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REVIEW

Functional Bioink and 3D Bioprinting Tissue Scaffold Applications for Spinal Cord Injury

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Abstract

Spinal cord injury (SCI), commonly resulting from sudden trauma such as traffic or sports accidents, leads to severe disruption of axonal connections and loss of sensory and motor function below the injury site. Despite numerous therapeutic efforts, effective strategies for neural repair remain limited. Tissue engineering has emerged as a promising approach for axonal regeneration, particularly through the design of three-dimensional (3D) polymeric scaffolds that can restore the structural and functional integrity of the injured spinal cord. This review focuses on recent advances in biomaterials and scaffold designs developed for SCI repair, emphasizing the role of nanocomposite systems that combine graphene oxide (GO), synthetic polymers such as PLGA–PEG, and bioactive ceramics like hydroxyapatite (HA). These hybrid materials offer improved biocompatibility, mechanical matching with spinal tissue, and enhanced cellular adhesion and guidance cues for axonal growth. The synergistic integration of these components enables the fabrication of multifunctional scaffolds capable of supporting stem cell differentiation and neurotrophic factor delivery. By critically summarizing the key parameters influencing scaffold performance, such as microarchitecture, surface modification, and mechanical compliance, this work outlines a framework for developing next-generation 3D nanocomposite scaffolds for SCI regeneration. The proposed approach highlights how GO/PLGA–PEG/HA systems can bridge the gap between experimental tissue engineering and clinically translatable neuroregenerative therapies.

Keywords Spinal cord injury · Axonal regeneration · Scaffold · Tissue engineering · Graphene oxide · Synthetic polymers · Hydroxyapatite

Introduction

Spinal cord injury (SCI) is a disease of the central nervous system (CNS that currently lacks successful treatment. According to the National Spinal Cord Injury Association Resource Centre report [1], approximately 450,000 people are living with SCI in the United States. There are approximately 8000 new SCIs each year; the majority (82%) involve males between the ages of 16-30 years. These injuries are caused by motor vehicle accidents (44%), violence (24%), falls (22%), sports (8%), or other situations (2%); hence, there remains a significant need for effective therapies. Therefore, there is a significant need to find a cure for injuries to the nervous system. Current tissue engineering research is focused on creating an environment to support axonal regeneration at the site of injury. The development of a biological scaffold that helps prevent neural tissue degeneration and wound formation is an ongoing research area. Graphene, a two-dimensional, sp^2-hybridised sheet of carbon atoms, has recently gained attention in neurosurgery and neural tissue engineering [2]. Due to the unique properties of graphene, such as zero-gap semiconductor features [3], high thermal conductivity, high surface area-to-volume ratio, chemical modifiability allowing functionalization of biotherapeutic molecules [4], and biocompatibility, enormous biomedical potential has been found for bioscale applications in SCI [5]. Two-dimensional graphene films have been used for the study of neuronal growth in vitro. However, these have not been well translated into *in vivo* applications. Therefore, recent research has investigated a novel three-dimensional (3D) material based on graphene that could be

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used as an *in vivo* scaffold. Recent studies have demonstrated the production of self-assembled 3D hydrogels by reducing GO under various conditions. Since these reduced graphene hydrogels exhibit excellent mechanical strength and high electrical conductivity, they are promising candidates for neural regeneration and SCI repair [6].

SCI is a serious clinical problem that leads to permanent neurological deficits and offers only limited functional recovery with current surgical, pharmacological, and rehabilitation approaches. Therefore, interest in regenerative medicine and biofabrication techniques has increased in recent years, with 3D bioprinting technology [7], in particular, emerging as a promising method for producing personalized scaffolds that mimic the complex structure of spinal cord tissue. Functional bioinks are one of the most critical components in this field, and various materials such as alginate, gelatin, hyaluronic acid, collagen, polyethylene glycol derivatives, and nanocomposite hydrogels are being investigated for their potential in terms of biocompatibility, mechanical durability, and cellular support. However, spinal cord tissue-specific bioink formulations are still limited, and key challenges such as vascularization, long-term cell viability, and functional integration remain. In this context, this study aims to summarize the current advances in functional bioink and 3D bioprinted-based tissue scaffolds for SCI and discuss the current shortcomings in the field.

Structure of the Nervous System

The nervous system can be divided into two main parts: the central nervous system (CNS; brain and spinal cord) and the peripheral nervous system (PNS; somatic and autonomic divisions) [8]. The repair processes of these two systems are different. The spinal cord is a soft, gelatinous structure extending from the brainstem to the lumbar region. It is approximately 38–43 cm long and roughly thumb-width in diameter. The spinal cord sits in the hollow canal that runs within the vertebral canal formed by the 33 vertebrae of the spinal column. Spinal nerves exit segmentally along the cord and innervate specific body regions. The spinal cord and brain form the central nervous system. The spinal cord contains afferent (sensory) and efferent (motor) pathways. Afferent axons carry sensory information to the brain, whereas efferent axons convey voluntary and autonomic motor outputs to the periphery [6]. The spinal nerves are supported by various glial cells. For example, oligodendrocytes insulate axons via myelin sheaths and enable rapid transmission of action potentials, whereas astrocytes provide metabolic support and release growth factors [9]. SCI is most often due to acute trauma (e.g., motor vehicle accidents or sports injuries). Vertebrae may dislocate or fracture and penetrate the spinal cord, leading to loss of sensory and motor function below the level of the lesion. Therefore, there is a significant need to find a cure for injuries to the nervous system [10].

Central and Peripheral Nerve Regeneration

Problems with regeneration in the central nervous system are the result of various processes that take place after injury. These processes are designed to restore the blood-brain barrier and prevent further (secondary) tissue damage, which creates an unfavorable environment for nerve regeneration. Nerve damage increases the activity of astrocytes (glial cells), and thus creates a glial scar. Astrocytes migrate, proliferate, grow, and produce a glial scar rich in extracellular matrix (ECM) proteins (especially CSPGs) and myelin-associated inhibitors released by oligodendrocytes. Activated astrocytes secrete proteoglycans, including chondroitin sulphate proteoglycans (CSPG), known to be potent inhibitors of axonal growth. Glial scars can prevent axon regrowth through the formation of chemical and physical barriers. Effective regeneration requires a permissive substrate, guidance cues, and space for axonal extension. The goals of all regeneration strategies in the CNS are generally to alleviate reactive gliosis while promoting axon growth and tissue regeneration [11]. Peripheral nerve damage is typically caused by traumatic injury. It can be a result of complications of orthopedic surgery. The process of peripheral nerve regeneration works in several phases. In the first phase, multiple pathophysiological events occur, defined as Wallerian degeneration. This process is linked to myelin sheaths and the breakdown of nerve fibers due to cells called macrophages. Apart from their phagocytic properties, these cells play an important role in the process of nerve fiber reconstruction called regeneration [12]. They produce various cytokines that stimulate Schwann cell (SC) proliferation and also the production of neurotrophic substances such as NGF (Nerve Growth Factor). These substances reach the cell body by retrograde axonal transport, and their products stimulate the synthesis of genes responsible for axonal regeneration. Schwann cells form Büngner's tubes or characteristic bands where axons grow [13].

The axon growth rate is estimated to be approximately 0.5–1 mm/day [14]. It is said that a few millimetres can be overcome within a few days; however, reutilisation of target tissues may take months. It is important to note that mature neurons do not undergo mitosis. Therefore, it is the goal of a nerve guidance conduit (NGC) to support the regrowth of axons from existing cells to distal targets [15]. Often, the defects of peripheral nerves are more than 2 cm in length, so the implant must be designed to provide directional axon growth from proximal to distal nerve stumps for synaptic connections. Allografts and autografts, such as cutaneous nerves or blood vessels, are often used as nerve conduits. Among materials of non-biological origin, natural

and synthetic polymers are the most common for producing peripheral nerve prostheses. These materials include polymers such as silicone, collagen, alginate, laminin, chitosan, hyaluronic acid, polylactide (PLA), polyglycolide (PGA), PLA copolymers with PGA and polycaprolactone (PCL), and others [16]. Despite advances, surgical repair and implant performance still fall short of clinical demands in many cases. Specifically, for autografts from other parts of the body, it is difficult to obtain sufficient donor nerves, and this lack of available tissue can lead to functional impairment [17]. Other biomaterials, including various polymers used in the fabrication of nerve conduits, may be limited by the incompatibility of mechanical and electrical properties, as well as reduced biocompatibility [18].

In contrast, in recent years, nanomaterials have become promising candidates for various tissue engineering applications. Nanomaterials are materials that realize structural configuration and morphology in the nanometric range (i.e., at least one dimension less than 100 nm). Due to their wide range of properties, nanomaterials can be designed to interact with cells and proteins with greater specificity [19].

Neural Scaffolds and Biomaterials for Nerve Regeneration

Nerve Scaffolds

The physical scaffold of tissue-engineered nerve grafts, referred to as the neural scaffold for short, serves to:

- Guide axonal growth from the proximal to the distal nerve stump
- Providing adequate mechanical support for regenerating nerve fibers
- A channel for the diffusion of neurotropic and neurotrophic factors secreted by the damaged nerve cavity and a channel wall for the exchange of nutrients and waste products
- Prevent infiltration of fibrous scar tissue that inhibits axonal regeneration
- Create an optimal microenvironment (niche) for nerve regeneration by accumulating and releasing endogenous/exogenous biochemical cues [20].

For clinical use, it is hoped that the neural scaffold can be easily fabricated, sterilized, and simply implanted into the body by microsurgical techniques. So far, various natural or synthetic biomaterials, combined with well-defined fabrication techniques, have been used to prepare neural scaffolds with different structures, in which the tubular NGC represents the fundamental design [21].

Requirements for an Ideal Scaffold

An ideal neural scaffold for SCI repair must satisfy multiple biological and physicochemical requirements, primarily biocompatibility, biodegradability, permeability, biomechanical strength, and appropriate surface properties [22]. SCI presents unique challenges such as glial scar formation, axonal loss, poor vascularization, and inhibitory microenvironments, which scaffolds must address to support functional regeneration [23, 24]. These characteristics are governed by the scaffold material, fabrication method (including 3D bioprinting), and both micro- and macro-architecture, which together enable proper axonal guidance, glial alignment, vascular integration, and functional connectivity in the injured spinal cord [25, 26].

Biocompatibility Biocompatibility refers to the ability of a scaffold to interact with neural tissue without eliciting an adverse reaction while supporting cell adhesion, proliferation, and differentiation. For spinal cord injury applications, biocompatibility is critical for preventing additional damage to the injured tissue and promoting axonal growth [27]. Biocompatibility of neural scaffolds can be evaluated in three aspects: blood compatibility, ensuring that contact with blood does not trigger hemolysis, coagulation, or thrombosis; histocompatibility, ensuring that scaffolds do not induce toxic or inflammatory responses (e.g., necrosis or genotoxicity) in surrounding tissues; and mechanical compatibility, meaning that scaffold elasticity and stiffness should match spinal cord tissue to minimize mechanical mismatch [28]. For example, hydrogels such as collagen and hyaluronic acid exhibit excellent histocompatibility, whereas conductive polymers and nanocomposites are being explored to combine mechanical and electrical compatibility, both of which are essential for functional neural regeneration [29].

Biodegradability Biodegradability is a key requirement for scaffolds in spinal cord injury repair, since the implanted material must degrade in a controlled manner without provoking toxic by-products or inflammatory responses. Early studies in neural tissue engineering employed non-degradable materials such as silicone and polyethylene. Although they achieved partial success in peripheral nerve repair, they required secondary removal because of long-term complications such as toxicity and limited tissue remodeling [30]. For SCI applications, degradable and absorbable biomaterials such as collagen, chitosan, hyaluronic acid, and synthetic polymers like PLA and PCL are now preferred [31]. These materials allow scaffolds to provide temporary mechanical support and cellular guidance while gradually resorbing within the host tissue. Importantly, the degradation kinetics should be tuned to match the rate of axonal regeneration; overly rapid degradation may fail to protect

and guide regenerating axons, while slow degradation can cause compression, chronic inflammation, or foreign body reactions. Ideally, the scaffold should remain structurally stable throughout the critical phases of axonal regrowth and then degrade with minimal swelling, thereby ensuring both structural support and safe integration with host tissue [11].

Permeability A soft tissue-engineered neural scaffold should have sufficient permeability for nutrient and gas exchange [32]. It should prevent the build-up of pressure due to fluid retention and maintain fluid exchange between the regenerating spinal cord and surrounding tissues. Permeability also influences the formation of the fibrin matrix and axonal sprouting during the early stages of spinal cord regeneration [33]. Therefore, optimized scaffold permeability helps inhibit fibroblasts and glial scar formation while enabling mass transport, nutrient diffusion, and capillary ingrowth. Manufacturing techniques such as fiber spinning, addition of pore-forming agents, and 3D bioprinting parameters affect permeability by altering the porous structure of neural scaffolds. Furthermore, the hydrophilic nature of scaffold biomaterials is another critical factor that influences the permeability of neural scaffolds, ensuring efficient nutrient delivery and waste clearance in regenerating spinal cord tissue [34].

