm and myoblasts, forming multinucleated myotubes. Although graphene increased the expression of transcription factor (Pax6) and Nestin in iPSCs, it did not induce the growth of new neurites in mature SH-SY5Y cells [54]. Further, Guo et al. conducted microbial fermentation on porous 3D graphene to create a porous network of BC, forming a conductive nanofiber scaffold with new micropores. NSCs adhered to the scaffold surface, quickly differentiated into neurons, and organized into neural networks [55]. Furthermore, GO can be coated on PLCL microfibers. Reduced GO (rGO), resulting from the in-situ reduction of GO, imparts a scaffold with conductive properties (about 0.95 S/cm), accelerating the formation of neuronal networks [56]. Compared to GO, the unique one-dimensional structure of CNTs enhances their electrical and thermal conductivities. Kuzmenko et al. developed a conductive ink by

Table 2 Fabrication and properties of bioscaffold-free conduits

Material composition	Regenerative effect	Animal model	Reference
Dermal fibroblasts	Generation of abundant myelinated axons	Rat, dog	[60]
Human dermal fibroblasts	Promoting the regeneration of 10-mm nerve injuries	Rat	[61]
Stem cells derived from deciduous teeth	Promoting functional reconstruction	Rat	[62]
Mesenchymal stem cells (from human gingiva)	Promoting functional restoration	Rat	[63]
Decellularized nerve matrix	Effectively promoting axon regeneration and myelination	Rabbit	[64]

combining cellulose nanofibers and CNTs (CNFs–CNTs) with nanosized topological features (diameter <1 mm) and adequate conductivity (0.38 S/cm). Neuronal cells can attach to and proliferate on the surface of the CNF–CNT composite [57]. Most importantly, the addition of CNTs significantly enhanced the differentiation capacity of neuronal cells [58].

Autologous nerve graft materials

Autologous nerve transplantation is a common clinical approach for the treatment of segmental nerve defects. However, harvesting nerve grafts through this method is surgically complex and can lead to increased pain and sensory loss. An alternative to autologous nerve grafting is scaffold-free nerve grafting, which involves the fabrication of functional 3D tissues without using biomaterials (Fig. 6). This technology can employ 3D bioprinters for the fabrication of intricate tissues that were previously unattainable using conventional techniques, allowing cells to produce tissue structures without any external support (Table 2). Scaffold-free tissue engineering involves the development of tissue constructs guided by nondestructive imaging techniques, such as micro computed tomography (micro-CT) or magnetic resonance imaging (MRI) scanning [59]. Mitsuzawa et al. demonstrated that bioscaffold-free conduits obtained by combining 3D bioprinting with fibroblasts effectively promoted sciatic nerve regeneration in rats. Additionally, these bioscaffold-free conduits stimulated the production of numerous myelinated axons in large models (dogs). Furthermore, the regenerated nerve fibers played a crucial role in reinnervating hypothenar muscles [60]. This technology has also been valuable for promoting the repair of 10-mm nerve injuries [61].

In a manner analogous to human dermal fibroblasts, human stem cells can also serve as building blocks for the construction of neural scaffolds. dos Santos et al. discovered that decellularized scaffolds, crafted from the stem cells (shed) of human deciduous teeth, can significantly enhance the reconstruction of impaired nerve function [62]. Likewise, using a segmental defect model, Zhang et al. showed that this neural scaffold effectively facilitated the reconstruction of facial nerve function in rats [63].

Electrospinning technology can be utilized to fabricate biomaterial-free neural scaffolds, which can bypass immunogenicity and other associated complications. The nerve conduit (xDNME) engineered by Kong et al., derived solely from porcine decellularization without additives, showed remarkable biocompatibility and transcended interspecies barriers. This conduit regulated the compromised neural microenvironment and facilitated synaptic regeneration. The xDNME conduit demonstrated excellent electrical signal transmission capabilities and significantly promoted axon regeneration and myelination. Most importantly, its ability to reconstruct damaged nerves was comparable to that of autologous transplantation, and it avoided immunogenicity and other complications [64].

Neural scaffolds for the repair of different nervous systems

Nerve damage can significantly impact the structure and function of motor, sensory, and mixed nerves in the human body. Motor nerves primarily comprising efferent neurons with myelin sheaths and motor end plates transmit signals for muscle contraction. Myelin is crucial for rapid signal transmission and varies depending on the sensory receptor type. Sensory nerve endings are equipped with receptors that perceive various stimuli. Signals from incoming neurons stimulate the formation of distinct myelin sheaths. Mixed nerves containing both sensory and motor fibers also feature myelin, facilitating bidirectional communication between the CNS and peripheral tissues. Myelin is vital in nerve function reconstruction, enhancing signal transmission and overall neural function restoration.

Peripheral nerves

Peripheral nerve injury, a prevalent form of neurotraumatic injury, is increasing. Autologous nerve transplantation is the current clinical approach for the treatment of peripheral nerves, but it may lead to donor site complications and