Biomechanical Properties Adequate mechanical strength should be considered when designing neural scaffolds for spinal cord injury repair. A tubular spinal cord scaffold should have a Young's modulus comparable to that of spinal cord tissue to resist physiological loads during axonal regeneration and tissue remodeling. To fulfill this requirement, the biomechanical parameters of central nervous system tissues have been studied, although data from peripheral nerves are often used as a reference due to limited direct measurements in spinal cord tissue.

To allow bending without kinking, neural scaffolds must maintain sufficient flexibility. Achieving the optimal balance between flexibility and stiffness is critical in SCI applications: overly rigid scaffolds may cause additional tissue damage or impede integration, whereas overly flexible ones may fail to support regenerating axons. The biomechanical properties of spinal cord scaffolds are influenced not only by the material composition but also by scaffold design factors such as wall thickness, lumen diameter, and filling with bioactive hydrogels to promote axonal guidance and structural stability [35].

Surface Properties Surface properties play a dominant role in governing interactions between a neural scaffold and spinal cord neurons and glial cells, particularly through surface functionalization. The longitudinally oriented surface texture of spinal cord scaffolds has been shown to direct axonal growth, regulate glial cell behavior *in vitro*, and enhance spinal cord regeneration *in vivo* [36].

Nanofiber scaffolds have recently attracted increasing interest in spinal cord tissue engineering due to their unique surface chemistry, providing a high surface area for cell adhesion and growth, and topographical cues that guide axonal outgrowth and glial alignment in SCI applications [37].

Material Manufacturing and Porosity

Scaffold biomaterials for spinal cord placement are manufactured by dissolving the polymerized monomer into macromers via chemical, thermal, or photo-crosslinking reactions in an aqueous or organic solvent. Crosslinking efficiency can be enhanced by adding specific reagents or catalysts. Most spinal cord scaffolds are fabricated via injection molding or 3D bioprinting. Porogens are incorporated into polymer blends to generate pores within the scaffold structure. Commonly used porogens include sodium chloride and ice crystals, gas bubbles foamed with peroxides or air, and gelatin-based composites. In all cases, the polymer solidifies around the porogen, which is subsequently removed to yield the final porous scaffold. Pore size and interconnectivity can be tuned by adjusting crystal size, growth orientation, or hydrogel water content to match spinal cord tissue requirement [38, 39]

A continuous, highly interconnected porous structure mimicking the mechanical properties of native spinal cord tissue provides a favorable microenvironment for axonal regeneration. Scaffold porosity is crucial for cell adhesion, migration, and phenotype maintenance, and also facilitates vascularization of the otherwise avascular scaffold, thereby promoting functional recovery after spinal cord transection. Porosity further enhances scaffold integration and mechanical stability at the spinal cord–implant interface [40, 41].

Macroengineering

Nerve tracts in the human spinal cord have a diameter of 100-1000 µm. The role of scaffold macroengineering is to design channels that optimize axonal growth potential along the alignment of fascicular groups [39]. Reported channel sizes are up to 200 µm in agarose and 450 µm in PLGA scaffolds [42]. While numerous studies discuss scaffold design in theory, quantitative comparisons of specific architectural parameters are critical for advancing the field. A seminal study by Wong et al. [43] provided a direct, quantitative comparison of poly(ε-caprolactone) scaffolds with five distinct architectures, including solid, tubular, multi-channel, and open-pathway designs. Their findings were revealing open-pathway designs not only demonstrated superior regenerative capacity but, more importantly, revealed that closed

designs (e.g., solid, tube) negatively impacted the ambient tissue and doubled the size of the defect. This underscores that a poorly chosen architecture can be actively detrimental to repair. Further investigating the critical parameter of channel size in multi-channel scaffolds, another study constructed PLGA scaffolds with 450 µm and 660 µm aligned channels via injection molding [44]. When seeded with Schwann cells, these scaffolds promoted robust axonal outgrowth. However, a key quantitative observation was the formation of a fibrotic ring around regenerated neurons, a common impediment to functional regeneration. Crucially, follow-up analysis demonstrated a direct relationship between channel diameter and outcomes: a larger channel size (660 µm) significantly reduced both the area of this fibrous tissue and the number of regenerating axons. This trade-off highlights a central challenge in scaffold design: optimizing one parameter (e.g., channel size to reduce fibrotic encapsulation) can inversely affect another (e.g., axonal count), emphasizing the need for a balanced and application-specific approach to these quantitative parameters (Figure 1A) [41].

Microengineering

In the CNS, large, myelinated axons have a diameter of 15–20 µm. To reconstitute functional connections in spinal cord injury, the scaffold must regionally align groups of damaged neurons at the axonal and growth cone level. Scaffold microengineering aims to create micro-scale features

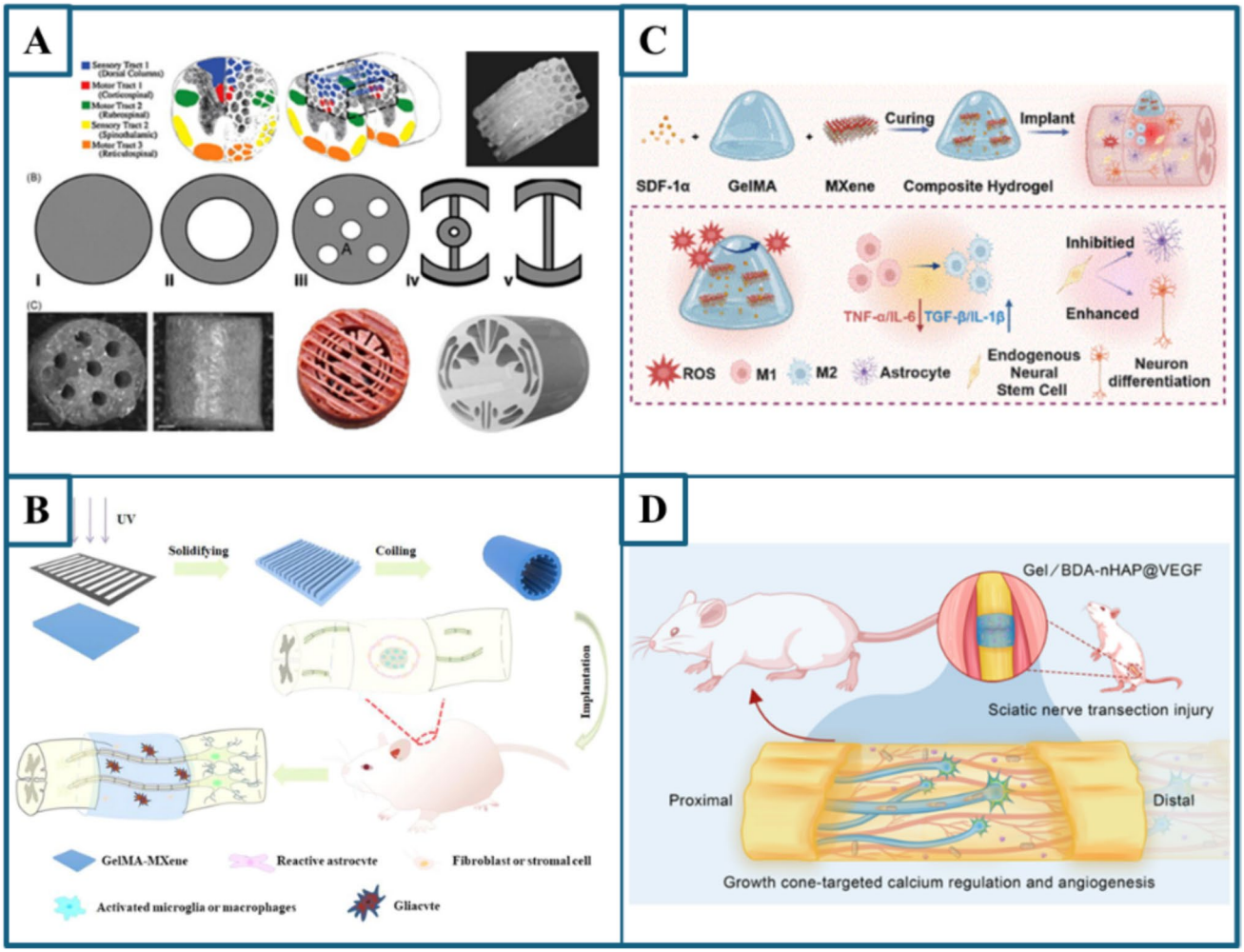


Fig. 1 **A** Macromarchitectural design. Various injection molding strategies for aligning fascicular bundles to the spinal cord scaffold. (a) Agarose casting templated on polystyrene fibers, a hemisection model, produces linearly aligned channels of 200 µm diameter. (b) Cylinder, pipe, multi-channel, and open-pathway designs, poly(ε-caprolactone) casting. (c) PLGA multi-channel scaffold, dorsal and ventral channels, cast on parallel metal wiring [45]. **B** Schematic summary, the fabrication and application of GelMA-MXene hydrogel nerve conduit with microgroove structure is shown [46]. **C** Schematic summary, the development of MXene-based conductive hydrogel targeting endogenous NSCs for SCI repair is demonstrated [47]. **D** Schematic summary, the BDA-modified hydroxyapatite nanorod and VEGF-loaded hydrogel system was shown to enhance functional recovery by promoting growth cone elongation and angiogenesis in peripheral nerve injury [48]

(a few microns in at least one dimension) that guide axonal adhesion and directional growth within the injured spinal cord. In the context of the spinal cord, microstructures enhance axonal alignment and provide precise guidance cues. It is conceptually useful to consider the reorganization of nerve fascicles into three-dimensional axon bundles, whereas individual axons tend to elongate along a two-dimensional plane. Micro-grooves influencing axon interaction and alignment can be fabricated on polymer surfaces via laser etching [49].

Axons also preferentially grow along the length of micro- and nanofibers of biomaterials such as collagen and synthetic polymers (PLGA, PCL). Polymer fibers are produced by electrospinning to achieve either random orientation of larger caliber fibers or parallel alignment of nanometric fibers (50 nm–30 µm). This technique uses electric charge to draw and elongate liquid polymer fibers from a syringe pump, allowing the creation of scaffold channels that mimic native axonal densities and promote directional axonal regeneration in SCI [50].

Neural Scaffolding Structures

A neural scaffold structure and scaffold material are critical factors determining the effectiveness of neural scaffolds in promoting peripheral nerve regeneration. The neural scaffold can be easily fabricated into a simple structure, namely an NGC with a single hollow lumen. However, there has also been a focus on the development of more complex scaffold structures. The NGC lumen can exhibit complex internal architecture, into which physical fillers are introduced into the NGC lumen or a multi-channel NGC is created to replace a single hollow lumen NGC. These structural modifications have been achieved using various well-defined fabrication techniques. Most studies have investigated the application potential of these more complex scaffold structures compared to the basic skeleton structure [51].

Single-Cavity Lumen NGCs

Single-cavity lumen NGCs are mostly used for the repair of small sensory nerve injuries with a nerve gap of less than 30 mm, such as digital nerve lesions [52].

Multi-channel NGCs

The basic structure of neural scaffolds has been modified by adding one or more intraluminal channels to the NGC lumen to form a multi-channel NGC. This approach is based on mimicking the architecture of neural fascicles. Multi-channel NGCs can be fabricated by using an injection molding technique, following the same procedures as for the preparation of single-lumen NGCs, but with different molds [53].

Moreover, compared to single-cavity lumen NGCs, multi-channel NGCs provide more surface area for cell attachment and local release of growth factors, and thus better support nerve regeneration in a larger nerve spacing by stabilizing the fibrin matrix. However, the extra internal structure can also affect some important properties of the neural conduit, such as permeability, bending and deformation properties, swelling, and degradation. In summary, it remains to be clarified whether the multi-channel structure is indeed superior to the single-lumen structure [54].

NGCs with Physical Luminal Fillers

To enhance peripheral nerve regeneration within the single-cavity lumen NGC, a series of biomaterial-based fillers with different physical forms (such as fibers, filaments, gels, or sponges) is incorporated into the lumen to enhance attachment, proliferation, and migration of Schwann cells and axonal development. Some studies have shown that fibers or filaments inserted into the NGC lumen improve internal contact guidance and increase the permeability of the neural conduit, suggesting that this modified scaffold structure is superior to the basic scaffold structure in terms of nerve repair performance. In addition, the insertion of fibers or filaments into the NGC lumen also increases the cross-sectional area of regenerated nerve tissues, enhances the formation of myelinated axons, and improves sensory functional recovery [55]. With the development of nanotechnology, fibrous scaffolds at the submicron and nanoscale levels are becoming potential candidates for use as tissue-engineered neural grafts.