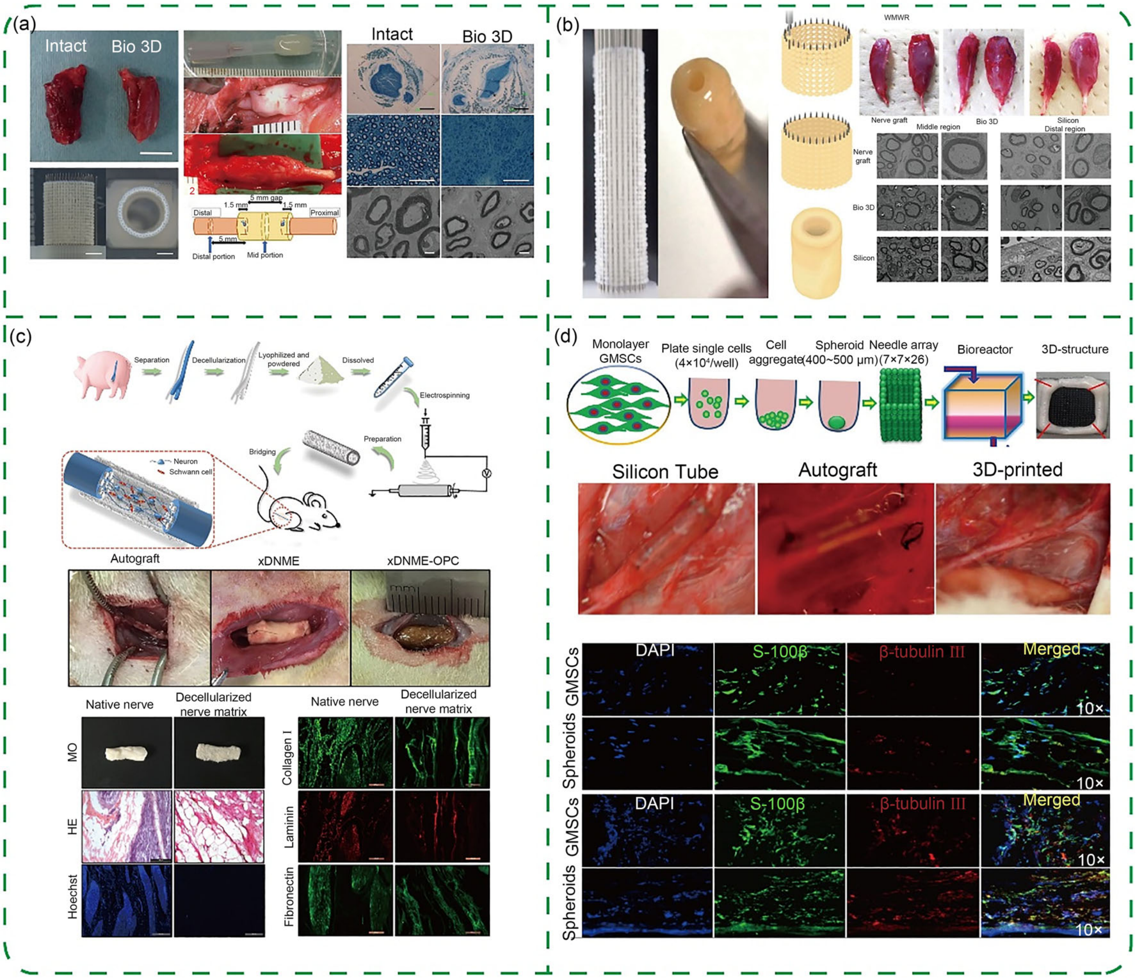


Fig. 6 Design and development of autologous nerve grafts: **a** biological scaffold-free catheters comprising autologous dermal fibroblasts (reproduced from Ref. [60], Copyright 2019, with permission from the authors, licensed under CC BY-NC); **b** fibroblast-based 3D nerve conduits (reproduced from Ref. [61], Copyright 2019, with permission from Wiley Periodicals, Inc.); **c** xDNME-based 3D nerve conduits (reproduced from Ref. [64], Copyright 2022, with permission from Elsevier B.V.); **d** GMSC-based 3D neural scaffold (reproduced from Ref. [63], Copyright 2018, with permission from the authors, licensed under CC BY 4.0). GMSC: gingiva-derived mesenchymal stem cell; xDNME: pure porcine decellularized nerve matrix

limitations in transplant sites [65]. Theoretically, providing an excellent nerve regeneration microenvironment is critical to promote axonal regeneration in peripheral nerve damage (Fig. 7) [66]. Li et al. demonstrated that multifunctional 3D-printed scaffolds combined with SCs promoted directional axon growth along the scaffold orientation, accelerating myelin formation and significantly increasing myelinated nerve density in mouse models [67]. Another innovative approach proposed by Lacko et al. involves magnetic template technology that arranges soluble magnetic alginate microparticles (MAMs) into a tubular structure within a hydrogel, forming a neural scaffold [68]. Therefore, designing neural scaffolds for peripheral nerve repair must focus on maximizing their ability to promote blood vessel and scar tissue formation while regulating the inflammatory response at the nerve site [69].

As an alternative to autologous nerve transplantation, NGCs must mimic native nerve tissues in terms of structure, mechanical properties, electrical conductivity, and protection. The 3D printing technologies discussed in Sect. "3D printing" can produce multifunctional NGCs [70]. Ye et al. used DLP technology to create GelMA multichannel NGCs, supporting the growth and migration of PC-12 cells while inducing neuron formation [71]. In another study, Chen et al. developed a robust bioactive dECM–PDA-coated PCL conduit that mimicked the structure of mussels. This conduit exhibited mechanical resilience, making it more resistant to tearing during surgery and handling. PDA improved hydrophobicity and enhanced the dECM coating.

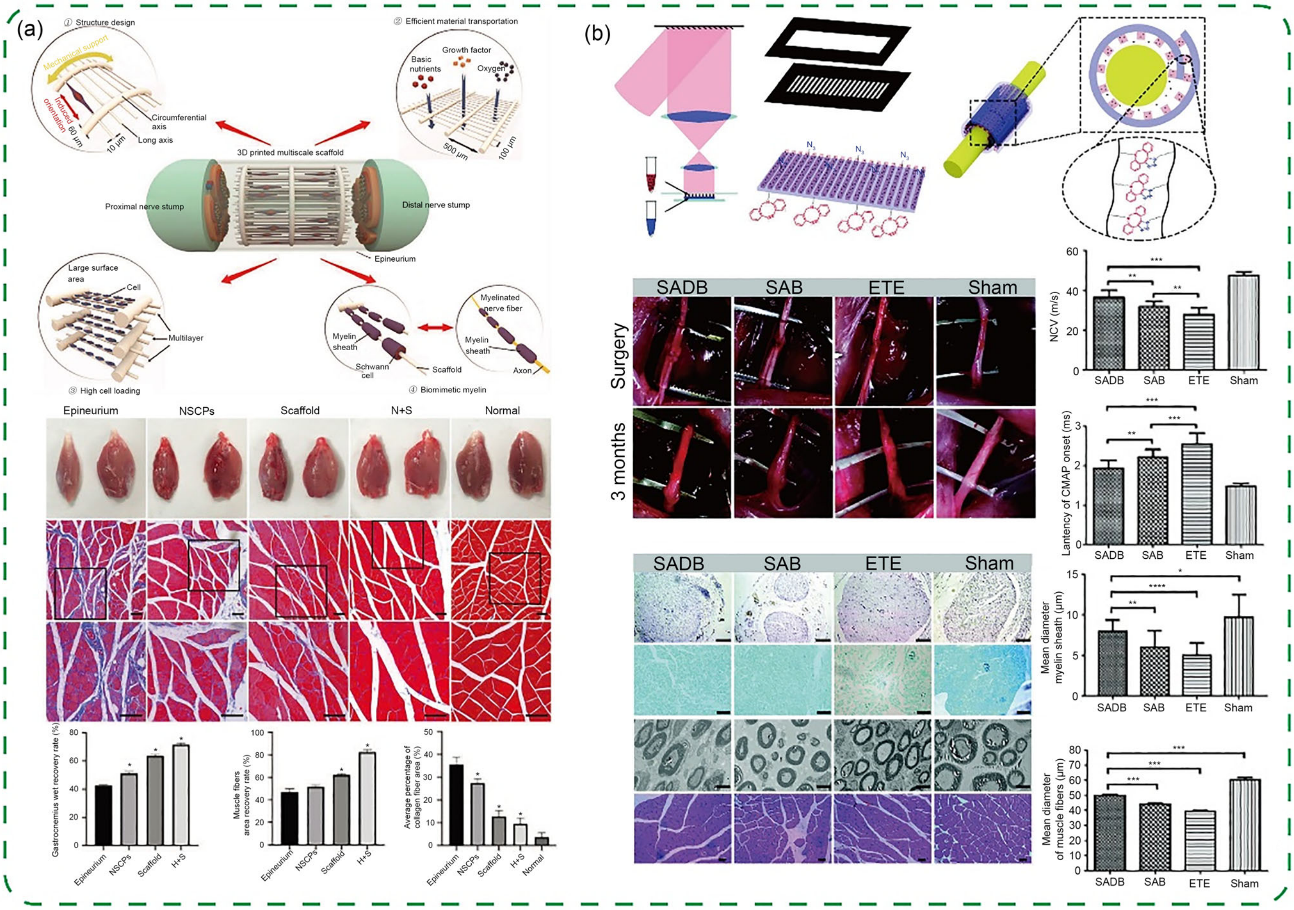


Fig. 7 3D-printed neural scaffolds promoting peripheral nerve regeneration: **a** combination of Schwann cells and multiscale scaffolds promoting nerve regeneration (reproduced from Ref. [67], Copyright 2021, with permission from Wiley–VCH GmbH); **b** self-adhesive bandages repairing peripheral nerve injuries (reproduced from Ref. [80], Copyright 2020, with permission from the authors, licensed under the terms of the Creative Commons Attribution License)

Most importantly, the conduit upregulates neuronal markers (Nestin, MAP2, TUJ-1, and ERK) [72].

Compared to other methods for manufacturing porous neural scaffolds, EHD jet technology provides the flexibility to control scaffold fiber size and spatial structure based on specific clinical requirements by adjusting process parameters. Vijayavenkataraman et al. utilized this approach to fabricate PCL-based NGCs with varying pore sizes and pore structures. Among them, NGCs with a pore diameter of (125 ± 15) μm exhibited excellent porosity (over 60%) and mechanical properties resembling those of natural peripheral nerves. Their degradation rate also matches the pace of nerve regeneration [73]. To further enhance PCL-based NGCs, the authors incorporated different concentrations of polyacrylic acid (PAA) to construct PCL–PAA NGCs. These scaffolds displayed an electrical conductivity of 1×10^{-6} S/cm and an ultimate tensile strength ranging from 6.5 to 11.7 MPa, comparable to the properties of natural human nerves. Compared to pure PCL scaffolds, the PCL–PAA NGCs provided better support for cell growth and neural differentiation [74]. Additionally, the same method was used to construct a PCL–rGO scaffold, which utilized the high surface area of rGO to promote the growth of PC-12 cells. The rGO incorporation increased the softness of the scaffold, making it more favorable for neural differentiation [75]. Furthermore, these functional neural scaffolds can upregulate the expression of growth-related protein 43, tubulin, and neurofilament protein.

Using the high surface-to-volume ratio and electrical conductivity of rGO, Uz et al. combined it with gelatin to create nerve conduits and scaffolds with specific microstructures. rGO not only provides 3D microstructures for MSC attachment and growth but also promotes MSC differentiation and paracrine signaling into SCs in response to electrical stimulation [76]. Park et al. synthesized r(GO–GelMA) by polymerizing GO and GelMA to fabricate conductive hydrogel-based NGCs ((8.7 ± 1.6) mS/cm). The hydrogel exhibited excellent flexibility, mechanical stability ((57 ± 13) kPa), durability (up to 500 compression cycles), and permeability. Moreover, the nonbiotoxic r(GO–GelMA) NGC significantly enhanced the repair of 10-mm peripheral nerve injuries by promoting nerve cell growth, aiding myelination and facilitating tissue functional reconstruction [77]. Furthermore, Qian et al. conducted a long-term (18 months) study on the biosafety of GO-loaded PCL-layered scaffolds in a rat model of peripheral nerve defects. GO exhibited excellent long-term biological safety in vivo and facilitated the repair of large neural defects via multiple regulatory mechanisms involving SCs and astrocytes [78].