Neural Scaffold Production Methods

Cells and tissues have a three-dimensional (3D) organization in the body. Therefore, scaffolds for SCI repair must be prepared with appropriate methodologies to enable cell distribution, axonal guidance, and tissue integration in a 3D space [56]. Various techniques such as freeze-drying, electrospinning, phase separation, 3D printing, particle leaching, and fiber bonding have been employed for scaffold fabrication. Among these, electrospinning and particle filtration have been particularly explored in neural tissue engineering and SCI models due to their ability to produce porous and aligned structures that support neural regeneration [57]. Below, the main fabrication techniques of neural scaffolds are summarized [58].

ElectroSpinning Method

Electrospinning uses an intense electric field between two oppositely charged electrodes to fabricate polymeric fibers ranging from nanoscale to microscale. One electrode is

immersed in the polymer solution, and the other is placed in the collector. The electric field stretches the polymer solution into fibers, whose thickness depends on the distance between the droplet point and the collector [59]. Importantly, this technique allows the fabrication of aligned nanofibers that mimic the longitudinal orientation of spinal cord axons. Such structural guidance has been shown to promote axonal regeneration and improve functional recovery in SCI models, making electrospinning one of the most promising approaches in neural tissue engineering [60].

Particle Filtration Method

One of the most common methods used to prepare scaffolds for tissue engineering studies is the particle leaching method. Porogens such as salt, wax, or sugar are used to create pores or channels within the scaffold. To achieve the desired size, salt particles are ground and poured into a mold, followed by the polymer solution. After solvent evaporation, the salt is leached out with water to obtain a porous scaffold structure. The pore size can be controlled by the amount, size, and shape of the porogen. This technique has a porosity of approximately 94–95% and produces scaffolds with suitable crystallinity and a pore size of about 500 µm. Its main advantage is that a smaller amount of solution is required to produce the scaffolds, although pore shape and inter-pore connectivity cannot be precisely controlled [61]. This method has been applied to neural tissue engineering and SCI models to produce scaffolds with interconnected pores, which facilitate nutrient transport, axonal ingrowth, and vascularization, all critical for supporting neural regeneration. Although some factors, such as pore shape and inter-pore connectivity, are difficult to precisely control, ongoing advances aim to overcome these limitations and improve scaffold performance for SCI repair.

Properties of Ideal Materials for Neural Tissue Engineering Applications

It is very important that the material used as a scaffold has excellent cytocompatibility, biomechanical, and electrical properties. It should be biodegradable and exhibit good biocompatibility with extremely low levels of inflammatory, immunogenic, and cytotoxic responses. A material without good cytocompatibility may not contribute to the growth of damaged nerves and may cause severe inflammation or infection. Furthermore, high electrical conductivity is required for scaffolds used in neural tissue engineering to mimic the electrical properties of nerves and also to stimulate neuron interactions [62]. Some biomaterials that can be used as scaffolds to treat damaged nerves include PLGA, PLA, polyesters, fibronectin, collagen, and laminin. While autografts provide a suitable microenvironment for neurovascular regeneration, they also have some disadvantages. Most of the time, there is an insufficient amount of donor nerve tissue when autografts are used. On the other hand, the use of autografts triggers the loss of healthy nerves and may cause paresthesia or anesthesia in the donor area. Allografts can be used as needed, but they can cause an inflammatory reaction and lead to failure of the nerve graft. Other biomaterials, such as polymers, also have unfavorable mechanical properties. They can form glial scar tissue around the implant and reduce the chance of complete recovery. The scaffold should provide sufficient mechanical strength to prevent scaffold failure during patient movements and physically support neural tissue regeneration. At the same time, the scaffold should be elastic enough to reduce stress at the injured point. Moreover, an ideal scaffold should be porous because it allows the nerve fiber growth and keeps the cells alive until the completion of the treatment period [63].

Synthetic Polymers

The use of natural polymers in the spinal cord takes advantage of their inherent properties; their natural roles, to some extent, form the basis of their function or modification, whereas the use of synthetic polymers offers a wider scope for designing and controlling the properties of the material. The synthetic polymers used to date for spinal cord applications are either polyester-based biodegradable materials such as PLA and PGA, biodegradable hydrogels based on PEG, or non-biodegradable hydrogels based on methacrylate. The first spinal cord scaffolds, commonly used in clinical practice for peripheral nerve and skin transplant repair, were made of the same materials. Rapid advances in hydrogel chemistry have produced materials with mechanical properties that are better suited to the spinal cord. The properties of these highly hydrous, soft polymers suggest that their chemistry and structure can also be tuned, given their similarity to spinal cord tissue. Functionalized synthetic polymers include surface charge modification for cell adhesion and neurotrophic gradients. Table 1 shows a list of synthetic polymers and examples of their applications in the spinal cord [64].

Poly α-hydroxy Acid Polymers In previous research, much of the work in scaffold design has focused on the use of biodegradable synthetic polymers, particularly poly(α-hydroxy acids) such as PLA, PGA, and their copolymer PLGAs. These polymers contain polyester bonds formed between lactic and glycolic acid, which are hydrolyzed in vivo to release the lactic acid and glycolic acid, thereby

Table 1 Main synthetic polymers used in animal models of spinal cord injuries and their examples

Synthetic polymers	Structure	Examples of applications for spinal cord repair	References
Biodegradable			
PLA	Poly (D, L lactic acid)	Single and seeded with Schwann cells, multi-channel scaffolds	[65]
PGA	Poly (glycolic acid)	PLA: single-channel	[66]
PLGA	Poly (lactic-co-glycolic acid)	Freeze-dried macroporous foam scaffold containing BDNF Freeze-dried macroporous scaffold with BDNF-secreting Schwann cells PLGA: seeded with Schwann cells, multi-channel scaffolding Multi-channel scaffold seeded with nerve stem cells Protein release from chitosan scaffolds	[67]
PCL	Poly-ε-caprolactone	Nanofibre spinning for axonal growth guidance.	
PCLF	Poly-ε-caprolactone fumarate	Multi-channel piers	[68]
Hydrogels			
PEG	Polyethylene glycol	Intravenous solution Three-dimensional axon growth matrix Magnesium sulphate intravenous PEG	[69]
Non-biodegradable hydrogels			
pHEMA	Poly (2-hydroxyethyl methacrylate)	pHEMA sponges Fibre scaffolds Neurotrophic gradients	[70]
pHEMA-MMA	pHEMA-co-methyl methacrylate	PGLA microspheres and pHEMA-NGF NGF release from polymer bilayer	[71]
pHPMA	Poly [N-(2 hydroxypropyl methacrylamide)]	pHPMA colloid gel Colloid gel with CNTF and BDNF	[72]
PAN/PVC	Poly(acrylonitrile-covinylchloride)	Schwann cell-seeded scaffold with cord infusion of BDNF and NT-3	[73]

dissolving the material. The pH around the grafted area thus becomes acidic. These compounds are a good initial choice for spinal cord placement and have a long history of FDA-approved clinical use as absorbable suture material [74].

PLA

Upon demonstrating the resorbability and biocompatibility of PLA with Schwann cells and the spinal cord when PLA was used to make scaffolds, the University of Miami group implanted a single channel, Schwann cell-loaded scaffold into a transected rat spinal cord. The PLA materials used were structurally unstable, degradable, and collapsible, and were found to support axonal and vascular growth [75].

PLGA

PLGA, a synthetic copolymer composed of polylactic acid and polyglycolic acid, is biocompatible and biodegradable. The degradation rate of the copolymer can be controlled by changing the ratio of polylactic acid to polyglycolic acid. Neural tubes fabricated from PLGA were implanted into a completely transected rat spinal cord. Axonal regeneration was observed in the neural conduits. However, it was noted that the degradation of PLGA produces glycolic and lactic acids that lower the local pH and may inhibit the process of tissue repair [76].

Synthetic Hydrogels

Polyethylene Glycol (PEG) PEG is a water-soluble, non-toxic polyether compound known to be resistant to protein adsorption and cell adhesion. These properties make the PEG polymer highly resistant to recognition by the immune system after implantation. In addition, PEG helps to seal cell membranes after injury. Depending on the cross-links formed, PEG hydrogels can be engineered with various degradation rates and used as drug delivery vehicles. Moreover, they can be further modified to enhance cell adhesion. PEG has excellent qualities for spinal cord repair, including being highly hydrophilic, biocompatible, non-immunogenic, and neuroprotective. However, depending on the polyether backbone, it can degrade in the body [77].

As a carrier material, PEG features an active hydroxyl group at its ends, allowing it to be combined with various drug molecules to create effective drug delivery systems. The version of PEG with two hydroxyl groups is most used due to its ability to be modified with other functional groups, thereby expanding its potential applications. One such application is the creation of functional hydrogels through photopolymerization, where PEG interacts with other monomeric macromolecules or particles. This ability to modify PEG via photopolymerization makes it a highly

adaptable material for a diverse range of biomedical uses [78]. Additionally, PEG's biocompatibility and non-immunogenic properties make it particularly suitable for delicate biomedical procedures, including implantation, controlled drug release, and spinal cord regeneration. For example, to address the need for a supportive yet non-inflammatory scaffold in spinal cord repair, Mahoney et al. synthesized hydrolysable PEG-based hydrogels modified with PLA and PGA. The key advantage of this design for SCI applications is the material's high resistance to protein adsorption, a property stemming from its neutrality and high molecular mobility. This is critical because it prevents the non-specific attachment of cells and proteins that can lead to glial scar formation, a major barrier to neural regeneration following injury [79].

Graphene

Graphene is a two-dimensional monolayer of sp^2 hybridized carbon atoms with exceptional electrical, mechanical, and thermal properties. Agrawal et al. [80] identified graphene, the thinnest material recognized with the Nobel Prize in Physics. Following this discovery, graphene research has steadily increased across a wide range of applications, from electronics to tissue engineering. Carbon-based materials (graphene, nanotubes, nanofibers, etc.) have garnered significant interest in the biomedical field due to their unique properties, including biocompatibility, excellent mechanical strength, and electrical conductivity. Particularly in the field of neural tissue regeneration, such as SCI, these materials play a key role in functional bioink formulations and tissue scaffolds produced by 3D bioprinters. Their high electrical conductivity can be utilized to facilitate neural signal transmission in the lesion area, while their superior mechanical properties ensure that implanted structures can withstand the physical stresses of spinal cord tissue. Quantitative parameters such as conductivity (S/m), porosity (%), and Young's modulus (MPa) are critical for evaluating the effectiveness of designed structures on neural cell migration, axonal outgrowth, and tissue integration. Therefore, the integration of carbon-based nanomaterials is promising for the development of innovative and functional 3D bioprinting strategies for spinal cord repair.

Graphene and its derivatives have emerged as highly promising nanomaterials for neural tissue engineering due to their exceptional electrical conductivity, mechanical strength, and large surface area, which can be leveraged to create conductive bioinks that support neural cell function and tissue regeneration. While the cytocompatibility of graphene-based materials is a critical factor for their use in SCI scaffolds, the current discussion remains largely focused on general biomaterials research, with limited direct connection to quantitative parameters for neural repair. Kotchey et al. [81] demonstrated a degradation pathway for GO using hydrogen peroxide and horseradish peroxidase (HRP), suggesting a potential strategy to control the in vivo lifetime of graphene-based scaffolds. The biodegradation rate is a crucial quantitative parameter, as it must be tuned to match the timeline of neural tissue regeneration to provide temporary support without inducing long-term foreign body effects.

Similarly, studies on cytotoxicity need to be contextualized within the specific cell types relevant to SCI, such as neurons and glial cells. Sasidharan A. et al. found that carboxylated graphene's increased hydrophilicity correlated with minimal cytotoxicity [82].

The mechanism of toxicity is also a key consideration. Hu W et al. [83] discovered dose-dependent cytotoxicity in human somatic cells, attributing it to the direct physical damage from GO sheets. For a functional spinal cord scaffold, the relevant quantitative parameters would include the critical safe dosage threshold (e.g., μg/mL) for neural progenitor cells and the material's nano-topography, which must be designed to minimize physical damage while promoting axonal guidance.

In summary, while these studies provide a foundational understanding of graphene's biocompatibility, their direct relevance to functional spinal cord repair is limited. Future work must bridge this gap by explicitly linking material parameters (e.g., degradation rate, surface charge, sheet size) to quantitative functional outcomes in SCI models, such as the degree of axonal regeneration across the lesion and the recovery of electrophysiological function [84].