In addition to incorporating conductive GO, nerve cell factors or drugs can be added to stimulate neuronal regeneration. Lee et al. designed PLGA core–shell nanoparticles loaded with neurogenic factor (NGF) and embedded them in a porous PEG-DA scaffold [79]. The nanocomposite scaffold not only improved PC-12 neuronal cell adhesion but also extended the average length of neurites. Further, Zhang et al. developed a self-adhesive drug-loaded bandages (SADB) for wrapping damaged nerves and promoting their regeneration. This structure relied on a click reaction to create a self-adhesive effect, firmly binding parallel hydrogel layers. SADB released drugs in a targeted manner and improved the growth of SCs [80]. In addition, nanoparticles made of poly(ethylene glycol) and poly(3-caprolactone) (MPEG-PCL) were employed to repair nerve defects and reconstruct nerve function through the Hippo pathway [81].

Peripheral nerve repair processes must address myelin reconstitution alongside the stimulation of neuronal regeneration. Inadequate differentiation of SCs and macrophages at the injury site can significantly hinder myelination regeneration, resulting in poor neurological recovery. To recruit and promote the polarization of macrophages, Wang et al. developed a Cs-AAD ternary polymer hydrogel loaded with tacrolimus (FK506) to mitigate immune rejection post-transplantation. The anti-inflammatory properties of FK506 attract macrophages, thereby increasing the CD206-to-TNF-α ratio, modulating interleukin 10 (*IL-10*) mRNA expression levels, and creating a neuroprotective environment [82]. However, nerve conduits comprising nanofibers arranged randomly around anchored microfiber bundles have been constructed. The oriented microfibrils recruited and polarized macrophages, facilitated SC migration, and promoted axon elongation. Implanting the conduit stimulated myelin structure formation, enhanced electrophysiological signals, and promoted the functional reconstruction of the sciatic nerve. In addition, it helped alleviate muscle atrophy and promoted the reconstruction of sciatic nerve function [83]. To improve the local environment, melatonin can be employed to regulate autophagy at damaged nerve sites. Melatonin aids in breaking down damaged nerve fragments by reducing the expression levels of Atg3–5–7, Beclin1, and LC3A–B, providing energy for the growth of new nerves [84]. However, the efficacy and feasibility of these neural conduits in clinical trials remain unclear.

CNS

The CNS plays a crucial role in coordinating and connecting various organs in the human body, and its damage can result in functional impairments. Prolonged inflammation at CNS injury sites can lead to local bleeding and edema, causing long-term disability. Further, the accumulation of neutrophils and macrophages can release soluble factors, including cytokines, proteolytic enzymes, and oxidative metabolites, worsening the inflammatory microenvironment. Addressing the acidic postinjury environment, Xi et al. developed pH-responsive electrospun fibers to modulate the immune response. This scaffold significantly altered immune cell subtypes, reduced acute inflammatory responses, and expedited scar tissue formation, promoting blood vessel and nerve regeneration for functional recovery [85]. Similarly, Yuan et al. designed an immunomodulatory dynamic structural hydrogel tailored to cell adaptability. Incorporating adipose stem cells within the hydrogel formed a conducive environment for neural differentiation and axonal growth, facilitating the repair of spinal cord injuries by activating the PI3K–Akt signaling pathway (Fig. 8). Furthermore, the hydrogel simultaneously released damage-associated molecular patterns (DAMPs) and IL-10 to mitigate inflammation in macrophages and microglia [86]. This dual-release capability supported axonal growth without leaving scars, effectively enhancing hindlimbs motor function in spinal cord injury (SCI) mice [87].

CNS regeneration is a complex and challenging process with limited treatment options [88]. Liu et al. developed an alginate–gelatin scaffold loaded with stem and glial cells to promote myelination formation [89]. To mimic the complex CNS structure, Koffler et al. designed a PEG-DA–GelMA hydrogel scaffold with dimensions matching those of the spinal cord. PEG-DA–GelMA promoted astrocyte responses aligned with the developing neural axis, facilitating axon extension and connection to the injured spinal cord, ultimately restoring signal transmission [90].

To enhance the conductivity of CNS-regenerated hydrogels, Gao et al. introduced PEDOT:LS (sulfonated lignin) into GelMA–HAMA hydrogels comprising GelMA and hyaluronic acid methacrylate (HAMA). The hydrogels effectively promoted axon regeneration and myelination, successfully repairing a completely transected rat spinal cord and restoring hindlimb movement. Excessive deposition of glial scars was also mitigated, which enhanced regenerative outcomes [91]. Similar to the hydrogel matrix, functional bioinks can promote axonal regeneration and

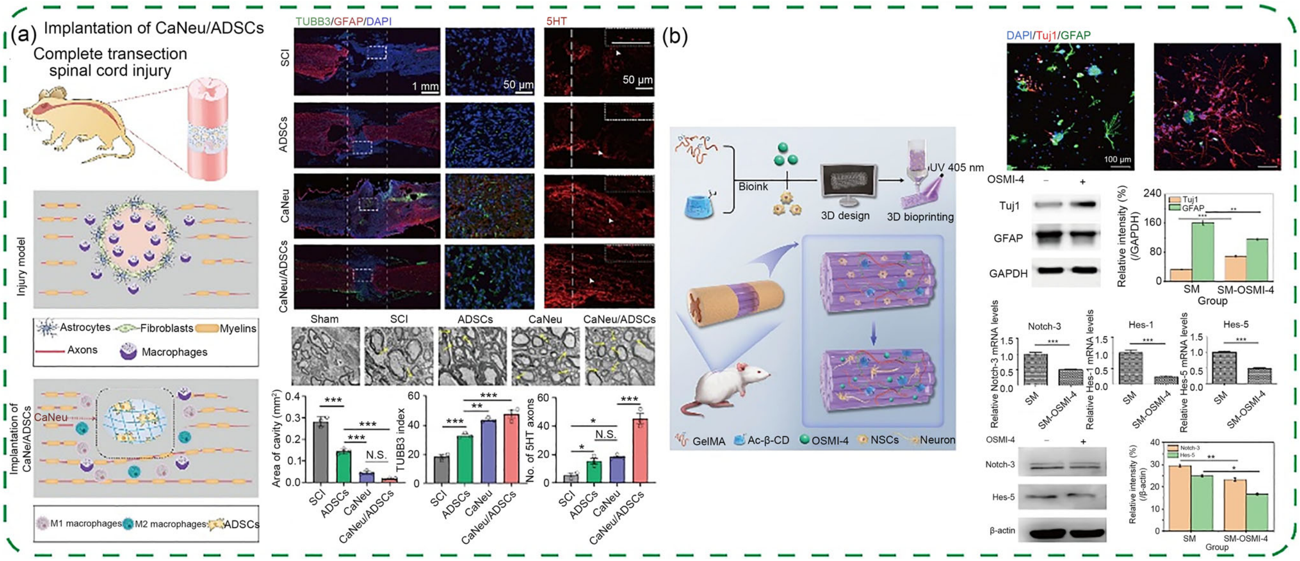


Fig. 8 3D-printed neural scaffolds promoting CNS regeneration: **a** cell-adaptive neurogenic (CaNeu) hydrogel transport of ADSCs promoting neuronal regeneration (reproduced from Ref. [86], Copyright 2021, with permission from the authors, licensed under CC BY-NC-ND); **b** methacrylate gelatin–acrylate β-cyclodextrin supramolecular bioink that transports NSCs and OSMI-4 (reproduced from Ref. [92], Copyright 2022, with permission from Acta Materialia Inc.). CNS: central nervous system; ADSC: adipose-derived stem cells; NSCs: neural stem cells; OSMI-4: O-GlcNAc transferase inhibitor

reduce glial scar deposition. Liu et al. developed a bioink comprising GelMA and acrylated β-cyclodextrin, which was loaded with a small-molecule inhibitor. The controlled release of OSMI-4 within this bioink inhibited the Notch signaling pathway, significantly enhancing the neuronal differentiation of NSCs [92]. Moreover, novel biocompatible bioinks such as hydroxypropyl chitosan (HBC)/hyaluronic acid (HA)/Matrigel (MA) hydrogels comprising chitosan, hyaluronic acid derivatives, and matrix gels, facilitated cell–material interactions and formed conducive microenvironments for neurons. The HBC/HA/MA hydrogels promoted neuron regeneration and axon outgrowth and improved motor recovery in SCI model rats [93]. In addition to drug delivery, the integration of pluripotent stem cells with growth factors can assist 3D-printed scaffolds with accelerating axon regeneration [94]. Liu et al. prepared a 3D-CC-BDNF (3D collagen/chitosan scaffolds integrated with brain-derived neurotrophic factor) neural scaffold, where the inclusion of BDNF or spinal cord neuronal progenitor cells (SNPCs) reduced the formation of cavities and scars at the lesion site, improving motor function [95].

Applications of 3D neural scaffolds in different tissues

Connective tissues

Nerve repair during connective tissue regeneration requires the creation of new biomaterials that promote osteoblast and neuronal growth and differentiation (Fig. 9) [96]. Fitzpatrick et al. constructed a silk-HAP bone cement enriched with bioactive agents that regulate osteoinduction, angiogenesis, and innervation, thereby enhancing its versatility. This innovative material stimulated the proliferation and differentiation of HUCMSCs, EMSCs, and NSCs, making it suitable for dental, oral, and maxillofacial surgeries [97]. When designing neural scaffolds, not only biomaterial components but also the impact of the spatial structure on neural differentiation must be considered. Hsiao et al. explored the effects of 3DP-PLAS neural scaffolds with different gap widths on stem cell differentiation. Their findings revealed that the scaffolds with a 150-μm gap were more likely to induce cell orientation, promoting the expression of morphology-related proteins (Nestin and MAP2) [98].

To realize synchronization nerve regeneration during the healing of connective tissue defects, Zhang et al. constructed an rGO composite hydrogel scaffold (rGO–GelMA) loaded with SCs and BMSCs. In a rat model, the rGO (0.05%)–GelMA hydrogel implantation site exhibited a low inflammatory response, with the surface filled with nascent collagen fibers. Most importantly, the regeneration site exhibited significantly increased expression levels of nascent proteins, including osteogenic and neuronal proteins (Fig. 9) [99]. In another study, Ma et al. synthesized SCSs by promoting the silicification of the collagen matrix with choline chloride. In a rat defect model, the SCSs promoted new bone formation in the distal femur while stimulating innervation