The foundational step in utilizing graphene lies in its successful integration into printable bioinks. The creation of bioengineered tissue substitutes requires advanced biomaterials and precise fabrication technologies that allow the production of cell-laden scaffolds capable of mimicking native tissue architecture. Gelatin methacryloyl (GelMA) hydrogels have emerged as one of the most promising candidates for 3D bioprinting due to their favorable biocompatibility, biodegradability, and tunable mechanical behavior. Lavín-López et al. formulated a novel hybrid hydrogel system by combining GelMA with a high-purity graphene derivative (BioGraph) to develop bioinks suitable for extrusion-based 3D printing. The incorporation of BioGraph at concentrations up to 0.1% w/v supported smooth extrusion, minimized clogging during printing, ensured efficient photo-crosslinking under the tested light intensities, and preserved high-resolution scaffold architectures with good shape fidelity. Photocrosslinking analysis revealed that increasing BioGraph levels slowed the curing process and reduced matrix stiffness. Cell culture experiments demonstrated that BioGraph concentrations $\leq 0.1\%$ w/v maintained the viability and proliferation capacity of rat adipose-derived mesenchymal stem cells (r-AMSCs). In vivo subcutaneous implantation of acellular scaffolds in rats triggered a local macrophage-driven

immune response accompanied by collagen encapsulation, without adverse effects on surrounding tissues. Interestingly, scaffolds containing low BioGraph content (0.025% w/v) enhanced macrophage interaction, promoted scaffold degradation, and further supported r-AMSC proliferation in vitro. Collectively, these findings indicate that GelMA–BioGraph hybrid hydrogels provide enhanced rheological and biological properties, making them highly suitable for extrusion-based bioprinting applications targeting both soft tissues (such as neural and hepatic systems) and harder structures (such as bone) [85].

Building on the development of basic conductive bioinks, research has advanced to incorporate topographical cues to guide neural growth directionally. In order to facilitate neuronal differentiation and tissue regeneration, Cai et al. designed a conductive hydrogel containing MXene with a micro-grooved structure to function as a nerve guide channel. MXenes, a new class of two-dimensional nanomaterials, are particularly attractive for SCI repair due to their superior electrical conductivity, hydrophilicity, and stability, as well as their proven biodegradability *in vivo*. When integrated into hydrogels, MXenes form composites that provide both a biocompatible matrix and efficient pathways for electrical signal transfer between neural cells. Nevertheless, many MXene-based hydrogels reported so far have shown limited ability to direct cell orientation. To overcome this, they fabricated a GelMA–MXene composite hydrogel film with aligned microgrooves, produced by UV crosslinking through a patterned mask. GelMA was selected for its excellent biocompatibility and well-established support of cell proliferation, while the addition of MXene enhanced its conductivity [86–88]. The resulting construct not only supported robust cell growth but also guided axonal alignment across injury gaps. *In vivo* experiments demonstrated that implantation of this structured conduit promoted spinal cord regeneration and significantly improved hind limb motor recovery in rats. (Figure 1B) These findings suggest that micro-grooved GelMA–MXene hydrogel conduits represent a promising strategy for clinical SCI repair [46].

The next frontier in graphene-based biomaterials is the development of multifunctional, "smart" systems that respond to external stimuli to provide on-demand therapy. SCI remains one of the most severe neurological conditions, and zinc ions (Zn^{2+}) have been identified as key players in the repair process. However, achieving a sustained and localized release of Zn^{2+} at the lesion site is still a major challenge. To address this, a novel near-infrared (NIR)-responsive MXene-based hydrogel decorated with ZnO quantum dots (QDs) has been developed. Characterization through TEM and EDS confirmed the uniform distribution of ZnO QDs on the MXene nanosheets. Enzymatic assays demonstrated that ZnO–Ti_3C_2 nanostructures exhibited catalase-like activity, efficiently scavenging reactive oxygen species (ROS). Further biological analysis revealed that ZnO–Ti_3C_2@H supported mitochondrial protection by reducing ROS with clearance efficiencies of up to 80%, thereby limiting neuronal apoptosis and facilitating neurovascular regeneration. Under NIR irradiation, the hydrogel locally increased the temperature to approximately 42.9 °C, which promoted accelerated Zn^{2+} release at the injury site. Behavioral assessments and histological studies demonstrated enhanced functional recovery, reflected by improved locomotor scores approaching normal levels. Overall, this multifunctional material, integrating ROS regulation and on-demand Zn^{2+} delivery, presents a promising therapeutic platform for SCI repair. [89].

Perhaps the most sophisticated application of conductive 2D nanomaterials is their use to recruit and modulate the body's own stem cells for in situ regeneration, bypassing the need for complex cell transplantation. Endogenous neural stem cells (NSCs) are increasingly recognized as promising candidates for SCI repair. Nevertheless, their recruitment to lesion sites and subsequent differentiation into functional neurons are strongly limited by the hostile post-injury microenvironment, which is typically marked by hypoxia, oxidative stress, inflammation, and acidic conditions. To address this challenge, researchers developed a conductive hydrogel incorporating stromal cell-derived factor-1α (SDF-1α) and MXene nanosheets. This multifunctional material exhibited several therapeutic effects: (i) controlled release of SDF-1α promoted the recruitment of endogenous NSCs toward the injury site; (ii) MXene nanosheets mitigated oxidative stress and modulated macrophage polarization toward a pro-regenerative M2 phenotype, thereby creating a more favorable environment for NSC survival and neuronal differentiation; and (iii) the hydrogel's electrical conductivity, conferred by MXene, further enhanced neuronal differentiation of NSCs. Transcriptomic analyses also indicated that MXene nanosheets supported SCI recovery through activation of PI3K–Akt and calcium signaling pathways. To the best of current knowledge, this is the first report of an MXene-based multifunctional hydrogel designed to activate endogenous NSCs for SCI therapy. By simultaneously improving cell recruitment, neurodifferentiation, and microenvironmental modulation, this approach highlights a novel and clinically relevant strategy for functional spinal cord regeneration (Figure 1C) [47].

Collectively, these studies underscore the transformative potential of graphene and related 2D nanomaterials in bioink design, evolving from passive conductive components to active, intelligent systems capable of guiding regeneration, modulating the hostile microenvironment, and harnessing the body's own innate healing capacity for functional recovery after spinal cord injury.

Biocompatibility and Multifunctionality: Degradation, Drug Delivery, and Barrier Applications

The successful translation of graphene-based scaffolds from bench to bedside hinges not only on their functional performance but also on addressing critical issues of long-term biocompatibility, controlled degradation, and advanced therapeutic delivery. A primary concern for any non-biodegradable nanomaterial, including graphene derivatives, is their potential long-term toxicity. The investigation by Kurapati et al. [90] on the enzymatic biodegradation of GO provides a critical foundation for its potential use in safe and effective spinal cord repair scaffolds. A significant hurdle in deploying nanomaterials like GO in neural applications is their long-term biocompatibility and predictable clearance from the implantation site. This study directly addresses this by demonstrating that recombinant eosinophil peroxidase (EPO) can effectively degrade three distinct GO variants with varying oxygen content over a 90-hour period. The quantitative data from Raman spectroscopy and transmission electron microscopy, which show clear structural breakdown, are not merely observational; they are essential parameters that define the degradation kinetics and biocompatibility profile. Therefore, the role of this research transcends basic material science. It offers a targeted strategy to potentially mitigate chronic inflammation or foreign body responses by ensuring that the GO-based bioink or scaffold does not persist indefinitely after fulfilling its initial supportive role. This controllable biodegradation mechanism is vital for designing next-generation 3D bioprinted constructs for SCI, where the scaffold must provide temporary structural and biochemical support for neural regeneration and then safely degrade to be replaced by native neural tissue.

Beyond functioning as conductive scaffolds, graphene-based materials can be engineered as sophisticated multifunctional platforms for targeted therapy. Afzal et al. [91] successfully incorporated GO nanosheets into zinc oxide (ZnO) nanoparticles to create a composite material with potential for neural drug delivery. X-ray diffraction (XRD) analysis confirmed the presence of a hexagonal structure in the doped material, with an observable increase in peak broadening. Under transmission and field emission electron microscopy, both undoped and ZnO-doped GO displayed distinct morphologies, such as nanoparticles, nanorods, and flakes. UV-visible spectroscopy revealed an increase in the absorption spectrum of the doped material. The study identified the formation of hydrogen bonds between the functional groups of GO and ZnO as a limiting factor for achieving maximum drug loading efficiency. It was found that the drug loading efficiency of GO-doped ZnO was approximately 89% higher than that of pure ZnO (82%), a critical parameter for ensuring a sufficient therapeutic dose of neuroprotective agents is delivered to the injury site. However, this trend reversed during the drug release phase, suggesting the composite could provide a more sustained release profile, which is vital for long-term treatment strategies in SCI repair.

Finally, graphene's versatility extends to creating protective barriers to prevent post-surgical complications. In a separate approach focused on creating protective barriers, Park et al. [92] produced a self-contained "paper-like" material formed by binding rGO and polyoxyethylene sorbitan laurate (Tween) via noncovalent interactions. The Tween-coated rGO paper was highly stable in water, biocompatible, non-cytotoxic to mammalian cell lines, and inhibited non-specific binding of bacteria. These properties are highly relevant for SCI applications, as such a material could serve as a biocompatible scaffold or an insulating membrane to prevent scar tissue formation and microbial infection post-implantation.

In summary, these studies expand the role of graphene from a primarily conductive element to a multifaceted material where biodegradation kinetics, drug release profiles, and barrier properties become integral, designable parameters for creating safe and effective next-generation neural scaffolds.

Hydroxyapatite (HA)

Hydroxyapatite (HA), which is naturally found in bone tissue and distinguished by its biocompatibility, has traditionally been used in bone regeneration; however, recent studies have revealed its potential in neural tissue engineering [93]. In particular, HA particles in the form of nanorods have attracted attention for their ability to support neural stem cell differentiation and axonal regeneration. Beyond its bone-like properties, hydroxyapatite also shows promise as an active therapeutic agent for nerve regeneration owing to its ion-exchange capacity. Controlled differentiation strategies allow stem cells to be directed toward specific neuronal lineages by modulating developmental cues, yet current methods remain insufficient for rapidly generating distinct neuronal subtypes required in disease modeling and therapy. Recent findings indicate that HA nanorods, owing to their unique chemical characteristics, can enhance the neuronal commitment of neural stem cells. Once internalized, lysosomal degradation of these nanorods elevates intracellular Ca^{2+} levels, which in turn promotes the accelerated formation of GABAergic neurons. Mechanistically, the transient calcium surge triggered by HA nanorods activates c-Jun, leading to repression of TLX3, a gene that normally regulates the balance between GABAergic and glutamatergic fate. This dual-level effect observed at both molecular signaling and functional neuronal levels demonstrates that HA nanorods can effectively bias stem cells toward GABAergic identity. Since GABAergic neurons are central to numerous physiological functions and implicated in several neurological disorders, these results highlight the potential of HA nanorods as a promising

tool for precise and efficient stem cell-based neuroregenerative therapies [94].

Beyond its intracellular effects described above, HA-mediated Ca^{2+} release has also been exploited in extracellular therapeutic constructs for nerve repair. NSC transplantation has emerged as a leading therapeutic option for neurodegenerative disorders, yet its clinical translation remains limited by the inherently slow pace of spontaneous differentiation. While biological growth factors can promote NSC maturation, their use is restricted by high production costs, instability *in vivo*, short half-life, and procedural complexity. Recent studies highlight HA, a key mineral component of bone, as a promising inorganic alternative. When presented in nanorod form, HA accelerates both the differentiation of NSCs into neurons and their functional maturation. In culture, NSCs exposed to HA nanorods rapidly developed into electrophysiologically active neurons within five days, significantly faster than the two weeks typically required under standard conditions. Transcriptomic analysis further indicated that the neuroactive ligand–receptor interaction pathway plays a central role in this process. These findings suggest that inorganic biomaterials such as HA can act as cost-effective, stable, and practical bioactive cues, offering a potential strategy to regulate NSC fate and improve neural repair outcomes in neurodegenerative disease therapies. Hao et al. demonstrated that the intracellular Ca^{2+} signal provided by HA nanorods through lysosomal degradation accelerates subtype-specific neurogenesis by modulating the molecular pathways (c-Jun/TLX3) that determine the fate of GABAergic neurons. Additionally, they showed that this controlled Ca^{2+} signaling provided by HA plays a critical role in the axonal regeneration process in mature neurons [95].

Ca^{2+} homeostasis plays a pivotal role in the repair process following peripheral nerve injury (PNI), particularly in facilitating growth cone extension during axonal regeneration. Despite its importance, there are only a few biomaterials capable of providing a stable and long-term source of Ca^{2+} at the site of injury. In a recent strategy by Li et al., hydroxyapatite nanorods (nHA) functionalized with amine groups and further modified with biotinylated dextran amine (BDA) were integrated into a hydrogel carrier together with vascular endothelial growth factor (VEGF). This multifunctional construct, referred to as BDA-nHAp/Gel, was designed to deliver sustained Ca^{2+} release while simultaneously supporting angiogenesis. The gradual release of Ca^{2+} ions from the modified nHAp elevated intracellular Ca^{2+} concentrations within growth cones, thereby enhancing neurite elongation through activation of PI3K-PAK and MAPK signaling cascades. In parallel, VEGF stimulated vascularization during the early stages of nerve repair, creating a favorable microenvironment for neurogenesis. Collectively, this dual-action therapeutic platform provides a promising approach for achieving effective structural and functional recovery after PNI. PNI repair requires sustained Ca^{2+} signaling to support continuous growth cone advancement, yet current therapeutic materials rarely address this need. The BDA-functionalized nHAp-based hydrogel offers a novel solution by ensuring localized, long-term Ca^{2+} availability in combination with VEGF-mediated angiogenesis. Through this synergistic design, the system fosters both axonal regrowth and vascular support, presenting a new paradigm for high-quality peripheral nerve regeneration. This innovative study demonstrates how HA can be integrated into hydrogel scaffolds as a functional component, transforming them into targeted and continuous sources of Ca^{2+}. When synergistically combined with VEGF, this system provides a highly effective strategy for peripheral nerve repair by supporting both neurogenesis and angiogenesis simultaneously. These positive effects of HA have also been observed with different formulations (Figure 1D) [48].