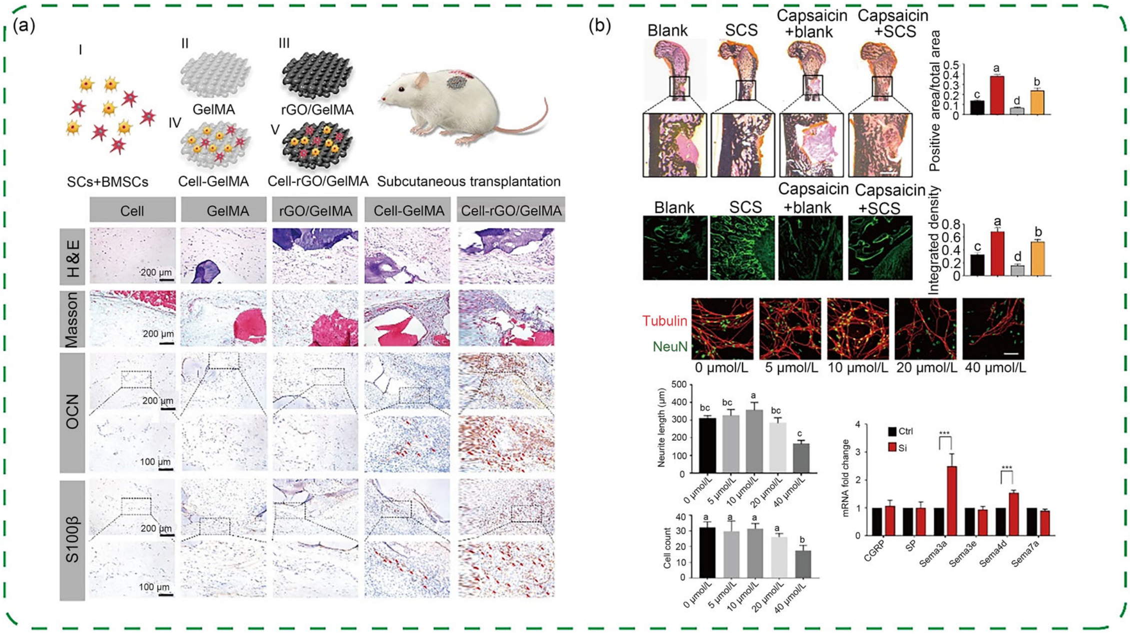


Fig. 9 3D-printed neural scaffolds stimulating nerve regeneration to accelerate bone repair: **a** rGO–GelMA hydrogel enhancing osteogenesis and neural differentiation (reproduced from Ref. [99], Copyright 2023, with permission from The Royal Society of Chemistry); **b** siliconized collagen scaffold stimulating osteogenesis, nerve and angiogenesis (reproduced from Ref. [100], Copyright 2021, with permission from the authors, licensed under CC BY-NC-ND). rGO: reduced graphene oxide; GelMA: gelatin methacrylate

and angiogenesis at the wound site. This innervation and angiogenesis can be attributed to the activation of mTOR pathway-related signaling protein expression [100]. In addition to common bioactive ingredients, MSC-CM can promote nerve regeneration. Raoofi et al. incorporated MSC-CM into PCL scaffolds, achieving reduced recruitment of neuronal and satellite cells via the controlled release of neurotrophic factors. It further significantly enhanced the expression of repair-related genes (*BDNF*, *S100*, and *NGF*) and accelerated the sciatic nerve repair process [101].

Neurological disease modeling

The primary feature of most neurodegenerative diseases (NDs) is the progressive accumulation of abnormal proteins in specific brain regions, resulting in cellular dysfunction and subsequent brain damage. The lack of appropriate disease models has hindered the research progress in the treatment of CNS diseases. Animal models cannot accurately represent the process of human neurodegeneration, and commonly used cell culture systems cannot mimic complex neuronal systems in vitro [102, 103]. To expedite research on NDs, model systems more suitable for studying the pathogenesis of NDs must be developed . For instance, 3D brain organoids constructed from pluripotent stem cells can mimic complex tissue environments [104]. Adine et al. used M3DB to fabricate biofunctional tissue organoids innervated with distinct SG-like cell compartments. In radiation injury and healthy SG models, the epithelium and neurons can adhere to and rapidly grow on the organoid surface [105]. In addition, somatic cell-reprogrammed iPSC technology can be used to construct 3D organoids, which provide an environment with a biomimetic brain structure [106]. Consequently, these organoids can serve as screening and molecular mechanism models for studying novel neurotherapeutics.

Similar to organoid models, 3D-printed nerve regeneration scaffolds can be used to evaluate the potential applications of innovative pharmaceutical formulations. Consequently, a 3D GF model was developed to study dopaminergic (DA) neurons in vitro, and the cellular and molecular mechanisms of NDs were investigated. In this model, the average length of regenerating neurites was significantly extended by effectively promoting the expression of tubulin, TH, and NeuN [107]. Additionally, Bordoni et al. prepared a conductive reversible embedded hydrogel disease model using CNFs, alginate, and single-walled carbon nanotubes (SWCNTs) [108].

While these studies provide valuable disease models, achieving a mature model of the disease remains challenging.

Degenerative diseases

Since the long-term treatment of chronic diseases requires continuous medication, 3D printing implant platforms with controlled drug release functionality have been extensively investigated [109, 110]. Dong et al. developed multifunctional soft helical microtweezers composed of GelMA-based hydrogels loaded with magnetoelectric nanoparticles (MENPs). The microtweezers achieved neuronal cell delivery and automatically degraded after MENP release was completed. Within a localized area, the hydrogel acted as an electrical stimulator of neuron-like cells, promising for neuronal cell stimulation in a less invasive and gentler manner [111]. In another study, Liu et al. constructed a canalicular scaffold loaded with bilayer cells (PC-12 and BMSC), which could secrete bile acids to induce the regeneration of cholangiocytes, thus promoting nerve and bile duct regeneration [112].

In addition, a single 3D-printed neural scaffold can be combined with other stimuli to treat neurodegenerative diseases, such as LLLT or photoacoustic stimulation. LLLT positively affects the rehabilitation of neurological disorders. In particular, the red laser stimulates the growth of NSCs attached to the surface of neural scaffolds, significantly enhancing the populations of neuronal cells and inhibiting the expression of glial cell markers [113]. Furthermore, photoacoustic agents can accelerate the repair of neurological diseases. Zheng et al. combined PEG-modified CNTs (a high-efficiency photoacoustic agent) with SF to form a soft photoacoustic functional scaffold (CNT–silk scaffold). The scaffold enabled the nongenetic activation of neurons and promoted neuronal regeneration. In a rat dorsal root ganglion model, the CNT–silk scaffold enhanced the expression of neurotrophic factors by on-demand photoacoustic stimulation, resulting in a 1.74-fold increase in axonal growth [114].

Nerve regeneration stimulation strategies

Morphological simulation

The design of neural scaffolds must adhere to the following principles: (1) presence of large (100 μm) pores or channels, (2) internal structure that can guide the direction of adherent cell differentiation, and (3) a complete interconnection channel between cell migration and tissue integration (Table 3) [115]. Huang et al. constructed a 3D scaffold with longitudinal gradients and microchannels to synergistically enhance nerve directional regeneration and extension. Their scaffold effectively promoted nerve regeneration distal to the defect, enabling the growth of new axons enveloped in myelin sheaths and facilitating the repair of sciatic nerve defects larger than 10 mm (15 mm). Consequently, it accelerated the recovery of sensory and motor functions in the defect model [116]. The research team implemented a staged strategy to construct a directional cryo-O-CCH–PCL scaffold comprising collagen–chitosan and electrospun PCL, which promoted axon regeneration and SC migration [117]. Moreover, the scaffold surface topography similar to that of neural tissues

Table 3 Design strategies for neural scaffolds

Design strategy	Characteristics	Reference
With continuous biochemical gradients and longitudinal physical cues	Achieving synergistic promotion of nerve regeneration and directional elongation in vitro	[116]
Stagewise strategy (directionally freezing orientated collagen–chitosan (O-CCH) filler, electrospinning poly(ε-caprolactone) (PCL) sheaths)	Protecting regenerating axons from compressive stress while providing sufficient space for nerve regeneration	[117]
Oriented multilayer printing pattern	Mimicking the geometry and microstructure of neural tissues, directing axonal regeneration, and supporting the growth of induced pluripotent stem cell-derived neurons	[118]
3D printing guided by scanning and 3D modeling of human nerve sample tissue	Constructing a nerve repair scaffold consistent with different types of neural structures in the nerve tract	[121]
Microchannels with tunable cross section and porosity	Guiding neuronal processes at injury sites to accelerate neural repair; constructing complex scaffold geometries from the images of patient neural structures	[122]
Thin-walled microtubule arrays, parallel microtubule configurations with high void spaces and membranous walls	Mediating cell orientation and axon pathfinding	[123]
Microchannels	Directing guided sciatic nerve regeneration	[124]
Secondary gelation step (granular hydrogel)	Supporting the development of neuronal and astrocyte colonies	[129]

was constructed using directional multilayer printed patterns, directing axon regeneration and supporting neuronal derivation [118].

Myelin plays a crucial role in neuron protection and signaling in functional nervous system reconstruction after nerve injury. The integrity of myelin depends on the presence of oligodendrocytes and myelin sheaths, which are influenced by the stiffness, size, and coating of the artificial scaffold [119]. Oligodendrocytes can sense artificial axons at the implanted site, and designing highly compliant and thicker artificial axons can guide oligodendrocyte differentiation and myelin formation. Moreover, coating implanted axons can direct oligodendrocyte interactions to achieve specific differentiation. Developing directional gradient structural scaffolds with high mechanical properties enables better simulation of complex natural tissue structures and environments, aiding cell migration and signal transmission [120]. Further, MRI technology can be employed to obtain numerous nerve microstructures within a nerve bundle, facilitating the construction of 3D-printed scaffolds that match the human nerve defect site [121]. Furthermore, nerve repair scaffolds with adjustable cross sections and porosities can be customized based on the images of damaged nerve structures [122]. Li and Gao designed a directional thin-walled microtubule structure that mimics the structure of peripheral nerve sheaths. SCs could be oriented along these channels, and the regenerated neurites extended in the direction of the central axis of the microtubules [123]. Similarly, Zhu et al. used fast sequential 3D printing with DLP to fabricate NGCs featuring microchannels and cannulas to guide the regeneration of the mouse sciatic nerve [124].

Stem cell differentiation simulation

Various stem cells, including NSCs and EMSCs, have the potential to differentiate into neurons and neuroplastic cells, offering a promising avenue for cell replacement therapy in the presence of damaged nerve cells. These stem cells can be combined with natural or synthetic matrix materials to construct monolayers or 3D scaffolds, allowing researchers to study neural development, simulate nervous system diseases, and uncover the underlying disease mechanisms [125]. In addition, human dental pulp stem cells (hDPSCs), which exhibit a high degree of vascularity and neural differentiation, can contribute to repairing damaged peripheral nerves. Differentiated hDPSCs guide dorsal root ganglion neurites and promote myelination. Notably, hDPSCs exert paracrine effects, facilitating the conversion of nerve and ECs into neurons and SCs, respectively [126].

A combination of mesenchymal stem cells (MSCs) has been shown to be effective in treating degenerative eye diseases, such as optic inflammation, glaucoma, and diabetic retinopathy. However, MSCs face challenges related to targeting and low cell viability during tissue repair. MSCs are often combined with bio/nanomaterials to address these issues and enhance cell function and differentiation [127]. When paired with exogenous neurotrophic drugs, stem cells can significantly induce neuron regeneration, leading to the sustained repair of nerve defects and the reconstruction of long nerve gaps [128]. As a material for 3D cell culture, hydrogels have a dense polymer network structure that hinders nutrient exchange and cell–cell interactions. To overcome this limitation, Hsu et al. developed hyaluronic acid hydrogel particles that promote the derivation of cortical neurons and significantly extend nascent neurites [129]. Moreover, Sultan et al. used SF hydrogels to encapsulate brain-derived neurotrophic factors secreted by an abundant population of human mesenchymal stem cells (hMSCs) to treat brain injuries. This approach significantly reduces neuronal death in the hippocampus and promotes neurological rehabilitation [130].

Design strategies for 3D-printed neural scaffolds

As mentioned in the previous section, 3D printing-based neural scaffolds show immense potential in regenerating various tissues. Nevertheless, the use of neural scaffolds has primarily been limited to the treatment of bone and degenerative diseases. When designing 3D-printed neural scaffolds, suitable biomaterials must be selected, and locally controlled release cycles must be established based on the specific clinical requirements of patients. In this section, we introduce the critical factors for 3D neural scaffold design to guide researchers in the interdisciplinary fields of clinical medical nerve regeneration.