HA, a calcium-rich compound that can also be derived from natural sources such as fish bones (e.g., tuna heads), is well recognized for its role in bone regeneration. Chitosan, a biodegradable polysaccharide with excellent biocompatibility and osteoconductive properties, has also been investigated as a carrier for targeted therapeutic delivery in SCI. Ma et al. produced nanoparticles composed of chitosan and hydroxyapatite and demonstrated that they remain stable at low temperatures. Treatment with the chitosan–HA composite improved functional recovery in SCI rats, as reflected by significantly higher Basso Beattie Bresnahan (BBB) scores compared with the control and chitosan-only groups. Furthermore, administration of these composite nanoparticles reduced lesion cavity size and improved tissue organization at the injury site, suggesting enhanced neural repair. Overall, this work highlights the potential of chitosan–HA nanostructures in spinal cord repair and demonstrates an efficient biomedical application of fish-derived hydroxyapatite. The ability of chitosan–HA nanoparticles to promote functional recovery (BBB scores) and reduce lesion volume in an SCI model strongly supports the broad applicability of HA-based composite materials in neural regeneration. However, the potential toxic effects of HA nanoparticles on neural tissues should not be overlooked, underscoring the importance of comprehensive biocompatibility assessments [96].

HA nanoparticles (HANP) are widely employed in tissue engineering and biomedical applications; however, their potential neurotoxic effects remain poorly understood. Eldeeb et al. investigated the potential adverse neurological effects of HANPs and evaluated whether chitosan nanoparticles (CNPs) and curcumin nanoparticles (CUNPs) could provide protective benefits. Exposure to HANPs led to a marked decrease in several key neurotransmitters, including acetylcholine, dopamine, serotonin, epinephrine, and norepinephrine. Moreover, HANPs downregulated cortical genes associated with mitochondrial biogenesis, particularly

PGC-1α and mitochondrial transcription factor A (mTFA). The results also demonstrated pronounced neuroinflammatory responses, elevated apoptosis, lipid peroxidation, oxidative DNA damage, and increased nitric oxide levels, accompanied by reduced antioxidant enzyme activities and glutathione content. Interestingly, co-administration of CNPs and/or CUNPs significantly alleviated these detrimental effects, restoring neurotransmitter levels, mitochondrial function, and oxidative balance while reducing neuroinflammation and DNA damage. Combined supplementation with both CNPs and CUNPs proved more effective than either treatment alone, suggesting that these nanomaterials may serve as promising neuroprotective agents against HANP-induced toxicity. This study has shown that HANPs can cause neurotoxicity, neurotransmitter imbalance, oxidative stress, and neuroinflammation when used in high doses or without proper control. However, these toxic effects can be mitigated with agents such as chitosan and curcumin nanoparticles. These findings highlight the critical importance of dose optimization, surface modification, and combination with neuroprotective agents to ensure the safety of HA-based therapeutic systems [97].

In conclusion, hydroxyapatite plays a multifaceted role that extends beyond being a mere structural scaffold material in neural regeneration. Owing to its chemical composition, it can act as an effective inorganic growth factor, accelerating neural stem cell differentiation, guiding the formation of specific neuronal subtypes, and supporting axon regeneration through controlled Ca^{2+} release. These effects make it a valuable component for 3D bioprinted smart scaffolds in combination with hydrogels and other polymers (such as chitosan). However, careful optimization of nanoparticle size and application parameters, considering potential neurotoxic effects, is a prerequisite for its safe and effective use in clinical settings.

Support Cells

Using neural scaffolds alone to repair peripheral nerve damage has met with variable success, but in this way, small peripheral nerve gaps can be reconstructed. When the length of nerve gaps increase, neural scaffolds alone will not be sufficient unless supportive cells or growth factors are incorporated. Support cells implanted into the injured nerve can produce growth factors or ECM molecules to facilitate nerve regeneration. Schwann cells, neural stem cells, embryonic stem cells, and bone marrow stromal cells, among others, are the most studied support cells.

Schwann Cells

Schwann cells are the main glial cells in the peripheral nervous system and play an important role in the function and survival of neurons. In response to nerve injury, Schwann cells undergo rapid changes in phenotype, and their basal lamina provides a conduit for axons to regrow. Schwann cells also produce different growth factors such as nerve growth factor, brain-derived neurotrophic factors, insulin-like growth factor and erythropoietin in the sensory system and glial-derived growth factor in the motor system. In addition, Schwann cells have the capacity for phagocytosis, which is involved in the initial clearance of myelin debris. These properties make Schwann cells the first and most widely used supportive cells in peripheral nerve regeneration [98].

Sources of Schwann Cells *Allogeneic Schwann cells:*

In the 1980 s, allogeneic Schwann cells were selected as supportive cells for the repair of peripheral nerve injuries. Because allogeneic Schwann cells are rejected by the host, they must be given at the same time as immunosuppressive drugs. Without immunosuppression, foreign Schwann cells are rejected, but the morphology of nerve grafts can be restored by host-derived Schwann cells [99].

Syngeneic Schwann cells:

Due to immunologic rejection concerns associated with allogeneic Schwann cells, syngeneic Schwann cells present a more viable option for clinical application. Mosahebi et al. investigated this by comparing the effects of allogeneic and syngeneic Schwann cells on repairing a 10 mm mouse sciatic nerve gap. Their critical finding was that allogeneic Schwann cells were rejected, whereas syngeneic Schwann cells were detected 6 weeks after implantation. While both cell types supported a similar axonal regeneration distance, the quality of regeneration was superior in the syngeneic group, which exhibited a significantly greater number of axons. [100]. This study underscores that although allogeneic and syngeneic Schwann cells can both facilitate repair, the absence of immune rejection with syngeneic cells leads to a more robust and sustained regenerative outcome, which is a crucial quantitative measure for successful spinal cord repair.

Autologous Schwann cells:

Although immunosuppressive drugs can eliminate rejection of allogeneic cells by the host, their clinical use is still controversial. Since the 1990 s, autologous Schwann cells have been tested as supportive cells. For example, a neural scaffold seeded with autologous Schwann cells effectively reconstructed the rat median nerve gap and proved to achieve results on par with autologous nerve grafting. [101].

Implantation of Schwann Cells as Support Cells In addition to direct injection of Schwann cells into the neural scaffold placed between the proximal and distal nerve stumps, implantation of Schwann cells can also be accomplished by pre-adding Schwann cells onto the neural scaffold via

injection or co-culture. Recently, several new techniques for implanting Schwann cells have been developed. For example, Schwann cells were seeded into cell-polymer constructs that were implanted into the backside of nude mice for six weeks and then re-seeded for implantation into a sciatic nerve cavity in nude mice. This attempt provides a novel approach to create a construct containing a high density of living Schwann cells without supporting foreign material or tubular structure. So far, Schwann cells have been incorporated into neural scaffolds composed of various biomaterials of natural or synthetic origin. At the same time, neural scaffolds with different structures have also been attempted to incorporate Schwann cells. To investigate the effect of implanted Schwann cells on peripheral nerve regeneration, implanted Schwann cells need to be monitored for cell survival, migration, differentiation, and myelin production. Marker molecules such as beta-galactosidase (lacZ) or green fluorescent protein (GFP) genes provide effective approaches to monitor the status of Schwann cells in the host. According to monitoring studies, Schwann cells in the host are not only responsible for myelination of nerve fibers but also for the secretion of growth factors. In a tissue-engineered nerve graft composed of Schwann cells, growth factors produced by Schwann cells stimulated nerve regeneration. Moreover, the porosity of NGCs, the wall thickness, and the density of Schwann cells can modulate the concentration of growth factors produced. Even though implanted Schwann cells provide bridging of peripheral nerve gaps, several studies have debated the permanent function of Schwann cells in peripheral nerve regeneration. One reason may be that nerve gaps are not long enough to show the obvious advantages of Schwann cells or too long to optimize cellular and material components. Another reason may be that Schwann cells and NGCs have insufficient biocompatibility [102].

Embryonic Stem Cells (ESCs)

After peripheral nerve injury, many neurons die due to malnutrition. Since the number of neurons cannot be easily expanded by in vitro culture, the application of primary cultured neurons in neural tissue engineering is challenging. ESCs have great potential for unlimited proliferation and differentiation into neurons under various protocols and are therefore a good candidate as supportive cells in cell-based therapies for nerve injuries and disorders. Differentiation of ESCs can be modulated by stimulation with growth factors. Retinoic acid (RA) and nerve growth factor have been found to be potent enhancers of neuronal differentiation. [103].

Use of ESCs for Peripheral Nerve Regeneration While extensive research has focused on the implantation of ESCs for the treatment of CNS disorders, much of the potential of ESC-based therapy for PSS injuries remains unknown. In this study, ESC-derived neural progenitor cells were implanted into a 10 mm sciatic nerve gap and induced significant axonal regrowth and nerve repair. The implanted cells survived for up to 3 months and differentiated into myelinated cells. The nerve stumps showed almost normal diameter with a longitudinally oriented, densely packed Schwann cell–like arrangement. Electrophysiologic recordings confirmed the return of functional activity to the nerve cavity. [104].

Neural Stem Cells (NSC)

NSCs have the potential to differentiate into the three major cellular elements of the nervous system, including neurons, astrocytes, and oligodendrocytes, and can proliferate indefinitely while undergoing rapid cell growth in response to nerve injuries.

The characteristics of NSCs, such as multipotential differentiation, strong plasticity, high migratory ability, ease of isolation and in vitro culture, and low immunogenicity, make NSCs an attractive source of supportive cells for tissue-engineered nerve grafts. [105].

Use of NSCs for Peripheral Nerve Regeneration Chitosan NGCs with seeded NSCs were used as a tissue-engineered nerve graft to bridge 10 mm nerve gaps in rabbits. Nerve regeneration 12 weeks after implantation was like that of an autologous nerve graft. Genetically modified NSCs can also be used as a source of neurotropic factors. NSCs engineered for the overproduction of a neurotrophic factor derived from a glial cell lineage known to protect motor neurons were implanted into the distal nerve. Implantation of this neurotrophic factor can significantly increase nerve action potential amplitude, axonal area, and axon number [106].

Mesenchymal Stem Cells (MSCs)

Bone marrow mesenchymal stem cells (MSCs), also called bone marrow stromal cells, are pluripotent stem cells localized in the stromal compartment of the bone marrow, where they support hematopoiesis and differentiate into mesenchymal lineages. MSCs have increasingly wide applications in cell-based therapies for various diseases, including neural injury and disorders. This is because they can be easily obtained by aspiration of bone marrow and are widely expanded by in vitro culture.

The capabilities of MSCs include:

– Differentiate into mature cells with therapeutic potential for regenerative medicine and to populate established tissue.

- Secrete growth factors or other soluble mediators.
- The protein is presented as a vehicle for drug delivery, i.e., gene therapy [107].

Growth Factors

Growth factors refer to proteins and polypeptides produced by various cells that can regulate cell proliferation and differentiation. Since nerve regeneration is modulated by many complex interactions between cells, ECM molecules, and growth factors, the presence of growth factors at nerve injury sites plays a vital and complex role in controlling survival, proliferation, migration, and differentiation of various cell types involved in nerve regeneration. It has been shown that after nerve injury, endogenous growth factors secreted by nerve cells can promote axon regeneration, but this supportive effect cannot be sustained due to a marked decrease in cellular growth factor production over time. Therefore, a continuous, controlled supply of growth factors becomes an important research topic. Besides the neurotrophic actions provided indirectly by support cells within the neural cytoskeleton, exogenous growth factors can be directly added to the neural cytoskeleton and serve as an essential component of tissue-engineered nerve grafts [108]. Here, some of the growth factors commonly used in neural tissue engineering will be described in terms of their effects.

Neurotrophic Factors (Neurotrophins)

Neurotrophic factors play an important role in functional recovery after SCI as they protect nerve cells from apoptosis and promote axonal regeneration. Neurotrophic factors can be divided into neurotrophins, ciliary neurotrophic factors, glial cell line-derived neurotrophic factor family, and other growth factors or cytokines. Neurotrophins develop a range of neural responses by binding to specific cell surface receptors, which are divided into two classes: p75 and the tyrosine kinase receptor family (TrK). The most used neurotrophic factors are nerve growth factor (NGF), neurotrophin-3 (NT-3), and brain-derived neurotrophic factor (BDNF) [109]. Discovered in 1950, NGF is expressed at low levels in healthy peripheral nerves and is upregulated in the distal stump upon nerve injury. Recently, NGF has been shown to have the ability to enhance peripheral nerve regeneration. BDNF promotes motor neuron survival and promotes axonal outgrowth of motor and sensory neurons. Endogenous BDNF is required for peripheral nerve regeneration and remyelination after nerve injury. BDNF needs to be delivered locally and in high concentrations to produce promoting effects on nerve regeneration [110]. Gu et al. developed a tissue-engineered neural scaffold using a hybrid strategy that combines traditional biomaterials with a bioactive Schwann cell-derived extracellular matrix (SC-ECM). A key quantitative aspect of their method was the use of chitosan channels and 15 µm silk fibroin (SF) fibers to facilitate ECM deposition by Schwann cells before a decellularization process. The scaffold's efficacy was quantitatively evaluated in a 10 mm critical-sized defect in a rat sciatic nerve model. This specific gap size is a crucial parameter for assessing potential translation to larger SCI lesions. Results showed that the SC-ECM–modified scaffold achieved regenerative outcomes comparable to an acellular nerve graft (ANG), the current ECM-based gold standard, and significantly superior to a plain chitosan/SF scaffold. Critically, morphological and electrophysiological parameters such as myelin thickness and nerve conduction velocity demonstrated that the SC-ECM provides essential bioactive cues that actively promote axonal regeneration and remyelination, moving beyond mere structural support. The scaffold's safety was also confirmed. This study underscores the potential of hybrid scaffolds for neural repair. While the chitosan/SF provides the critical architectural parameters (e.g., channel structure, mechanical support) to bridge a lesion, the integrated SC-ECM adds the necessary biological signals to guide cell behavior. This synergistic approach addresses a key limitation in SCI treatment by creating a multifunctional microenvironment that could be adapted for the complex challenges of spinal cord regeneration, making it a promising strategy for future research [111].

While many studies discuss the potential of various factors, few have successfully translated them into scaffold-based applications for SCI. However, some key studies demonstrate the critical role of specific neurotrophic factors, with their efficacy often defined by precise quantitative parameters. For instance, Stokols et al. discovered that implanting an agarose scaffold loaded with BDNF into a rat spinal cord model significantly guided regenerating axons, promoting a directed linear growth pattern crucial for bridging lesions [112]. Similarly, NT-3, a factor essential for neuronal survival, growth, and synaptogenesis, has shown unique promise. It is notably considered the only factor capable of promoting regeneration in the corticospinal tract (CST), a key pathway for voluntary movement after SCI. NT-3 primarily exerts its biological effect by binding and activating the TrkC receptor.

The therapeutic potential of NT-3 was effectively demonstrated in a functional application by Sarker et al. They engineered porous NGCs made from PHEMA-MMA that were filled with a collagen matrix impregnated with NT-3 to bridge a critical 10 mm gap in a rat sciatic nerve. The quantitative outcome was clear: at the 8-week post-implantation point, the NT-3-loaded scaffolds supported significantly superior nerve regeneration compared to both hollow NGCs and NGCs containing only a plain collagen matrix [113]. This highlights how the strategic incorporation of specific

quantitative factors into a bioprinted scaffold's design is directly linked to its functional efficacy in neural repair.

Other Growth Factors with Neurotrophic Effects

In addition to the 4 members of the neurotrophins family, some growth factors such as glial cell line-derived neurotrophic factor, ciliary neurotrophic factor and acidic and basic fibroblast growth factor are also applied for peripheral nerve regeneration due to their neurotrophic function [114].

Glial cell line-derived neurotrophic factor (GDNF) was initially isolated from a rat B49 glioblastoma cell line supernatant. GDNF proved to be a potent survival factor for midbrain dopaminergic neurons and other neuronal populations [115].

Ciliary neurotrophic factor (CNTF) belongs to the family of neuropoietic cytokines (neurokines). The function of CNTF is to promote neurotransmitter synthesis and axon growth in certain neuronal populations, including astrocytes [116].

Fibroblast growth factors (FGFs) belong to the heparin-binding growth factors family. They are potent promoters of angiogenesis and help directly or indirectly in the healing of injured nerves. They stimulate mitogenesis in a wide variety of cell types, including endothelial cells, fibroblasts and Schwann cells. The best-known members of the FGF family include acidic FGF (aFGF) and basic FGF (bFGF). FGFs have been found to act as neurotrophic factors to promote neuronal survival and regeneration *in vitro* [117].

Bioink Innovation for Bone Tissue Engineering Applications

Bone Tissue Engineering Applications

Bone damage frequently occurs in daily life due to various causes, such as traffic accidents, aging, and bone tumors. In the treatment of such damage, the autograft procedure, considered the "gold standard," has been used for many years. This method involves transplanting the patient's own bone tissue to the affected area and generally yields successful results. However, there are limitations and challenges associated with autografts. These include the potential lack of sufficient healthy tissue in the recipient area and additional injury at the donor site. Due to these limitations, rapid advancements in technology and science have driven the development of alternative methods for bone tissue regeneration. Innovations in cellular therapies, bioengineering approaches, and biomaterials are among the significant developments in this field. For example, 3D printing technologies in bioengineering allow for the creation of customized bone implants, while stem cell therapies can help reconstruct bone tissue. Additionally, the use of biomaterials and bioinks is showing promising results in bone tissue engineering. These alternative methods aim to enhance both the treatment processes and outcomes, offering more effective and personalized solutions for bone tissue repair. Advances in technology and scientific research have the potential to provide broader application opportunities and more successful treatment methods in the field of bone tissue engineering [118].

Traditional Approaches

The most common traditional approach for tissue production involves seeding cells onto a biopolymer scaffold, which facilitates cell proliferation and differentiation to create tissue. In this method, cells are placed on a scaffold material designed to mimic the extracellular matrix, allowing them to grow and form tissue structures. However, there are several limitations associated with this approach. One major challenge is the adhesion of cells to the scaffold, which can affect the overall tissue quality and function. Additionally, the production of complex scaffolds can be technically demanding and costly. Another significant limitation is the insufficient supply of essential factors such as water, nutrients, growth factors, and gases, which are critical for tissue growth and development. This issue arises because the traditional scaffold-based methods lack vascularity, which is crucial for delivering these nutrients and removing waste products from the growing tissue. As a result, although these approaches have struggled to produce thick tissue layers and maintain tissue viability over time, they have laid the groundwork for further advancements in tissue engineering. Researchers are continuously working on improving scaffold materials and methods to address these limitations and enhance tissue regeneration outcomes [119].

Scaffold

Scaffolds are crucial components in modern bone tissue engineering approaches. They serve as a framework for cellular growth, proliferation, and provide the support necessary for new tissue formation. These scaffolds act like a substitute for the extracellular matrix, providing the structural and biochemical cues needed for cells to thrive and develop into functional tissue. To be effective, scaffolds must create an optimal microenvironment that facilitates cell adhesion, proliferation, and migration while maintaining a 3-D structure conducive to new tissue development [120].

For scaffolds to perform their role effectively, they need to possess a range of specific properties. Firstly, biocompatibility is essential; the scaffold material must be compatible with the human body to avoid any adverse reactions. Secondly, the scaffold must be biodegradable, allowing it to

break down gradually as the new tissue forms and matures, thus eliminating the need for surgical removal. Non-immunogenic and non-toxic are also critical to ensure that the scaffold does not trigger an immune response or release harmful substances into the body.

In addition to these fundamental properties, scaffolds must exhibit suitable porosity to allow for cell infiltration and nutrient exchange. The dimensions and spatial organization of pores are pivotal determinants of the efficacy of a scaffold in facilitating tissue regeneration. The chemical properties of the scaffold material should promote cell attachment and growth, while the mechanical properties must provide the necessary support to withstand physiological loads and stresses [121].

The architecture of the scaffold plays a pivotal role in tissue engineering. An appropriate architectural design ensures that the scaffold can support the delivery and retention of cells and biochemical factors. This design also facilitates the diffusion of essential nutrients and gases to the cells while allowing the removal of metabolic waste products. Moreover, the scaffold should exert specific mechanical and biological influences to regulate cell behavior, guiding the cells towards the desired differentiation pathways and enhancing the overall tissue formation process.

Advanced scaffolds may incorporate additional features such as growth factors, peptides, and other bioactive molecules that can further enhance cell proliferation and differentiation. These scaffolds can also be designed to mimic the natural extracellular matrix more closely, providing a more conducive environment for tissue regeneration. Through the integration of these advanced features, scaffolds in bone tissue engineering continue to evolve, offering improved outcomes for patients with bone defects and injuries [122].

3D Bioprinting

3D bioprinting is an additive manufacturing technique that constructs the desired architecture of an organ or tissue using bioink, which comprises cells, materials, and various chemical factors. This method involves depositing the bioink layer by layer. Bioink consists of live cells, biomaterials, and growth factors. The precise placement of these layers ensures that cells are positioned correctly, enabling the creation of complex tissue structures. Bioprinting is not limited to tissue production; it also finds extensive applications in research areas such as drug development, disease modeling, and personalized medicine. Particularly in the drug development process, bioprinting technology can be used to produce mini-organs or tissues from patient-specific cells. These mini-organs can be used to test the efficacy and toxicity of drugs, making preclinical research more reliable. In recent years, 3D bioprinting has gained significant attention in tissue engineering due to its ability to precisely control scaffold production and cell distribution. This technology offers a high-resolution range at the micron level (10-10000 μm), allowing cells and other components to be placed with fine details. This high precision has made bioprinting a preferred method in tissue engineering and regenerative medicine. Another advantage of bioprinting is its potential for personalized treatment. The use of bioink derived from the patient's own cells in the creation of tissues reduces the risk of rejection by the immune system. Furthermore, bioprinted tissues can be designed to align precisely with the patient's anatomy, rendering them an optimal choice for surgical procedures [123]. Gao et al. have developed a new 3D printing approach using high-strength, heat-sensitive supramolecular copolymer hydrogels for the treatment of osteochondral defects. This copolymer hydrogel, created through the synthesis of hydrogen-bonding monomers, demonstrates excellent mechanical properties such as strong tensile strength, significant stretchability, and high compressive strength. Its rapid thermoreversible gelation and solubilization make it ideal for direct 3D printing. The successful creation of 3D printed biohybrid gradient hydrogel scaffolds was achieved by leveraging the shear-thinning characteristic of the copolymer hydrogel, which allows for continuous extrusion and immediate gelation upon deposition. These scaffolds, printed with transforming growth factor beta 1 and β-tricalcium phosphate in distinct layers, were found to enhance the attachment, spreading, and chondrogenic and osteogenic differentiation of human bone marrow stem cells in vitro. In vivo studies revealed that the 3D printed biohybrid gradient hydrogel scaffolds significantly accelerated the regeneration of both cartilage and subchondral bone. This cutting-edge 3D printing technique holds great potential for treating osteochondral defects (Figure 2A) [124].

Zhai et al. introduce a nanocomposite hydrogel structure produced using 3D bioprinting technology with polyethylene glycol diacrylate (PEGDA), laponite XLG nanoclay, and sodium hyaluronate (HyA). The PEG-clay hydrogel, crosslinked for enhanced stability, was designed to aid the bioprinting process by ensuring efficient oxygen and nutrient delivery to the growing cells. The addition of hyaluronic acid to the bioink improved cell viability, dispersion uniformity, and deposition efficiency. When cells were incorporated into the PEG-clay matrix, the constructs not only supported high short-term cell viability but also promoted osteogenic differentiation over time. This created an optimal environment for the differentiation of exogenous osteoblasts both in vitro and in vivo. This innovative 3D bioprinting approach holds great potential for advancing bone tissue regeneration (Figure 2B) [125].