Material choice

To realize the diverse clinical applications of nerve regeneration scaffolds, biocompatible materials suitable for 3D-printed neural structures must be carefully selected and developed. These materials must exhibit biocompatibility and robust mechanical strength and support cell growth while promoting differentiation and seamless integration with host tissues [131]. Further, conducting comprehensive research on various biodegradable and bioactive materials is essential to identify suitable biomaterials tailored to specific neurodegenerative applications.

Precise 3D printing technology

The enhancement of materials must be complemented by precise 3D printing technology, as technological advancement plays a crucial role in crafting intricate neural structures with exceptional resolution and precision. This review primarily introduces two fabrication methods for nerve regeneration scaffolds: electrospinning and 3D printing. Customization tailored to the unique requirements of individual patients is paramount for neuroregeneration applications [27]. Future research must concentrate on developing patient-specific 3D-printed neural scaffolds and structures that align with individual anatomical and functional specifications. The structure, pore size, and interconnectedness of the scaffolds must be thoughtfully considered to promote cell infiltration, nutrient exchange, and axonal growth. Moreover, innovative design strategies that mimic the native neural tissue and provide optimal support for neural regeneration must be developed.

Cellular integration

NSCs are pivotal in the process of nerve regeneration, and the optimization of their interactions with regenerative scaffolds significantly impacts the success of nerve regeneration [34]. Integrating suitable cells into neural scaffolds can greatly enhance their regenerative potential. We propose the selection of NSCs, SCs, and other pertinent cell types for integration into scaffolds to facilitate cell differentiation, tissue integration, and functional recovery. Most importantly, cell seeding and distribution within scaffolds must be optimized using innovative methods.

Combination of growth factors and neurotrophic agents

The incorporation of growth factors and nutrients into 3D-printed neural scaffolds is a viable approach to enhance the growth of nerve cells and promote regeneration [79, 80]. Combined with stem cell differentiation, it can rapidly stimulate cell proliferation and migration and promote axonal outgrowth. Moreover, controlled release mechanisms must be investigated for bioactive factors within scaffolds to identify the most effective combination and delivery method.

Multifunctional stimuli (light, sound, and electrical stimulation)

Developing strategies to facilitate the establishment of neural networks within 3D-printed scaffolds is critical to restoring functional connectivity. Therefore, functional materials must be incorporated into 3D-printed neural scaffolds to further enhance their regenerative potential. Functionalization encompasses light, sound, and electrical stimulation capabilities, and the integration of materials endowed with these functions into scaffolds can stimulate axon growth and enhance neural connections [52, 85].

In vivo testing and safety

Thorough and rigorous in vivo testing is essential to assess the clinical translation potential of 3D-printed nerve regeneration methods. This process involves assessing the biocompatibility, integration, and regenerative outcomes of the scaffolds using suitable animal models. Furthermore, comprehensive long-term studies that evaluate functional recovery, tissue remodeling, and potential adverse effects, including human trials, must be conducted.

Collaborative and interdisciplinary studies

Developing 3D-printed neural regenerative applications requires collaboration across various disciplines, including materials science, bioengineering, neuroscience, and clinical medicine. Such collaborations can leverage expertise in different fields, facilitate knowledge sharing, and accelerate the progress in neuroregeneration research. For instance, artificial intelligence can be used to design and optimize neural structures for 3D printing, enabling the development of patient-specific solutions and predictive models of treatment outcomes. Furthermore, it must be ensured that 3D-printed neural scaffolds comply with necessary guidelines and ethical principles.

Conclusions and perspectives

Although 3D printing can accurately create complex bionic structures, current methods may not be well-suited for mass production. Moreover, the reproducibility of 3D-printed neural scaffolds varies based on subtle printing conditions and biointerconnects. Hence, faster and more precise printing techniques must be developed to enhance the quality of printing structures and ensure the repeatability and scalability of 3D-printed neural scaffolds. Furthermore, standardized 3D printing protocols and quality control measures must be developed. The nerve regeneration process involves the coordinated actions of multiple cell types. To better simulate the natural in vivo microenvironment, various cell types can be incorporated into 3D-printed structures, such as SCs, NSCs, and ECs. It is paramount to ensure compatibility between different cell types and their synergistic promotion of nerve regeneration.

Future developments in 3D-printed scaffolds for nerve regeneration are expected to prioritize the improvement of their performance, biocompatibility, and clinical feasibility. Materials employed in 3D printing must conform to the surrounding tissue, offering controlled biodegradability to avoid long-term foreign body reactions. Although neural scaffolds can encapsulate cells and promote their survival and proliferation, they often suffer from low mechanical strength or inability to withstand the complex nervous system environment. Therefore, the precise selection of biomaterials tailored for nerve regeneration with enhanced biocompatibility and mechanical properties would be a game-changing improvement, which can pave the way for the promotion of axon regeneration. On the other hand, an in-depth exploration of the interactions between nerve cells and scaffolds is required. Although neural scaffolds can provide physical and chemical cues for nerve regeneration, the exact mechanisms that promote axonal growth remain unclear. Finally, the long-term stability and biodegradability of 3D-printed neural scaffolds must be carefully considered. While neural scaffolds are biodegradable, controlling the degradation rate is essential to ensure structural integrity over extended periods after scaffold implantation in vivo. Moreover, neural scaffolds must not induce toxicity or inflammatory reactions after degradation. Therefore, scaffold design must be optimized preclinically and clinically, evaluating their long-term safety and effectiveness in humans.

Overall, further development directions for 3D-printed neural scaffolds involve integrating new technologies and approaches to create more efficient and clinically feasible options for nerve repair. Researchers and doctors aspire to revolutionize the field of nerve regeneration, aiming to reduce or even eliminate patients' disease symptoms through ongoing research and treatment.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

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