This different study demonstrates that chitosan solution, along with its composite form containing hydroxyapatite, can be successfully combined with cells and printed using a bioprinter. The printed hydrogels exhibited viscoelastic

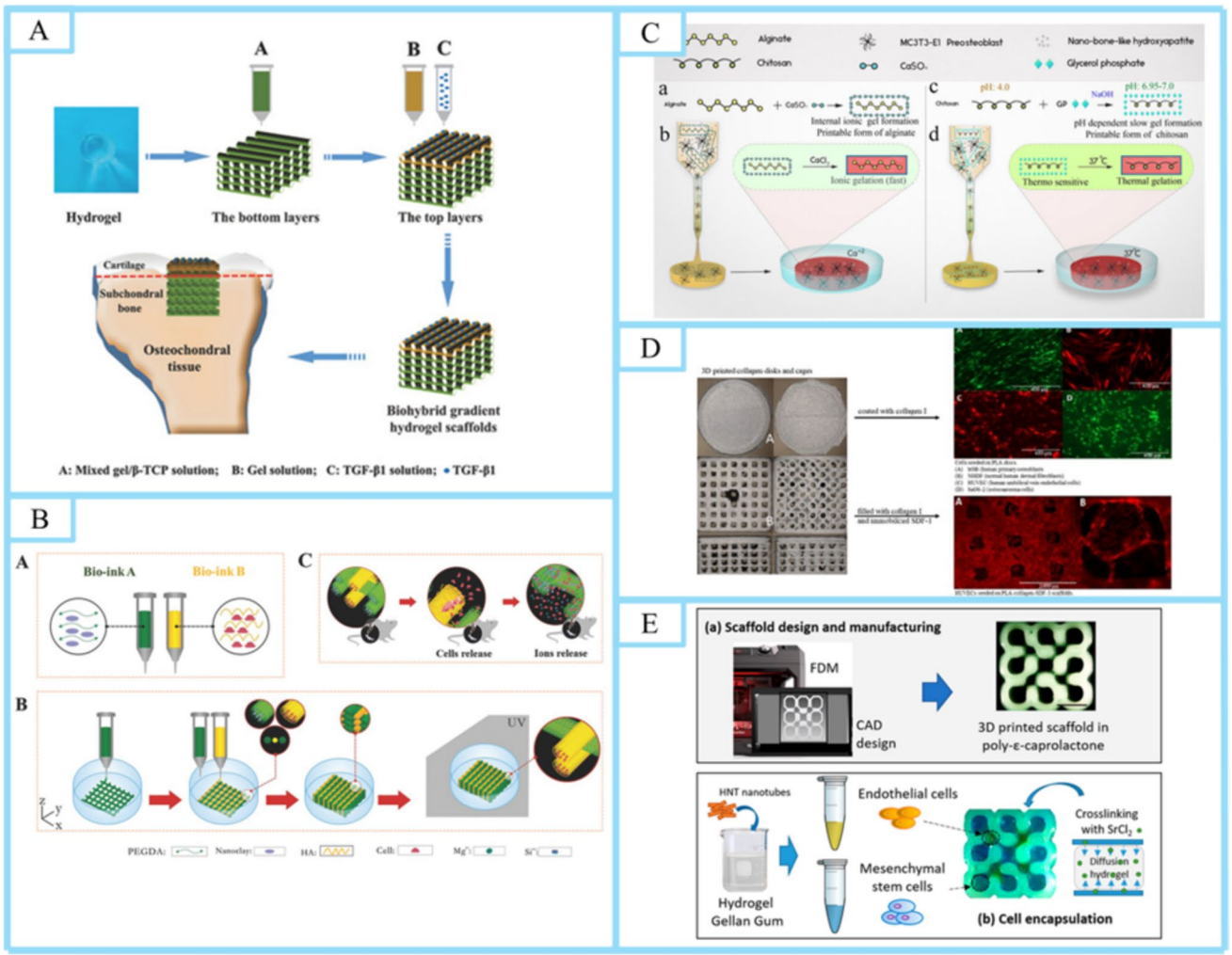


Fig. 2 **A** Schematic summary: hydrogels are synthesized by double-hydrogen-bond monomers. Gradient hydrogel scaffolds contain growth factors and β-tricalciumphosphate; they support chondrogenic/osteogenic differentiation of hBMSCs and have been shown to accelerate cartilage-bone regeneration in vivo [124]. **B** A step-by-step schematic representation illustrates the two-channel bioink system and the process utilized for 3D bioprinting and subsequent in vivo experiments. This includes: (a) the composition of bioink A and bioink B, (b) the 3D bioprinting process of ROB-loaded constructs employing the two-channel approach, and (c) a diagram depicting the in vivo experimental setup [125]. **C** Schematic diagrams illustrate the use of alginate and chitosan hydrogels as bioinks for 3D bioprinting in bone tissue engineering: (a) Alginate-HA solution is combined with $CaSO_4$ to create a printable alginate solution. (b) MC_3T_3-E_1 pre-osteoblast cells and nHA are added to the alginate-HA solution, then printed into a disk shape (6 mm diameter × 1 mm thickness) and placed in $CaCl_2$ solution for rapid ionic gelation. (c) Chitosan-HA solution (pH 4.0) is mixed with GP salt to form a printable chitosan solution (pH 6.95–7.0). (d) MC_3T_3-E1 pre-osteoblast cells and nHA are combined with the chitosan-HA solution, printed into disk shapes, and subjected to thermal ionic gelation at 37 °C and 5% CO_2 [126]. **D** Schematic summary, PLA 3D printed disks are shown to increase SDF-1 release by collagen and promote angiogenesis, demonstrating its biocompatibility [127]. **E** A schematic diagram illustrates the process of preparing the 3D construct. (a) Starting from a CAD design created with Autodesk Inventor Professional software, a 3D PCL scaffold is printed using a MakerBot Replicator 2X. (b) Cells are encapsulated in hydrogels made from gellan gum [128]

properties and maintained stability under physiological conditions, outperforming alginate hydrogels in this regard. The viscosity of the bio-solutions was found to be appropriate for three-dimensional bioprinting applications. After the printing process, cell viability and proliferation were observed across all groups. Additionally, cells printed within the chitosan-hydroxyapatite composite hydrogel showed enhanced expression of osteogenic markers. Following a 21-day culture period, cells within both chitosan and chitosan-hydroxyapatite hydrogels mineralized and differentiated into osteoblasts. The study concluded that chitosan is a more effective bioprinting material than alginate in terms of supporting cell proliferation and differentiation. These findings confirm that chitosan is a highly suitable bioprinting material with excellent printability for tissue engineering applications [126] (Figure 2C).

Liu et al. are investigating the components necessary for bone tissue engineering, including bone cells, three-dimensional scaffold materials, and osteogenic factors. To address the limitations of traditional methods, poly(lactic-co-glycolic acid) porous scaffolds were created using 3D printing technology. The printing parameters were carefully optimized to achieve scaffolds with an ideal structure. The study also explored changes in structure and porosity while examining the mechanical properties at different thicknesses. The findings present a novel approach in scaffold development for bone tissue engineering, offering potential improvements in cell placement and tissue repair support. Furthermore, the degradation rates of these 3D scaffolds under hydrolytic and enzymatic conditions were assessed, using techniques such as thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). These scaffolds, with their biocompatibility, biodegradability, and mechanical strength, supported the adhesion and proliferation of embryonic osteoblast precursor cells (MC_3T_3-E1) over time. The development of highly porous 3D scaffolds, combined with their detailed evaluation, could serve as a valuable reference for future studies and the optimization of scaffold fabrication for effective tissue regeneration [129].

Park et al. focus on the development of PCL scaffolds blended with PEG using a rapid prototyping system for 3D printing. The scaffolds were then treated to create small pores on their surface, improving their hydrophilicity. A series of tests was conducted on the resulting material, including scanning electron microscopy (SEM), water absorption, water contact angle measurement, WST-1, and Bradford assays. Additionally, cells cultured on the scaffolds were examined using confocal laser scanning microscopy (CLSM). The fabricated scaffolds showed small surface pores, which contributed to better hydrophilicity, enhanced cell proliferation, and increased protein content. These results demonstrate that incorporating small pores into the scaffolds can improve cellular interaction, making them a promising option for bone tissue engineering applications aimed at advancing human health [130].

Teixeira et al. investigate the effectiveness of surface modification of 3D printed PLA scaffolds using a polydopamine (PDA) coating, as a potential approach to enhance their bioactivity and promote the attachment of type I collagen (COL I) to the scaffold surface. The physical and chemical properties of PLA scaffolds coated with PDA, COL I, or both were assessed. Additionally, the response of porcine bone MSCs to these coatings was examined. The PDA coating improved collagen immobilisation on the PLA scaffold surface by 92%. The combination of PDA and COL functionalization provided the most favorable conditions for cellular response in the early stages (< 7 days). Furthermore, the PDA and COL-coated scaffolds supported robust extracellular matrix deposition during the first 14 days of cell culture. While MSC behavior on both uncoated PLA and PDA + COL scaffolds appeared similar by day 21, cells cultured on the PDA + COL scaffolds produced significantly higher alkaline phosphatase levels. These results indicate that the osteoinductive potential of 3D printed PLA scaffolds can be enhanced through the application of PDA and collagen type I coatings. This modification of polymeric scaffolds shows great promise for advancing bone tissue engineering [131].

Ritz et al. investigate the biocompatibility and endotoxin contamination of solid disks and porous cage-like structures created from PLA, which are either coated or filled with collagen. The analysis included microscopic evaluations and proliferation assays using several cell types on the PLA disks. The study also examined the release of stromal-derived factor (SDF-1) from collagen-filled cages and its effects on endothelial cells. The findings confirm that PLA is biocompatible and that endotoxin levels are well below FDA limits. Various cell types, such as osteoblasts, osteoblast-like cells, fibroblasts, and endothelial cells, were able to grow, spread, and proliferate on the PLA disks. The SDF-1 release from the collagen-filled PLA cages was consistent, supporting endothelial cell growth and promoting new blood vessel formation. These results highlight the potential of PLA scaffolds, manufactured using affordable desktop 3D printers, for use in medical applications, particularly in bone tissue engineering (Figure 2D) [127].

In a similar study, the use of fused deposition modeling (FDM) to 3D-print porous PCL scaffolds, which were subsequently modified with mineral additives commonly used in clinical and commercial settings: tricalcium phosphate (TCP), hydroxyapatite (HA), Bio-Oss (BO), and decellularized bone matrix (DCB). The print quality of the composite scaffolds was assessed, revealing that the PCL-TCP, PCL-BO, and PCL-DCB scaffolds exhibited a print quality of approximately 0.7, which was lower than that of PCL and PCL-HA scaffolds, both of which had a print quality of around 0.8. Incorporating the mineral particles did not significantly affect the compressive modulus of the scaffolds, with solid blocks showing values around 260 MPa and porous scaffolds ranging from 32 to 83 MPa. Raman spectroscopy confirmed that the scaffolds maintained the chemical integrity of their dopants after printing. The osteoinductive properties of the different scaffold composites were evaluated by culturing adipose-derived stromal/stem cells and assessing their differentiation into osteoblasts. Significant increases in calcium content (normalized to DNA) were observed in the PCL-TCP ($p < 0.05$), PCL-BO ($p < 0.001$), and PCL-DCB ($p < 0.0001$) groups compared to the PCL group. A similar trend in calcium content increase was observed in the PCL-HA group, though not statistically significant ($p > 0.05$). The expression of collagen type I was

found to be 10 times greater in the PCL-BO and PCL-DCB groups than in PCL alone ($p < 0.05$). Osteocalcin expression was also 10 times higher in the PCL-BO and PCL-DCB groups ($p < 0.05$), as measured by quantitative real-time PCR. These results suggest that PCL-BO and PCL-DCB hybrid materials may offer significant advantages over PCL-HA or PCL-TCP blends for bone healing applications [132].

Kuss et al. explore the integration of 3D printing technology with a hydrogel-based prevascularization approach to create prevascularized bone constructs. Human adipose-derived mesenchymal stem cells (ADMSCs) and human umbilical vein endothelial cells (HUVECs) were incorporated into bioactive hydrogels, and the impact of various culture conditions on vascularization in vitro was assessed. Additionally, composite structures were formed by 3D printing PCL and hydroxyapatite (HA) scaffolds, which were then coated with cell-laden hydrogels. The effect of co-culturing the cells on both vascularization and osteogenesis was evaluated. It was observed that the co-cultured ADMSC-HUVECs were able to form capillary-like networks within the porous 3D printed scaffolds. The co-culture system effectively enhanced vascularization in vitro but had little effect on osteogenesis. The prevascularized constructs were implanted subcutaneously into nude mice to assess their in vivo vascularization potential and the functionality of the engineered vessels. The hydrogel systems promoted the development of microvessels and lumens and facilitated the connection of human vascular networks with the murine host vasculature. These results highlight the promise of prevascularized 3D printed scaffolds with anatomical shapes in healing larger bone defects [133].

De Giglio et al. are investigating the design and production of multi-chambered structures using PCL and fused filament fabrication (FFF) 3D printing techniques. The design includes interconnected compartments, providing a suitable environment for the incorporation of stem cells and endothelial cells. The cells were encapsulated in an optimized gellan gum (GG)-based hydrogel matrix, which was crosslinked with strontium (Sr^{2+}) ions to enhance bioactivity, and assembled within compartments of various sizes. As a control, calcium (Ca^{2+})-crosslinked hydrogels were used to compare the osteogenic effects of Sr^{2+} ions. Results indicated that Sr^{2+} ions successfully diffused within the hydrogel matrix, improving its strength under compressive loading. The in vitro co-culture of human-TERT mesenchymal stem cells (TERT-hMSCs) and human umbilical vein endothelial cells (HUVECs), encapsulated within Sr^{2+}-containing GG-hydrogels and interconnected by compartmentalized scaffolds under osteogenic conditions, exhibited enhanced cell viability and promoted osteogenesis. This was evidenced by significant increases in alkaline phosphatase activity, osteopontin, and osteocalcin when compared to Ca^{2+}-crosslinked GG-PCL scaffolds. These findings highlight that compartmentalized co-culture systems of TERT-hMSCs and HUVECs can serve as an effective method for investigating and promoting osteogenesis (Figure 2E) [128].

In conclusion, 3D bioprinting technology offers significant potential for bone tissue engineering applications. Advances in bioinks and printing techniques enable the creation of customized, biologically compatible, and functional bone scaffolds. As this technology progresses, it could revolutionize the treatment of bone injuries by providing personalized solutions for patients. However, further research is needed in areas such as bioink formulations, cell interactions, and tissue integration for successful clinical translation.

Extrusion-Type Bioprinters

Willson et al. chose extrusion-type bioprinting machines for 3D bioprinting. This method is straightforward and capable of printing various materials as well as achieving high cell densities. It is also slower compared to other methods, has good vertical printing capability, is moderately priced, and has a resolution of 100 µm. This technique provides good structural integrity due to the continuous deposition of the bioink. Unlike other bioprinters, the extrusion-type bioprinter generates a continuous line of bioink rather than droplets by applying pressure, either pneumatically or mechanically, to release the bioink from a syringe. Extrusion-type bioprinters offer a wider range of bioinks, including high-viscosity materials such as hydrogels, copolymers, cell spheroids, and paste forms of bioinks. Unfortunately, the cell survival rate is lower compared to other types of bioprinters due to the substantial pressure applied. Generally, this type of bioprinter is preferred for the fabrication of aortic valves, branched vessels, and tumor models. Common extrusion-based printing methods can be categorized into three types: pneumatic, piston-driven, and screw-driven dispensing. In pneumatic dispensing, the required driving force is provided by air pressure, while in piston and screw-driven dispensing, vertical and rotational motion initiates the printing process. Three factors are considered in evaluating printability in extrusion-type bioprinters: adjustability of the viscosity, the phase of the bioink before and during bioprinting, and material-specific biofabrication properties. Additionally, the produced bioink should not cause clogging in the nozzle. Finally, bioinks with desired properties may not be printable, or not all printable bioinks may have the desired features [134].

Bioink for Tissue Engineering Applications

The pursuit of ideal bioinks for SCI repair has led to innovative strategies that move beyond providing mere

structural support to actively orchestrating the regeneration process. These strategies often focus on biomimicry, modulation of the pathological microenvironment, and direct instruction of embedded or host cells.

A fundamental approach involves recapitulating the native neural tissue's ECM to provide innate biological cues. In recent years, 3D bioprinting has gained considerable attention as an innovative tool in regenerative medicine. By recreating features of the extracellular matrix, this technology enables the fabrication of scaffolds that facilitate cell transplantation. One strategy to improve cellular adhesion, viability, and proliferation is the incorporation of decellularized extracellular matrix into bioinks. In this study, Santos et al. developed a bioink derived from lyophilized rat decellularized spinal cord tissue (DSCT) for use in 3D bioprinting of neural constructs. Evaluation through DNA quantification, hematoxylin and eosin staining, and DAPI staining confirmed that treatment with 1% sodium dodecyl sulfate for 9 hours efficiently eliminated cellular content from spinal cord samples. Cytotoxicity testing demonstrated that the decellularized scaffold was biocompatible with PC12 cells. The hydrogel formulation, consisting of DSCT, alginate, and gelatin, exhibited shear-thinning characteristics and a low G″/G′ ratio, providing suitable printability while maintaining post-printing cell viability. Importantly, the bioink supported long-term culture, with 93% of PC12 cells remaining viable four weeks after printing, and it promoted the expression of laminin-1 and neurofilament-M. These findings suggest that the proposed DSCT-based bioink is a readily accessible biomaterial with strong potential for applications in central nervous system tissue engineering [135].

In in vitro studies using brain-derived decellularized ECM (cortex, cerebellum, other regions), these biomaterials provided structural and biochemical cues, enhancing neural cell growth, phenotypic modulation, and cell rescue capacity in conditions mimicking ischemia. However, as natural ECM has low electrical conductivity and its mechanical properties are difficult to standardize, its direct applicability to clinical biosurrogates for SCI has been limited. While this study highlights how mimicking the biochemical richness of natural ECM is vital for neural regeneration, it also highlights a major limitation of this approach: it lacks the functional conductivity to support electrophysiological activity of the spinal cord. Furthermore, the difficulty of controlling and standardizing the mechanical properties of natural polymers of decellularized origin may limit their large-scale use in bioprinting applications [136].

However, effective regeneration in SCI also requires actively mitigating the inhibitory post-injury microenvironment, characterized by inflammation and a lack of supportive signaling. The hostile microenvironment that develops after SCI continues to hinder effective neural regeneration. Restoring a supportive niche through biocompatible scaffolds with multifunctional properties has therefore become a central focus in SCI repair strategies. In this work, Luo et al. engineered a hybrid hydrogel composed of Fmoc-modified chitosan (FC) and an Fmoc peptide (FI). The reversible π-π stacking of fluorenyl groups endowed the FC/FI hydrogel with excellent injectability and self-healing behavior, as confirmed through visual inspection and rheological analysis. To further enhance therapeutic potential, curcumin was incorporated into the system, yielding the FC/FI-Cur hydrogel, which provided controlled and sustained drug release. In vitro experiments demonstrated that the FC/FI-Cur hydrogel promoted neurite extension and supported Schwann cell migration from dorsal root ganglion (DRG) spheres, while also facilitating enhanced myelination. When applied to a rat SCI model, the hydrogel reconstructed extracellular matrix structures at the lesion site and modulated the inflammatory response by regulating infiltrating immune cell phenotypes. Moreover, it recruited endogenous SCs, which contributed to the remyelination of regenerating axons. These combined effects led to significant improvements in motor function recovery and electrophysiological performance. Overall, this study highlights the promise of multifunctional FC/FI-Cur hydrogels as both a therapeutic platform and a guiding strategy for advancing SCI regeneration [137].

Building upon these concepts, the most advanced bioinks aim to provide dynamic, cell-instructive signals that precisely guide stem cell fate and the formation of functional neural networks. Biomimetic neural substitutes fabricated via bottom-up 3D bioprinting approaches offer considerable potential for repairing neural tissue. Yet, a major challenge lies in directing neural stem cell (NSC) differentiation with high precision to secure functional outcomes. The behavior of NSCs is strongly influenced by their dynamic interactions and mechanotransduction processes within three-dimensional microenvironments. To tackle this issue, Yang et al. developed a dynamic bioactive bioink that integrates reversible Schiff-base linkages with biofunctional motifs, N-cadherin-inspired and BDNF-mimicking peptides, providing tunable mechanical and biochemical signals to guide NSC fate. Their findings show that the adaptable nature of the bioprinted living fibers reduces mechanical restrictions on NSCs, thereby promoting their mechanosensitivity, spreading, motility, and ability to remodel the surrounding matrix. Furthermore, the inclusion of N-cadherin- and BDNF-like peptides enhances NSCs' responsiveness to both mechanical and neurotrophic stimuli, which accelerates the self-assembly of functional neural networks within the 3D printed constructs. Importantly, these engineered networks contributed to notable recovery of motor and sensory functions in a rat model of complete spinal cord transection. Overall, this work highlights the necessity of carefully designing

cell-instructive bioinks to advance the performance of 3D bioprinted living systems for neural regeneration [138].

Structures were created that encapsulate NSCs in ECM-like hydrogel, mimicking compact nerve fiber bundles. These bioprinted constructs, evaluated on a rat SCI model (4 mm full transection), promoted angiogenesis and neurogenesis by modulating immune responses, and supported neural relay formation and circuit reorganization. As a result, a significant improvement in functional motor recovery was observed. This approach transformed bioprinted constructs from static scaffolds into "active, living structures", but engineering challenges remain, such as high cell density and long culture times. The major contribution of this approach is that it transforms the implanted construct from a static scaffold into a living entity that actively participates and evolves in the regenerative process. However, the manufacturing and processing challenges of such cell-viable inks, the constraints on the printability of cell density, and the possibility of long pre-transplant culture times are significant engineering problems that may complicate their practical application in the clinic [139].

CNT/GelMA fibers produced by rotational liquid bath electrospinning showed alignment, conductivity, and soft mechanical properties mimicking axons. In vitro, PC12 cell proliferation and directional adhesion were enhanced by ES; neuronal differentiation and axon-like extensions developed in NSCs. In vivo, fibers increased electrical conductivity, promoted axon regeneration and remyelination, and motor function was markedly restored in the T9 transection rat model. The possible toxic effects and long-term safety of CNTs, as well as the standardization of ES protocols, remain to be addressed. This study redefines the role of bioinks, positioning them as active therapeutic systems that are more than just a structural and electrical framework, but act as an 'interface' to deliver and amplify rehabilitative electrical stimulation. This synergistic combination of regenerative and rehabilitative approaches offers some of the most promising outcomes for functional recovery to date. However, ongoing concerns over the possible toxicological profile and long-term safety of CNT-containing inks necessitate extensive dosage and safety studies in preclinical models. Furthermore, standardization of electrical stimulation protocols (duration, frequency, intensity) is a critical prerequisite for reproducibility and large-scale adoption of this technique [140].

3D biobased conductive bioscaffolds fabricated with gelatin methacrylate (GelMA), hyaluronic acid methacrylate (HAMA), and PEDOT: LS supported high NSC viability (>90%) and markedly enhanced neuronal differentiation. Using a rat complete transection model, these conductive scaffolds restored motor function, reduced glial scar formation, and promoted axon growth and remyelination. Long-term biocompatibility and in vivo degradation of PEDOT: LS still need to be investigated. This innovative work embodies how imparting not only biocompatibility but also functional conductivity to bioinks can significantly enhance neuronal differentiation of neural stem cells and functional motor recovery. However, the long-term biocompatibility and in vivo degradation profiles of synthetic conductive components (such as PEDOT) are still not fully elucidated, posing a potential barrier to clinical translation. Furthermore, while this approach mainly offers a passive conductive scaffold for cells, the capacity to actively direct cells to form a three-dimensional and functional network within the bioink may be limited [141].

In conclusion, the evolution of bioink design for SCI is progressing from passive, biomimetic scaffolds to active, communicative systems that can modulate the microenvironment and precisely instruct cellular behavior to achieve functional repair.

Result and Discussion

This review highlighted tissue engineering approaches for spinal cord repair using GO/PLGA-PEG polymer/ HA and three-dimensional nanocomposite scaffold implantable using stem cells and growth factors at the site of spinal cord injury. Given the loss of sensory and motor control over the entire body below the site of injury after spinal cord injury, enabling axonal regeneration at the damaged site to connect with target neurons in the spinal cord will be a critical application for biomaterials approaches. Important bioengineering topics have provided an overview of some important applications and modifications of key materials for scaffold design, with fabrication techniques for macroengineering and microengineering.

Graphene, which has recently received much attention in biomedical applications, has high potential for regeneration of axons after spinal cord injury due to its high electrical conductivity and excellent biocompatibility with neurons. It is an ideal material for use in tissue scaffolding.

One of the most important problems in the design of nanocomposite scaffolds is to regulate the degradation rate of the scaffold. Accordingly, toxicity should be minimized. Tuning the biodegradation rate of scaffold base materials is a topic that needs to be investigated more extensively in the future. Moreover, controlling the conditions during the fabrication of composites to ensure homogeneous dispersion, alignment, and polymer-material interaction in a nanocomposite scaffold is still a major challenge. Although the functionalization of the graphene surface is being done to improve the interaction of graphene and the polymer matrix, the control of conditions such as graphene orientation, graphene contact remains a major challenge. Future work in

scaffold design may be directed towards providing more controlled conditions.

Conclusion

The creation of effective tissue scaffolds for spinal cord injury is a complex, multidisciplinary endeavor requiring an in-depth understanding of the nervous system's structure. With its intricate network of axonal pathways and neuronal connections, the nervous system demands scaffolds capable of mimicking its complexity. Innovative materials like GO/PLGA-PEG polymer combined with HA-based three-dimensional nanocomposites offer promising solutions. When paired with stem cells and growth factors, these scaffolds create an environment conducive to axonal regeneration, promoting functional recovery. By re-establishing connections with target neurons, these strategies address the vital challenge of restoring lost sensory and motor functions following spinal cord injury. Advancements in bioink technology for bone tissue engineering have greatly influenced the development of scaffolds for spinal cord repair. These bioinks, engineered to replicate native tissue environments, enable precise control over scaffold characteristics such as mechanical durability, biodegradability, and compatibility with cells. Methods adapted from bone tissue engineering are proving valuable for enhancing the fabrication of neural scaffolds, thereby improving their capacity to support neural regeneration. Additionally, embedding bioactive molecules within these bioinks can significantly enhance scaffold functionality, making them an essential tool in the realm of regenerative medicine. Despite these advancements, challenges remain. It is crucial to carefully regulate the biodegradation rates of scaffolds to avoid toxicity while maintaining their structural integrity throughout tissue regeneration. Materials like graphene, known for their excellent electrical conductivity and biocompatibility, show promise in promoting axonal growth. However, further research is needed to ensure their consistent performance. Future studies should focus on refining materials and developing scalable fabrication techniques. By combining knowledge from neural science and biofabrication, there is potential to revolutionize scaffold design, ultimately leading to significant improvements in the quality of life for those suffering from spinal cord injuries.

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Declarations

Conflict of interest The authors declare no competing interests.

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